

# Biochar Properties Influencing Greenhouse Gas Emissions in Tropical Soils Differing in Texture and Mineralogy

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## Abstract

The ability of biochar applications to alter greenhouse gases (GHGs) ( $\text{CO}_2$ ,  $\text{CH}_4$ , and  $\text{N}_2\text{O}$ ) has been attracting research interest. However, inconsistent published results necessitate further exploration of potential influencing factors, including biochar properties, biochar rates, soil textures and mineralogy, and their interactions. Two short-term laboratory incubations were conducted to evaluate the effects of different biochars: a biochar with low ash (2.4%) and high-volatile matter (VM) (35.8%) contents produced under low-temperature (350°C) traditional kiln and a biochar with high ash (3.9%) and low-VM (14.7%) contents produced with a high-temperature (800°C) Flash Carbonization reactor and different biochar rates (0, 2, and 4% w/w) on the GHG emissions in a loamy-sand Ultisol and a silty-clay-loam Oxisol. In the coarse-textured, low-buffer Ultisol, cumulative  $\text{CO}_2$  and  $\text{CH}_4$  emissions increased with increasing VM content of biochars; however,  $\text{CO}_2$  emission sharply decreased at 83  $\mu\text{g VM g}^{-1}$  soil. In the fine-textured, high-buffer Oxisol, there were significant positive effects of VM content on cumulative  $\text{CO}_2$  emission without suppression effects. Regarding cumulative  $\text{N}_2\text{O}$  emission, there were significant positive effects in the Mn-rich Oxisol. Ash-induced increases in soil pH had negative effects on all studied GHG emissions. Possible mechanisms include the roles biochar VM played as microbial substrates, a source of toxic compounds and complexing agents reducing the toxicity of soil aluminum and manganese, and the role of biochar ash in increasing soil pH affecting GHG emissions in these two contrasting soils.

## Core Ideas

- Biochar's volatile matter and ash constituents affect soil emission of  $\text{CO}_2$ ,  $\text{CH}_4$ , and  $\text{N}_2\text{O}$ .
- Biochar volatile matter raised  $\text{CO}_2$  emission up to a threshold in coarse-textured, low-buffer soil.
- Biochar volatile matter raised  $\text{CO}_2$  emission regardless of its content in fine-textured, high-buffer soil.
- Biochar ash decreased emissions of greenhouse gases regardless of soil texture.
- Al in Ultisol and Mn in Oxisol interacted with volatile matter and ash in influencing GHG emissions.

**B**IOCHAR (pyrolyzed biomass), when used as a soil amendment and for carbon sequestration, has the potential to improve soil and environmental quality (Gurwick et al., 2013). Although biochar shows great potential to increase carbon sequestration, its effects on greenhouse gas (GHG) emissions remain the subject of debate. Some studies reported that application of biochars decreased emissions of  $\text{CO}_2$ ,  $\text{CH}_4$  (Karhu et al., 2011; Liu et al., 2011), and  $\text{N}_2\text{O}$  (Karhu et al., 2011; Zhang et al., 2012); other studies showed increases (Junna et al., 2014; Zhang et al., 2010); and others showed no effect of biochar on GHG emissions (Wang et al., 2012; Zhang et al., 2012). According to a meta-analysis by Cayuela et al. (2014), the inconsistent effects of biochars on GHG emissions were influenced by several factors, notably biochar properties, biochar rates, soil texture and mineralogy, and their interactions.

Recent studies show that biochar properties, such as volatile matter (VM) and ash contents, have variable effects on GHG emissions. High-VM biochars have decreased  $\text{N}_2\text{O}$  emissions (Spokas, 2013) but increased  $\text{CO}_2$  and  $\text{CH}_4$  emissions (Deenik et al., 2010; Spokas, 2013). Toxic effects of volatile organic compounds on nitrifying and denitrifying microorganisms were proposed to explain the decreased  $\text{N}_2\text{O}$  (Spokas, 2013). Regarding increases in  $\text{CO}_2$ , there is some evidence that emissions are a result of rapid decomposition of labile organic compounds (Deenik et al., 2010). Meanwhile, the inhibitory effects of ethylene on methanotrophic bacteria resulted in increases in  $\text{CH}_4$  emission (Spokas, 2013). Yet other studies attribute decreased  $\text{N}_2\text{O}$  emissions to the reducing power of surface functional groups and dissolved organic substances from biochar (Ahmad et al., 2014), leading to decreases in  $\text{N}_2\text{O}$  emission through biochar-mediated reduction of  $\text{N}_2\text{O}$  to  $\text{N}_2$  (Cayuela et al., 2013). Biochar ash content is also related to decreased  $\text{CO}_2$  and  $\text{N}_2\text{O}$  emission (Cayuela et al., 2013; Spokas and Reicosky, 2009), but the mechanisms underlying these effects have not been elucidated. A possible mechanism may be associated with increases in soil pH to levels that inhibit microbial  $\text{CO}_2$  production and

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**Abbreviations:** DAI, day after biochar incorporation; FC, Flash Carbonization; GHG, greenhouse gas; TK, traditional kiln; SOC, soil organic carbon; VM, volatile matter.

denitrification, but previous work has shown that liming acid soils could enhance (Čuhel et al., 2010) or depress  $N_2O$  emission (Barton et al., 2013), with  $N_2O$  emissions increasing when soils were limed close to the neutral pH but decreasing when pH exceeded the neutral value (Mørkved et al., 2007).

In addition to conflicting effects of biochar VM and ash contents on GHG emissions, the interaction of soil texture and biochar has produced widely varying effects on GHG emissions (Cayuela et al., 2014). Some studies have reported that  $CO_2$  emissions were higher in coarse-textured than in fine-textured soils amended with biochar (Fang et al., 2014; Sun et al., 2014). On the other hand, Gomez et al. (2014) and Sigua et al. (2014) reported conflicting results. Results for  $CH_4$  emissions are equally inconsistent and difficult to interpret, with Xie et al. (2013) reporting higher  $CH_4$  emissions in coarse-textured than in fine-textured soils, contrary to the findings of Zhang et al. (2012). Similar discrepancies exist for  $N_2O$  emission: Xie et al. (2013) reported higher emission in coarse-textured than in fine-textured soils, but opposite findings were reported by Sun et al. (2014). A recent sophisticated study using soils ranging from coarse sandy loam to fine clay demonstrated that the effects of biochar on the three GHG emissions were complex, involving changes in various soil properties, notably soil C and N status, pH, bulk density, and aeration, all of which were generally influenced by soil texture (Stewart et al., 2013). The study found that although biochar C was used as microbial substrate in soil organic C (SOC)-poor, coarse-textured soils resulting in SOC negative priming, a SOC-rich, fine-textured counterpart produced positive priming.

