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Original Article

Biochar properties affecting carbon stability in soils contrasting in texture and mineralogy

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ABSTRACT

A pot experiment involving growing three consecutive corn crops in two contrasting tropical soils—a coarse-textured, Al-rich Ultisol and a fine-textured, Mn-rich Oxisol—treated with two eucalyptus wood biochars—at low (350 °C) and high (800 °C) pyrolysis temperatures—at weight per weight rates of 0%, 1%, 2% and 4%, was conducted to assess their effects on the soil organic carbon (SOC) stability (soil C remaining relative to initial soil C) 144 d after biochar application (after the third crop harvest). The low temperature biochar had higher volatile matter but lower ash and fixed C contents than its high temperature counterpart. In the Ultisol, the SOC stability significantly increased with both biochars at up to the 2% rate but beyond which it did not further increase, whereas, in the Oxisol, the SOC stability significantly decreased at all rates of high temperature biochar and at the 2% and 4% rates of the low temperature biochar. Proposed mechanisms underlying these contrasting responses of the two soils involve their different buffering capacities and their mineralogy in relation to the Al in the Ultisol and the Mn in the Oxisol interacting with the different contents of the three main biochar constituents (volatile matter, ash and fixed C) of the two biochars.

Introduction

Soil organic carbon (SOC) stability indicates soil C sequestration capacity which can be translated into mitigation of global warming and increased soil fertility (Lal, 2004). Biochar, which is pyrolized biomass used as a soil amendment and for carbon sequestration, has been shown to influence SOC stability but research results have been inconsistent regarding the direction of the influence during the past decade, with suppression (Cheng et al., 2008), stimulation (Kolb et al., 2009) and no effects (Santos et al., 2012) of biochar on SOC stability being reported. Based on the results reported in the literature, differences in soil types and climatic conditions appear to be key factors responsible for these inconsistencies. Most previous studies on biochar effects on SOC stability have been conducted in temperate soils (Cheng et al., 2008; Kolb et al., 2009; Santos et al., 2012). There have been some studies on subtropical soils (Fang et al., 2014a; Farrell et al., 2015), but no studies on tropical soils were identified.

Oxisols and Ultisols are highly weathered agricultural soils found widely in the tropics where they occupy 23% and 20% of the total agricultural area, respectively (Sanchez and Logan, 1992). Manganese is predominant in the Oxisols, and aluminum in the Ultisols (Hue et al., 2001). The deleterious effects of these elements on plant growth and microbial activities have been reported (for example, Nogueira et al., 2007). Biochar has been shown to be beneficial to soil fertility by alleviating these deleterious effects through decreasing soluble Al in an Ultisol. This was achieved through an increase in the biochar ash-derived soil pH and by biochar-Al complexation. Biochar also reduced Mn toxicity by decreasing tissue Mn in corn plants grown in an Oxisol although the soil soluble Mn increased (Butnan et al., 2015). A possible consequence of biochar-Al complexation is increased SOC stability. This was shown by a study in a temperate Al-rich soil which inferred that Al minerals may have enhanced stabilization of biochar C and native SOC through their complexation (Santos et al.,

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2012). Biochar-derived SOC stabilization effects, if proved true, are an additional benefit to soil fertility as well as to the environment (Lal, 2004). Similar biochar-derived SOC stability effects as influenced by soil Mn have not been reported. Increased soluble Mn content in an Oxisol treated with biochars was attributed to dissolution of Mn oxides by volatile matter (VM) constituent of the biochars, and the higher the VM content the greater the degree of dissolution (Graber et al., 2014; Butnan et al., 2015). Whether this interaction between soil Mn and biochar-derived VM affects SOC stability is not yet known.

In addition to mineralogy, soil texture can have significant effect on SOC stability (Olson et al., 2014). Increased clay content has been shown to enhance SOC stability (Bationo et al., 2007). However, the effect of different soil textures on SOC stability in biochar-amended soils is less clear. In addition, most previous studies were performed on temperate soils. For example, Fang et al. (2014b) have proposed that stronger interactions among native SOC, biochar C and soil minerals in a temperate finer-textured soil were a major mechanism underlying its higher SOC stability than that in a coarser-textured counterpart under similar climatic conditions. However, this proposed mechanism has not been validated by other studies. In addition, mechanisms of interactions among native SOC, biochar C and soil minerals may be different in tropical soils which have distinctive soil mineralogy from the temperate ones (Jien et al., 2015).

Not only clay minerals have a significant effect on the retention of SOC (Bationo et al., 2007) but also substances toxic to microbes (Labud et al., 2007). Variability in soil texture may have bearing on the adsorption of VM-derived substances toxic to decomposer microorganisms, which in turn may play a central role in SOC stability. The reported VM-derived toxic substances to microbes include tannin derivatives, ethylene, acetylene, polychlorinated dibenzo dioxins, furans and polycyclic aromatic hydrocarbons (Spokas et al., 2011; Adamczyk et al., 2013; Cayuela et al., 2014). Do VM-derived substances have the same microbial toxic potential when they are applied to different soil textures? Investigation of this question has yet to be conducted.

The hypotheses presented in this study were that there are interactions between biochars of different properties (notably the contents of fixed C, VM and ash) and tropical soils of different textures and mineralogy types (notably Al and Mn minerals) affecting SOC stability. As discussed earlier, biochars increase SOC stability in an Al-rich soil via complexation of biochar–SOC–Al, while the SOC stability of an Mn-rich soil decreases due to the dissolution of Mn oxides by biochar VM resulting in the destruction of soil aggregates. To test the hypotheses, the current study evaluated the effects of biochars possessing different major properties (fixed C, VM and ash contents) on SOC stability in tropical soils contrasting in texture and mineralogy.

Materials and methods

Soils

Two soils differing in texture and mineralogy were used—the Khorat (isohyperthermic Typic Oxyaquic Kandiustults) and the Wahiawa (kaolinitic isohyperthermic Rhodic Haplustox) soils. Site description, preparation, and initial properties of the two soils have been detailed in Butnan et al. (2015).

Biochars

Two biochars were produced from the same feedstock of the upper branches of eucalyptus trees (*Eucalyptus camaldulensis*) remaining after cutting for pulp production, but under different pyrolysis techniques: 1) Thai traditional kiln (TK) biochars and 2) Flash Carbonization™ (FC) biochars. The temperature used for production of TK was approximately 350 °C, while that of the FC biochar reached 800 °C. Details on the production, preparation and properties of both biochars were provided in Butnan et al. (2015). Major biochar properties, in terms of the fixed C contents measured as the percentage weight by weight (% w/w) relevant to this study for TK and FC, respectively, according to Butnan et al. (2015) are: VM (35.79% w/w and 14.65% w/w), ash (2.35% w/w and 3.85% w/w) and fixed C (61.86% w/w and 81.50% w/w).

Experimental design and data collection

A pot experiment was conducted from August 2011 to January 2012. The experiment involved soils of two types (the Khorat and the Wahiawa soils) amended with two types of biochar (TK and FC biochars) at four rates (0% w/w, 1% w