In the tropics, Oxisols and Ultisols with tendencies toward Al and Mn toxicity occupy large areas of agricultural land (Sanchez and Logan, 1992). Recent studies have shown that biochar can alleviate the phytotoxicities of soil Al (Hossain et al., 2010) and Mn (Butnan et al., 2015), but its concurrent effects on microbially mediated GHG emissions are not known. Previous work has shown that high concentrations of soil Al decreased  $CO_2$  production through inhibitory effects of Al on decomposition enzymes (Mutatkar and Pritchett, 1966) and the interaction of Al oxides and hydroxides with microbial substrates, such as phenolic compounds (Miltner and Zech, 1998a) and carbohydrates (Miltner and Zech, 1998b). High Al concentration can also reduce  $N_2O$  emission through its toxic effects on some  $N_2O$ -producing microorganisms, such as *Nitrosomonas europaea* (Nishina et al., 2009), *Aspergillus flavus* (Firestone et al., 1983), and *Escherichia coli* (Guida et al., 1991). To our knowledge, there is no evidence in the literature on soil Al affecting  $CH_4$  emission. Because some biochars can alleviate Al biotoxicity (Chan et al., 2007; Hossain et al., 2010), application of biochar may lead to increases in  $CO_2$  and  $N_2O$  emissions.

There is no evidence directly relating soil Mn content to GHG emissions. However, Miltner and Zech (1998b) reported that a Mn oxide (birnessite) increased the decomposition rate of carbohydrate from beech leaf litter. They speculated that Mn was an oxidizing agent for carbohydrate decomposition. In ammonium transformation processes, Mn is a catalyst for anaerobic ammonium oxidation (anammox) (Luther et al., 1997) and aerobic ammonium oxidation (Clément et al., 2005), transforming  $NH_4^+$  to  $N_2$ . Although biochar may have some potential in alleviating Mn toxicity, little is known about the mechanisms, which

may involve liming from the ash component, complexation from biochar-derived organic compounds (Butnan et al., 2015), or reducing power from biochar surface functionality (Graber et al., 2013).

It appears that there have been inconsistent results reported on the responses of GHG emissions to biochar application, which are purported to be due to interactions between biochar types, rates, and soil properties. We hypothesized that biochar properties, notably VM and ash content, would interact with soils contrasting in texture and mineralogy in affecting emissions of these GHGs. A high-VM, low-ash biochar stimulates the emissions of these GHGs, whereas a low-VM, high-ash counterpart depresses them. These effects are more pronounced in coarse-textured soils than in fine-textured soils. It was further hypothesized that VM and ash of biochars interact with soil Al and Mn in affecting GHG emissions. The objective of this study was, therefore, to evaluate the effects of biochars derived from similar feedstock but produced under different pyrolysis temperatures on GHG emissions (i.e.,  $CO_2$ ,  $CH_4$ , and  $N_2O$ ) in a coarse-textured Ultisol as compared with a fine-textured Oxisol.

## Materials and Methods

### Soils

Two soils differing in texture and mineralogy were used: (i) a loamy sand, Al-rich Khorat soil (isohyperthermic Typic Kandistults) and (ii) a silty clay loam, Mn-rich Wahiawa soil (kaolinitic isohyperthermic Rhodic Haplustox). The Khorat soil was collected from 0 to 15 cm depth at the Fruit Tree Research Station at Khon Kaen University, Thailand (16°27'50" N; 102°48'14" E). The Wahiawa soil was collected from 0 to 15 cm depth at the Poamoho Research Station, University of Hawaii (21°32'30" N; 158°05'15" W). The Khorat and Wahiawa were representative of highly weathered tropical soils used intensively in agriculture in northeastern Thailand and Hawaii, respectively. Both soils have a history of phytotoxicity due to Al in the Khorat (Butnan et al., 2015) and Mn in the Wahiawa (Hue et al., 2001). The soils were air-dried, sieved to pass through a 2-mm sieve, and stored in polyethylene containers at room temperature. The Khorat soil was shipped to the University of Hawaii at Manoa where the present study was conducted. Initial physical and chemical properties of the Khorat and Wahiawa soils have been reported in Butnan et al. (2015) (Supplemental Table S1).

### Biochars

Two biochars produced from the same feedstock but under different pyrolysis conditions were used in this study. The feedstock was the upper parts and branches of a eucalyptus tree (*Eucalyptus camaldulensis* Dehnh.) trimmings remaining after tree cutting for paper pulp production from the Mancha Khiri Forest Plantation Station, Khon Kaen province, Thailand. Biochar was produced under either Thai traditional kiln (TK) or Flash Carbonization (FC) techniques. The TK biochar consisted of eucalyptus branches, which were chopped in 30-cm lengths (diameters, 6–7 cm). They were subsequently pyrolyzed under low temperature (~350°C) with low  $O_2$  for 3 d and then cooled with water and air-dried for 4 d. The TK biochar was randomly sampled from the manufacturer to represent the bulk of the biochar produced and transported to University of Hawaii

at Manoa. The FC biochar was produced by the Hawaii Natural Energy Institute at the University of Hawaii at Manoa using the high-temperature FC process, under which the eucalyptus feedstock was subjected to a controlled flash fire (40 min) at 1 MPa, with peak temperature reaching up to 800°C. The eucalyptus branches were cleaved into quarters to fit in the FC canister. Both biochars were crushed and sieved to pass a 2-mm sieve and stored at room temperature. Three random samples from each biochar material were sampled and analyzed for proximate analysis and selected chemical properties. The initial properties of biochars can be found in Butnan et al. (2015) (Supplemental Table S2).

## Laboratory Incubation Experiments

Two laboratory incubation experiments were conducted separately to evaluate the effects of biochars on soil mineral N ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) contents (Experiment 1) and to determine GHG emissions ( $\text{CO}_2$ ,  $\text{CH}_4$ , and  $\text{N}_2\text{O}$ ) (Experiment 2) in the two studied soils. The two separate experiments allowed for destructive soil sampling for mineral N determination without affecting GHG determinations. They were performed in similar controlled conditions (air-conditioned laboratory at 25°C) during two periods (October–November 2011 for Experiment 1 and January–February 2012 for Experiment 2). A three-way factorial arrangement (2 soil types  $\times$  2 biochar types  $\times$  3 biochar rates) in a randomized complete block design with three replications was used in both experiments. One hundred grams of each soil amended with TK or FC biochar at 0, 2, and 4% (w/w) was mixed thoroughly and placed in wide-mouth polypropylene jars (125 cm<sup>3</sup> for Experiment 1 and 635 cm<sup>3</sup> for Experiment 2). The soil/biochar mixtures were watered with deionized water once a day to predetermined moisture contents (20.5 and 63.4% w/w for the Khorat and the Wahiawa soils, respectively) equivalent to 70% water holding capacity as determined by the cylinder method (Wilke, 2005). All treatments received a basal fertilizer application of  $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ ,  $\text{K}_2\text{SO}_4$ ,  $\text{MgCl}_2$ ,  $\text{ZnSO}_4$ , and  $\text{H}_3\text{BO}_3$  to achieve 300 mg P, 250 mg K, 100 mg Mg, 5 mg Zn, and 2 mg B kg<sup>-1</sup> soil. In Experiment 1, soil samples were collected at the end of the incubation (30 d after biochar incorporation [DAI]) for mineral N determination. The soil samples were kept frozen at -13°C until mineral N ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) analysis. In Experiment 2, GHG emissions were determined through gas samplings at 1, 2, 4, 8, 16, and 32 DAI. At each sampling time, a jar containing the sample was tightly capped for 24 h. A 10-mL syringe was used to draw the air from the jar, and the sample was immediately discharged into a previously evacuated 10-mL glass exetainer containing He gas. The exetainers were stored for 7 d before GHG ( $\text{CO}_2$ ,  $\text{CH}_4$ , and  $\text{N}_2\text{O}$ ) determination.

## Laboratory Analyses of Biochars, Soils, and Greenhouse Gases

Contents of biochar constituents (VM, ash, and fixed C) were determined using the proximate analysis method following ASTM 1762-84 (American Standard of Testing Material, 2001). Volatile matter, ash, and fixed C contents of TK biochar were 35.8, 2.35, and 61.9%, respectively; those of FC biochar were 14.7, 3.85, and 81.5%, respectively (Butnan et al., 2015).

Soil and biochar pH was determined in a 1:5 soil or biochar to deionized water ratio. The pH of the biochar was 6.52 and

8.92 for the TK and FC biochar, respectively. Mineral N ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) of soils and biochars was extracted with 2 mol L<sup>-1</sup> KCl and measured using a Discrete Autoanalyzer (EasyChem, Systema Scientific). Soil Al (in a 1 mol L<sup>-1</sup> KCl extract) and soluble Mn (in a saturated paste) data came from an accompanying study, which consisted of the same soils and biochar treatments (Butnan et al., 2015). Extractable Al and Mn contents were determined by inductively coupled plasma spectrophotometry on a Thermo Jarrell Ash Atom Scan 16 instrument.

For measurement of GHG emissions, a gas chromatograph (GC-2014, Shimadzu USA) equipped with a methanizer and a flame ionization detector was used to measure  $\text{CH}_4$  and  $\text{CO}_2$ , and an electron capture detector was used to measure  $\text{N}_2\text{O}$ .

## Data Calculations and Statistical Analyses

Volatile matter contents applied to soils of all treatments were calculated on a soil air-dried weight basis (Butnan et al., 2015). They are hereafter referred to as “added VM.” Cumulative emissions of  $\text{CO}_2$ ,  $\text{CH}_4$ , and  $\text{N}_2\text{O}$  were calculated as the sum of areas under the curves of each successive sampling interval.

A three-way ANOVA (F-test) based on randomized complete block design in a factorial arrangement was used to determine the effects of soil type, biochar type, and biochar application rate on soil properties and cumulative  $\text{CO}_2$ ,  $\text{CH}_4$ , and  $\text{N}_2\text{O}$  emissions. Mean comparisons were performed using Tukey's Studentized range test. Repeated-measures ANOVA under PROC GLM was applied to cumulative  $\text{CO}_2$  and  $\text{N}_2\text{O}$  emissions data to determine biochar treatments  $\times$  time interactions. Relationships of added VM and biochar-derived soil properties, the latter of which were published data of Butnan et al. (2015), with cumulative GHG emissions were determined using Pearson's correlation coefficient ( $r$ ) in the case of one-on-one independent relationships and robust stepwise-multiple regression with least-trimmed square estimation (Wilcox, 2001) using the ROBUSTREG procedure (Chen, 2002) in the case of combination effects of more than one interacting independent variable. All of these statistical analyses were performed using SAS software version 9.1 (SAS Institute). Graph plotting and fitting, regression analysis, and calculations of  $r^2$  were performed using Sigmaplot software version 12.3 (SYSTAT software, Inc.). Significant differences were at  $p \leq 0.05$ .

## Results

### Effects of Biochars on Soil Properties

Soil pH values were significantly different among treatments in the Khorat but not in the Wahiawa soil (Tables 1 and 2). In the Khorat soil, the difference was notable in the increased pH under the highest rate (4%) of FC biochar as compared with the control (Table 1). In the Khorat soil, application of both biochars significantly reduced Al concentrations compared with the control (Table 1). In contrast to Al concentration in the Khorat soil, concentrations of saturated paste Mn in the Wahiawa soil significantly increased with increasing rates of both biochars (Table 2). In both soils, mineral N concentrations decreased with increasing rates of both biochars (Tables 1 and 2). However, the significant decreases relative to the respective controls were found only under 4% TK biochar in the Khorat soil (Table 1) and 4% FC biochar in the Wahiawa soil (Table 2).

**Table 1. Properties of the Khorat soils amended with Thai traditional kiln and Flash Carbonization biochars.†**

Treatment‡	Soil properties		
	pH (soil/H <sub>2</sub> O = 1:5)	Al concentration	Mineral N
	mg kg <sup>-1</sup> soil		
Control	4.74 ± 0.07§b¶	24.82 ± 2.29a	4.61 ± 0.53a
2% TK biochar	4.72 ± 0.14b	4.84 ± 1.30b	3.75 ± 0.87ab
4% TK biochar	4.83 ± 0.32ab	1.23 ± 0.27bc	1.36 ± 0.17b
2% FC biochar	5.59 ± 0.05ab	0.33 ± 0.13c	2.39 ± 0.24ab
4% FC biochar	5.88 ± 0.31a	0.15 ± 0.05c	2.32 ± 0.13ab
p value	0.0227	<0.001	0.0134
F-test	*	***	*
CV (%)	8.01	20.95	30.61

\* Significant at the 0.05 probability level.

\*\*\* Significant at the 0.001 probability level.

† Adapted from Butnan et al. (2015).

‡ FC, Flash Carbonization; TK, traditional kiln.

§ Values are mean ± SE.

¶ Means within the same column followed by the same letter are not significantly different at  $p \leq 0.05$  (Tukey's Studentized range test).

Added VM was significantly negatively correlated with soil Al concentrations in the Khorat soil and with mineral N concentrations in both soils but was positively correlated with concentrations of saturated paste Mn in the Wahiawa soil (Table 3). pH levels were significantly negatively correlated with concentrations of Al and mineral N in the Khorat soil amended with FC biochar, but they were significantly positively correlated with concentrations of saturated paste Mn in the Wahiawa soil amended with the same biochar. In the Khorat soil amended with FC biochar, there was a positive correlation between concentrations of mineral N and Al. In contrast, in the Wahiawa soil amended with both biochars, negative correlations between concentrations of mineral N and saturated paste Mn were found (Table 3).

**Table 2. Properties of the Wahiawa soils amended with Thai traditional kiln and Flash Carbonization biochars.†**

Treatment‡	Soil properties		
	pH (soil/H <sub>2</sub> O = 1:5)	Saturated paste Mn concentration	Mineral N
		mg L <sup>-1</sup>	mg kg <sup>-1</sup> soil
Control	5.37 ± 0.08§	0.81 ± 0.01d¶	1.87 ± 0.09a
2% TK biochar	5.19 ± 0.11	1.87 ± 0.16c	1.65 ± 0.06ab
4% TK biochar	5.20 ± 0.22	3.18 ± 0.03b	1.51 ± 0.09ab
2% FC biochar	5.59 ± 0.05	3.06 ± 0.06b	1.47 ± 0.09ab
4% FC biochar	5.78 ± 0.19	4.65 ± 0.07a	1.24 ± 0.10b
p value	0.0784	<0.001	<0.001
F-test	ns#	***	***
CV (%)	4.56	4.76	3.57

\*\*\* Significant at the 0.001 probability level.

† Adapted from Butnan et al. (2015).

‡ FC, Flash Carbonization; TK, traditional kiln.

§ Values are mean ± SE.

¶ Means within the same column followed by the same letter are not significantly different at  $p \leq 0.05$  (Tukey's Studentized range test).

# Not significantly different (F-test).

## Effects of Biochar Applications on Greenhouse Gas Emissions

### Cumulative Greenhouse Gas Emissions

The Khorat soil produced generally lower cumulative CO<sub>2</sub> emission than the Wahiawa soil in all treatments, and the emissions in the Khorat soil were significantly lower than the Wahiawa soil in all treatments from 16 DAI onward (Fig. 1a vs. 1b). In the Khorat soil, there was a significant interaction effect of soil type × biochar type × biochar rate on cumulative CO<sub>2</sub> emission (Supplemental Table S3). At the early stage of the incubation (2 DAI) in the Khorat soil, 4% TK biochar produced significantly higher cumulative CO<sub>2</sub> emission than its lower rate (2%) (Fig. 1a). However, after 4 DAI, the higher rate showed a trend

**Table 3. Pearson correlation coefficients of concentrations of Al in the Khorat soil, saturated paste Mn in the Wahiawa soil, and mineral N in both soils treated with different biochars with added volatile matter and with soil pH ( $n = 9$ ).**

Parameter†	Volatile matter‡	Soil Al	Soil pH (1:5 soil/DI water ratio)
Khorat soil			
Soil Al <sub>TK</sub>	-0.924***	-	0.046ns§
Soil Al <sub>FC</sub>	-0.924***	-	-0.789**
Mineral N <sub>TK</sub>	-0.822**	0.611ns	-0.150ns
Mineral N <sub>FC</sub>	-0.800**	0.731*	-0.857**
	Volatile matter	Saturated paste Mn	Soil pH
Wahiawa soil			
Saturated paste Mn <sub>TK</sub>	0.992***	-	-0.247ns
Saturated paste Mn <sub>FC</sub>	0.995***	-	0.703*
Mineral N <sub>TK</sub>	-0.776**	-0.762**	-0.021ns
Mineral N <sub>FC</sub>	-0.879***	-0.883***	-0.453ns

\* Significant at the 0.05 probability level.

\*\* Significant at the 0.01 probability level.

\*\*\* Significant at the 0.001 probability level.

† Al<sub>FC</sub> and Al<sub>TK</sub>, Al concentration (mg kg<sup>-1</sup>) in the Khorat soil treated with Flash Carbonization biochar and Thai traditional kiln biochar, respectively; Mn<sub>FC</sub> and Mn<sub>TK</sub>, saturated paste Mn concentration (mg L<sup>-1</sup>) in the Wahiawa soil treated with Flash Carbonization biochar and Thai traditional kiln biochar, respectively; N<sub>FC</sub> and N<sub>TK</sub>, mineral N concentration (mg kg<sup>-1</sup>) in the Khorat and Wahiawa soils treated with Flash Carbonization biochar and Thai traditional kiln biochar, respectively.

‡ Added volatile matter (soil contents of biochar-derived volatile matter) (µg g<sup>-1</sup> soil).

§ ns, not significant.

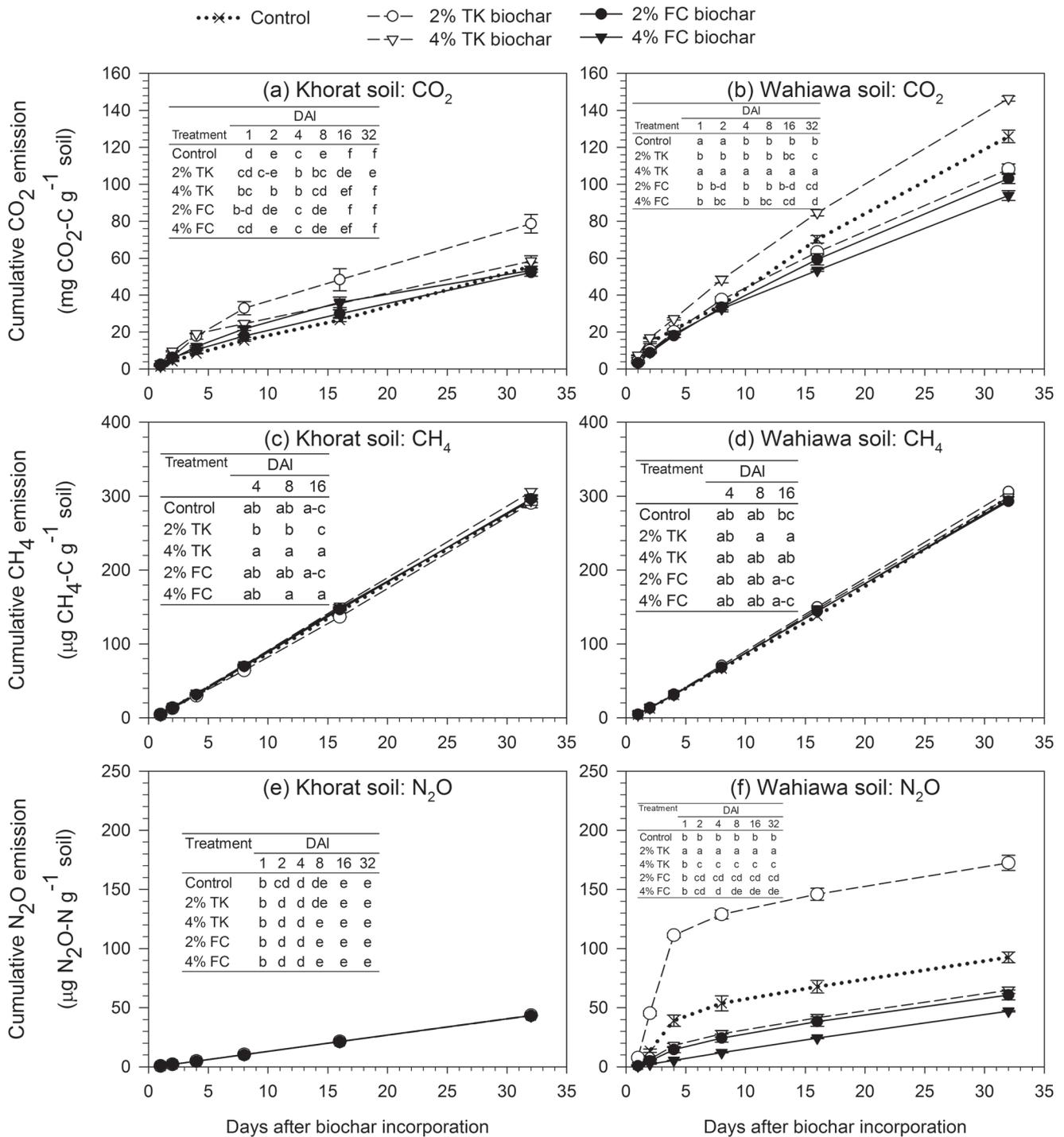


Fig. 1. Cumulative emission of greenhouse gases over 32 d after incorporation of Thai traditional kiln (TK) and Flash Carbonization (FC) biochars at 0% (control), 2%, and 4% w/w to the Khorat and Wahiawa soils. (a) Cumulative CO<sub>2</sub> emission in the Khorat soil. (b) Cumulative CO<sub>2</sub> emission in the Wahiawa soil. (c) Cumulative CH<sub>4</sub> emission in the Khorat soil. (d) Cumulative CH<sub>4</sub> emission in the Wahiawa soil. (e) Cumulative N<sub>2</sub>O emission in the Khorat soil. (f) Cumulative N<sub>2</sub>O emission in the Wahiawa soil. The inset tables show comparisons among treatments across soils within a time interval or a period of days after biochar incorporation (DAI). Similar letters within a DAI pertaining to a greenhouse gas are not significantly different ( $p \leq 0.05$ ; Tukey's Student range test). Vertical bars represent SEM.

of lower cumulative CO<sub>2</sub> emission than the lower rate, and the difference was significant at 32 DAI. Repeated measures analysis showed significant interaction of biochar treatments × time on cumulative CO<sub>2</sub> emission in the Khorat soil (Supplemental Table S4). The FC biochar generally produced lower cumulative CO<sub>2</sub> emissions than TK biochar, showing significant differences at 32 DAI when FC biochar at both rates produced lower cumulative CO<sub>2</sub> emissions than the 2% TK biochar. Contrary to the

Khorat soil, the Wahiawa soil showed higher cumulative CO<sub>2</sub> emission when amended with TK biochar at the 4% than at the 2% rate throughout the incubation period (Fig. 1b). In addition, FC biochar significantly depressed CO<sub>2</sub> emission below the control during the early (1–2 DAI) and final (32 DAI) periods of the incubation.

Biochar type and rate showed only small differences in cumulative CH<sub>4</sub> emissions and were similar regardless of the soil (Fig.

1c and 1d), producing significantly lower emissions only under 2% TK biochar during 8 and 16 DAI. In the Khorat soil, during 8 to 16 DAI, the higher rate (4%) of both TK and FC biochars produced significantly higher cumulative CH<sub>4</sub> emission than 2% TK biochar (Fig. 1c). On the contrary, in the Wahiawa soil, during 8 to 16 DAI, there was a trend of higher cumulative CH<sub>4</sub> emission in 2% TK biochar than its higher rate (4%) as well as both FC biochar rates. In addition, the 2% TK produced significantly higher cumulative CH<sub>4</sub> emission than the control at 16 DAI (Fig. 1d). However, at the end of the incubation (32 DAI), there were no significant differences in cumulative CH<sub>4</sub> emission in both soils.

In the Khorat soil, biochar had no significant effect on cumulative N<sub>2</sub>O emissions over the study period (Fig. 1e) and showed a linear increase in emissions. On the other hand, N<sub>2</sub>O emissions in the Wahiawa soil showed a curvilinear response, with emissions significantly higher in the Wahiawa control compared with the Khorat control. Cumulative N<sub>2</sub>O emissions in the Khorat soil under both TK biochar rates were lower than those of the respective treatments in the Wahiawa soil, but the difference was only significant under the 2% TK at 1 DAI onward (Fig. 1e and 1f). However, the FC biochar treatments showed lower emissions in the Khorat compared with the Wahiawa soils, but the significant differences were only found at a 2% rate from 8 DAI onward. Most notable was the large burst of N<sub>2</sub>O emitted from the Wahiawa soil amended with 2% TK biochar since 1 DAI, which lasted until 4 DAI, at which time cumulative N<sub>2</sub>O emission was more than 3-fold compared with all other treatments (Fig. 1f). This was supported by the repeated measures analysis, which showed significant interaction of biochar treatments × time on cumulative N<sub>2</sub>O emission in the Wahiawa (Supplemental Table S4). Both biochars at the highest rate and FC biochar at the lower rate (2%) produced significantly lower cumulative N<sub>2</sub>O emissions than the control from 2 DAI onward.

#### Biochar Added Volatile Matter Contents and Biochar-Derived Soil Properties Affecting Greenhouse Gas Emissions

Biochar VM and ash contents significantly affected GHG emissions in both soils. Whereas the former was represented by added VM to the soil, the latter was through its soil pH modification. In the Khorat soil, there were significant negative effects of added VM and biochar-derived soil properties (including soil pH and soil Al concentration) on cumulative CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O emissions, with the exception of effects of added VM on those of CO<sub>2</sub> and CH<sub>4</sub> and effects of Al on those of CH<sub>4</sub> (Table

4). Effects of added VM were apparent in the simple regression analysis for CH<sub>4</sub> emissions (Fig. 2b). Cumulative CO<sub>2</sub> emission stayed unchanged up to the added VM of 60 μg g<sup>-1</sup> soil; beyond this rate, it abruptly increased to the peak cumulative CO<sub>2</sub> emission at added VM of 83 μg g<sup>-1</sup> soil, beyond which the emission declined (Fig. 2a). Meanwhile, cumulative CH<sub>4</sub> emission increased with increasing concentrations of added VM in the Khorat soil (Fig. 2b).

In the Wahiawa soil, added VM positively affected cumulative CO<sub>2</sub> and N<sub>2</sub>O emissions (Table 5), whereas concentrations of saturated paste Mn negatively affected CO<sub>2</sub> and N<sub>2</sub>O. Similar to the Khorat soil, increasing soil pH resulting from biochar applications negatively affected cumulative CO<sub>2</sub>, CH<sub>4</sub> (Table 5), and N<sub>2</sub>O emissions when excluding the outliers of those pertaining to the low rate of TK biochar (Fig. 2d). Mineral N concentrations showed a significant positive effect on cumulative N<sub>2</sub>O emissions in the Wahiawa soil when excluding the outliers of the TK 2% w/w treatment from the regression analysis (Fig. 2c); however, it did not show a significant effect in the multiple regression analysis (Table 5).

## Discussion

The results of this study have highlighted the significant roles of labile constituents of biochars, VM, and ash in influencing the emissions of GHGs, which could manifest itself in the short-term (32 d). This is considered an important aspect of biochar in addition to its well-known characteristic of persistence leading to the long-term effects in soils.

### Volatile Matter and Ash Contents of Biochars Affecting CO<sub>2</sub> and CH<sub>4</sub> Emissions in Two Soils of Contrasting Texture and Mineralogy

#### Cumulative CO<sub>2</sub> Emission

The relationship of biochar-added VM to CO<sub>2</sub> emissions varied depending on soil type. The Khorat soil treated with high-VM TK biochar demonstrated higher CO<sub>2</sub> emissions than the unamended soil under both application rates (Fig. 1a). In contrast, the Wahiawa soil showed higher CO<sub>2</sub> emission relative to the unamended soil only under the 4% TK rate. Differences in CO<sub>2</sub> emissions may be associated with contrasting texture leading to different buffering capacities between the two soils. In general, labile organic carbon constituting VM was an extra source of CO<sub>2</sub> production because it contained rapidly decomposable

**Table 4. Multiple regression analysis of effects of biochar constituents and biochar-affected soil properties (Khorat soil) on cumulative CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O emissions over 32 d after biochar incorporation (n = 15).**

Greenhouse gas	y-intercept value	VM†	pH‡	Al§	N¶	r <sup>2</sup>
CO <sub>2</sub>	80.5	0.13 <sup>ns</sup> #	-5.32**	-0.32*	1.60 <sup>ns</sup>	0.802***
CH <sub>4</sub>	370	-0.23 <sup>ns</sup>	-11.89**	-0.43 <sup>ns</sup>	-2.31 <sup>ns</sup>	0.730***
N <sub>2</sub> O	44.2	-0.03**	-0.19**	-0.03***	0.16***	0.901***

\* Significant at the 0.05 probability level.

\*\* Significant at the 0.01 probability level.

\*\*\* Significant at the 0.001 probability level.

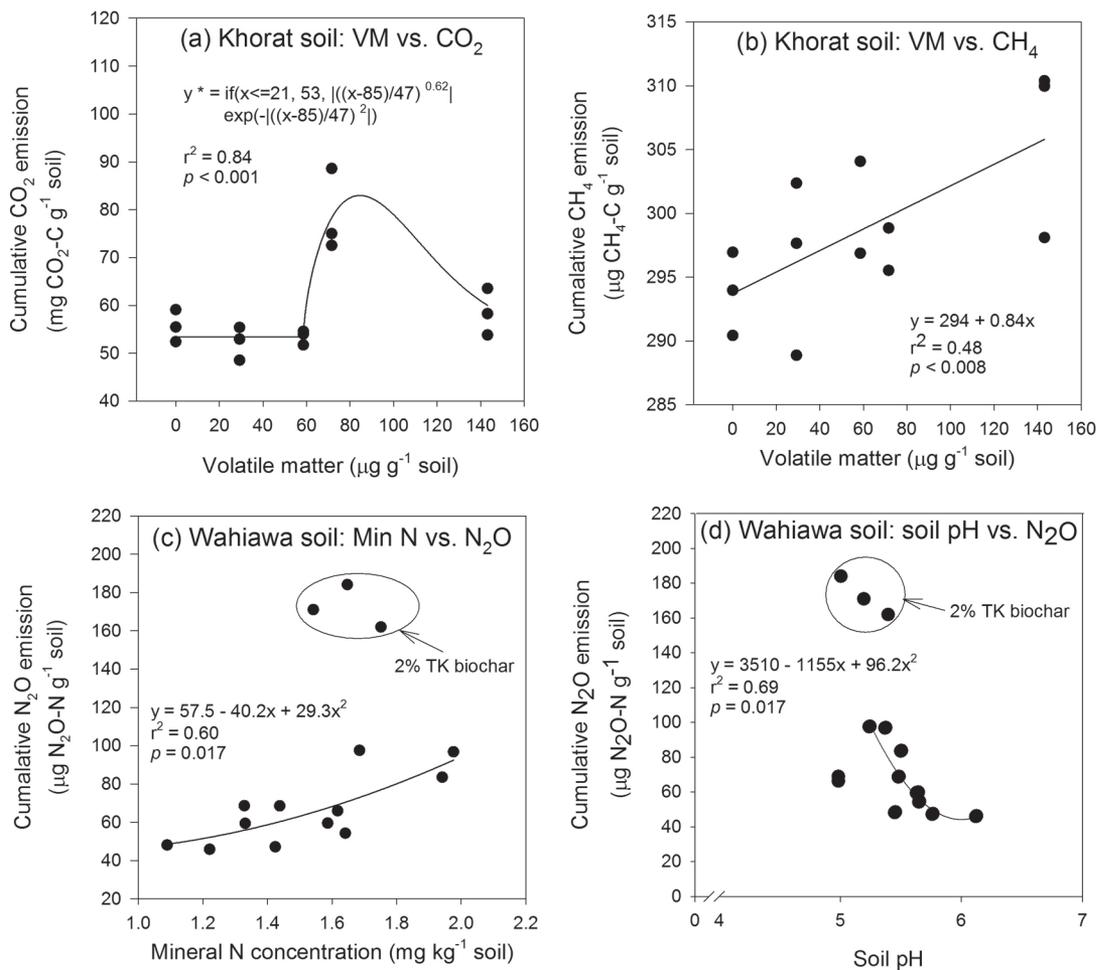
† Added volatile matter (soil contents of biochar-derived volatile matter) (μg g<sup>-1</sup> soil).

‡ Soil pH (1:5 soil/DI water ratio).

§ Soil Al concentration (mg kg<sup>-1</sup> soil).

¶ Mineral N concentration (mg kg<sup>-1</sup>) in the Khorat soil treated with Thai traditional kiln.

# ns, not significant.



**Fig. 2.** Relationships between (a) added volatile matter (VM) contents and cumulative CO<sub>2</sub> emissions in the Khorat soil, (b) added volatile matter contents and CH<sub>4</sub> emissions in the Khorat soil, (c) soil mineral N concentrations and cumulative N<sub>2</sub>O emissions, and (d) soil pH and cumulative N<sub>2</sub>O emissions. These cumulative GHG emissions were measured during 32 d after biochar incorporation. \*Weibull equation.

carbon (e.g., hydroxyl, carbonyl, carboxyl, and hemi-acetal functionalized compounds) (Deenik et al., 2010). However, in the clay-rich Wahiawa soil, adsorption of VM may have lowered VM bioavailability, whereas in the sandy Khorat soil VM bioavailability was not affected. Consequently, only at the highest biochar rate did the Wahiawa soil produce higher CO<sub>2</sub> emissions than the control.

The significant soil × biochar type × biochar rate interaction was also seen in the Khorat soil, demonstrating higher CO<sub>2</sub>

emissions under the lower (2%) than the higher rate (4%) of the high-VM biochar (TK) (Fig. 1a). The opposite was the case in the Wahiawa soil (Fig. 1b). Regarding the Khorat soil, dissolved organic matter (DOM) constituents of the VM not only served as a microbial substrate for CO<sub>2</sub> production but also alleviated Al bio-toxicity in this Al-rich soil. This is shown by the negative correlations between soil Al concentrations and added VM (Table 3). In addition, negative effects of Al concentrations on cumulative CO<sub>2</sub> emissions were found (Table 4). Bio-toxicity

**Table 5.** Multiple regression analysis of effects of biochar constituents and biochar-affected soil properties (Wahiawa soil) on cumulative CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O emissions over 32 d after biochar incorporation (n = 15).

Greenhouse gas	y-intercept value	VM†	pH‡	Mn§	N¶	r <sup>2</sup>
CO <sub>2</sub>	211	3.55***	-9.89*	-14.72***	-11.38 <sup>ns</sup>	0.968***
CH <sub>4</sub>	507	-1.46 <sup>ns</sup>	-35.54**	4.25 <sup>ns</sup>	-15.23 <sup>ns</sup>	0.518***
N <sub>2</sub> O	188	16.41***	-7.69 <sup>ns</sup>	-38.11***	-11.89 <sup>ns</sup>	0.945***

\* Significant at the 0.05 probability level.

\*\* Significant at the 0.01 probability level.

\*\*\* Significant at the 0.001 probability level.

† Added volatile matter (soil contents of biochar-derived volatile matter) (μg g<sup>-1</sup> soil).

‡ Soil pH (1:5 soil/DI water ratio).

§ Saturated paste Mn concentration (mg L<sup>-1</sup>) in the Wahiawa soil.

¶ Mineral N concentration (mg kg<sup>-1</sup>) in the Wahiawa soil treated with Thai traditional kiln.

# ns, not significant.

of soil Al to organic decomposition enzymes has been reported (Miltner and Zech, 1998b). Regarding Al alleviation, the processes of complexation and adsorption of Al onto biochar surface have been described by Qian and Chen (2014). However, there is no direct evidence of biochar-derived DOM reacting with Al in soil solution, although Hue et al. (1986) demonstrated clearly that soluble organic acids reduced activities of Al in soil solution through the formation of chelation complexes. Biochar and biochar-derived DOM may have stimulated CO<sub>2</sub> production by acting as oxidizing agents for decomposition of soil organic matter (Joseph et al., 2010). More recently, it has been reported that biochar surfaces (Dong et al., 2014) and biochar-derived DOM (Dong et al., 2014; Klüpfel et al., 2014) possessed quinone and hydroquinone functionality, which are both electron donors (reducing agents) and acceptors (oxidizing agents). Volatile matter, therefore, played the dual roles of C source and Al detoxification, which manifested in stimulation of CO<sub>2</sub> emissions under high-VM TK biochar in the Khorat soil.

Lower CO<sub>2</sub> emissions with increasing TK biochar rate found after 4 DAI may be due to VM toxicity in the coarse-textured, poorly buffered Khorat soil. There is some evidence supporting a toxic effect of VM at high contents. Specifically, ethylene (a constituent compound of biochar VM) has been identified as toxic to CO<sub>2</sub>-producing microorganisms (Smith, 1976; Spokas et al., 2010). The delayed toxicity of the higher biochar rate was caused by the time required for additional production of ethylene, a microbially mediated process, from other constituents of biochar. Considine et al. (1977) has shown that ethylene was formed from other organic C compounds (e.g., acetic, citric, vanillic, hydroxybenzoic, and ferulic acids) under anaerobic conditions within 10 d.

Contrasting results in biochar VM effects on CO<sub>2</sub> emissions in the Wahiawa soil highlight the importance of soil texture and mineralogy. In the clay-rich Wahiawa soil, increased sorption of VM could explain the lower CO<sub>2</sub> emissions in the TK biochar at 2% and the increased emissions when the biochar was added at 4%. This supports the idea that adsorption saturation needed to be reached before extra free VM was available for use as a source for CO<sub>2</sub> emission. In relation to soil mineralogy, VM could act as a reducing agent, rendering transformation of high concentrations of Mn oxides and Mn<sup>3+</sup> in the Wahiawa soil to more soluble Mn<sup>2+</sup> (Butnan et al., 2015; Graber et al., 2013). Biochar-derived labile organic C molecules (e.g., sugar acids, aliphatic organic acids, polyphenols, tannins, and humic-like substances) (Butnan et al., 2015; Graber et al., 2013) possess reducing power that may have electrically reduced Mn<sup>3+</sup> to Mn<sup>2+</sup>. In addition, functional groups on biochar surface (e.g., phenolic, hydroxyl, carboxyl, and amide) can perform similar reduction reactions (Ahmad et al., 2014). Added VM was shown to have positive correlations with saturated paste Mn (Table 3), indicating that increases in added VM content contributed to increases in soluble Mn concentrations. Soil pH increases have been found to enhance reducing capacity of some VM compounds, including catechol, gallic acids, and tannic acids (Hue et al., 2001). Addition of biochars resulted in pH increases in two possible ways, one due to ash contents and the other due to the reducing potential of VM, which increased pH as Mn<sup>4+</sup> or Mn<sup>3+</sup> were reduced to Mn<sup>2+</sup>. Dong et al. (2014) showed that Cr<sup>6+</sup> was reduced to Cr<sup>3+</sup> on increases in pH attributed to biochars. Similar process could have occurred

to Mn. Greater pH values of the Wahiawa soil by high-ash (FC) biochar significantly increased soluble Mn concentration, as shown by their positive correlation (Table 3). The coupling of the reducing power of VM and increases in pH increase the likelihood that Mn<sup>2+</sup> was the dominant Mn species in the Wahiawa soil.

In addition to possible pH effects on soil respiration, divalent cation Mn<sup>2+</sup> can have direct negative impacts on soil organic matter decomposition. First, Mn<sup>2+</sup> can be toxic to microorganisms decomposing organic substrates (Firestone et al., 1983; Nogueira et al., 2007). Second, Mn in its reduced state cannot oxidize organic C the same way as Mn<sup>3+</sup> (Schlosser and Höfer, 2002). Lastly, the propensity for Mn<sup>2+</sup> to form complexes with organic molecules as discussed by Hue et al. (2001) could protect organic molecules against degradation by soil microorganisms. The combination of these biochar-derived effects on Mn and pH leading to decreased CO<sub>2</sub> production explains their negative regression coefficient with cumulative CO<sub>2</sub>-C emission in the Wahiawa soil (Table 5).

In addition to the interaction of pH increase with Mn in reducing CO<sub>2</sub> emission, pH increases could have acted independently to alter the prevailing microbial composition of the initially acid Khorat and Wahiawa soils, with similar effects on GHG emissions. The microbial communities in these soils, which were adapted to acid conditions, were hence negatively affected by environmental changes (Fernández-Calviño and Bååth, 2010), as shown by a reduction in CO<sub>2</sub> production. Another possible role of biochar ash in decreasing CO<sub>2</sub> emission is sorption of CO<sub>2</sub> on mineral oxides, such as Al<sub>2</sub>O<sub>3</sub> and SiO<sub>2</sub> (Cornelissen et al., 2013).

Carbon dioxide emission is influenced in part by original soil C content, which varies among soil types (Buerkert et al., 2012). Overall, higher CO<sub>2</sub> emissions came from the higher-C Wahiawa soil (Fig. 1b) compared with the lower-C Khorat soil (Fig. 1a). Decomposition of native organic C induced by biochar is attributed to several possible mechanisms: (i) co-metabolism between biochar-derived labile organic C and microbial enzymes (Maestrini et al., 2014b; Singh and Cowie, 2014), (ii) biochar acting as foci (i.e., microsites with substrates where microorganisms colonize for their growth and activity) (Maestrini et al., 2014a), (iii) biochar's enhancement of soil fertility promoting microbial growth (Singh and Cowie, 2014), and (iv) biochar-derived organic molecules functioning as an oxidizing agent of native organic C in decomposition (Briones, 2012; Ennis et al., 2012; Joseph et al., 2010).

#### Cumulative CH<sub>4</sub> Emission

In general, biochar type showed no effect on CH<sub>4</sub> emissions in either soil. However, regression analysis suggests that added VM showed a significant effect on increasing CH<sub>4</sub> emissions in the Khorat soil, as shown partly by corresponding increases in cumulative CH<sub>4</sub> emissions with increases in added VM in this soil (Fig. 2b). Added VM may contribute to CH<sub>4</sub> production through two possible microbially mediated mechanisms. The first mechanism is related to production of CO<sub>2</sub> and acetic acid through aerobic decomposition of organic C compounds in soils (Poeplau and Don, 2014; Tsutsuki and Ponnampereuma, 1987), which could include biochar-derived VM. Carbon dioxide and acetic acid are precursors of CH<sub>4</sub> in the anaerobic processes

mediated by hydrogenotrophic and acetotrophic methanogens, respectively (Jain et al., 2004; Susanti and Mukhopadhyay, 2012). Under conditions prevailing in this study, anaerobic microsites were potentially generated by oxygen depletion through organic compound decomposition (Parkin, 1987). In addition, some anaerobic microsites could exist even under low soil moisture content and consequently generate CH<sub>4</sub> (Khalil and Baggs, 2005). Acetic acid is a form of VM readily available in biochars produced from different types of feedstock, including eucalyptus wood (Kim et al., 2013; Zhang et al., 2007). Moreover, acetic acid in biochar is derived from other VM compounds, notably ethanol and fumaric acid, through the microbially mediated mechanism termed “direct interspecies electron transfer” (Chen et al., 2014). Under this mechanism, electrons from ethanol are transported through biochar structure consisting of electron-donating to electron-accepting cells and finally passed to fumaric acid.

The second mechanism that could have contributed to increasing CH<sub>4</sub> emission from biochar-derived VM is inhibition of methanotrophs (i.e., microorganisms that oxidize CH<sub>4</sub> to CO<sub>2</sub>) by some VM compounds, notably ethylene, which is toxic to methanotrophs (Spokas et al., 2010). Furthermore, polycyclic aromatic hydrocarbons (e.g., benzene, toluene, ethyl benzene, *o*-xylene, *m*-xylene, and *p*-xylene, naphthalene, and furan), which are pyrolysis-derived products (Borchard et al., 2014), could have caused suppression of methanotrophic activity.

Regression analysis suggests that biochar ash-derived increases in soil pH had significant negative effects on CH<sub>4</sub> emission in both soils (Tables 4 and 5). Increasing soil pH may have resulted in suppression of methanogenic activity. Whereas increases in soil pH heavily depressed the activity of methanogens, the effects on methanotrophs was slight (Yu et al., 2013). Ranges of pH tolerance for methanotrophs and methanogens are between 5 and 7 (Le Mer and Roger, 2001), although methanotrophs are more tolerant to soil pH changes. However, the factor of microbial adaptation to soil environment comes into play, leading to different optimum pH values for maximum CH<sub>4</sub> consumption and production in these two types of microorganisms in different soils having different pH values (Taconi et al., 2007). Nevertheless, negative effects of soil pH, resulting from biochar applications, did not manifest themselves in temporal patterns of cumulative CH<sub>4</sub> emissions (Fig. 1c and 1d) because they were overridden by the stronger VM effects that promoted CH<sub>4</sub> emissions.

### Volatile Matter of Biochars Controlling N<sub>2</sub>O Production Processes in the Mn-Rich Soil

In the Mn-rich Wahiawa soil, Mn was likely involved in microbially mediated N<sub>2</sub> production processes. One possible pathway is through NH<sub>4</sub><sup>+</sup> oxidation, where Mn acts as an electron acceptor in both aerobic and anaerobic ammonium oxidation processes, resulting in N<sub>2</sub> production (Clément et al., 2005; Luther et al., 1997). However, addition of biochar-possessing VM at a level (i.e., 2% TK rate) sufficient to decrease Mn availability as an electron acceptor, through VM–Mn complexation, may suppress the NH<sub>4</sub><sup>+</sup> oxidation processes while enhancing N<sub>2</sub>O production through an alternate pathway of nitrification followed by denitrification processes. It was reported that VM-derived

organic molecules could act in a two-step process to decrease soil Mn availability and its ability to perform its electron acceptor function. In the first step, TK biochar-derived VM solubilized Mn oxides in the Mn-rich soil, resulting in increasing soluble Mn concentration relative to the control treatment (Table 2). In the second step, the biochar-derived VM further complexed with Mn, rendering the Mn unavailable to perform the electron acceptor function (Butnan et al., 2015). This hypothesis is supported by sharp increases in N<sub>2</sub>O emissions in the Wahiawa soil by the addition of VM-rich (TK) biochar at the low rate (2% w/w) (Fig. 1f). The immediate sharp increase taking place since the first day after biochar incorporation indicated that the reaction between VM compounds and Mn occurred rapidly. Graber et al. (2013) reported that DOC compounds derived from biochar could solubilize soil Mn within 24 h of their application to the soil. It was further postulated that this solubilized Mn would be further complexed by the DOC. However, at the highest rate of VM-rich TK biochar, it was hypothesized that VM-derived phenolics complexed with soil protein N, decreasing available NH<sub>4</sub><sup>+</sup> for nitrification and denitrification processes (Butnan, 2015). Unlike the Mn-rich, fine-textured Wahiawa soil, unchanged N<sub>2</sub>O emissions by biochar amendment in the coarse-textured Khorat soil may be due to the overriding negative effects of increased added VM, increased soil pH, and decreased soil mineral N over the positive effect of decreased soil Al. Additionally, unchanged N<sub>2</sub>O emissions in the Khorat soil were possibly influenced by the prevailing aerated conditions in this soil.

### Conclusions

The results of this study demonstrated that the more labile constituents of biochar, VM and ash, affected CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O emissions in a short-term incubation. Their effects on GHG emissions were also influenced by soil properties, notably their textural types. Whereas VM had both stimulating and inhibitory effects on CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O emissions in both contrasting textured soils, biochar ash demonstrated only inhibitory effects. In the coarse-textured, low-buffer soil, high-VM biochar at a particularly high rate (4%) suppressed CO<sub>2</sub> emission. Some toxic substance constituents of VM were proposed to inhibit the microbially mediated CO<sub>2</sub> production processes. Our results have led to explanations of various possible GHG production and emission mechanisms, microbially mediated, which were affected by the two critical biochar properties, VM and ash content. These mechanisms pertaining to VM substances involved their roles as microbial substrates, toxic agents, and complexing agents, reducing bioavailability of some nutrients and toxic elements. Meanwhile, those pertaining to ash constituents involved its capacity to increase soil pH and to directly adsorb some GHGs, CO<sub>2</sub>, and N<sub>2</sub>O. These proposed mechanisms may serve to explain the discrepancies in the literature regarding GHG emissions from soils with different properties treated with biochars. Validation of these mechanisms will require further investigations, including long-term incubations and GHG emissions at the field scale.

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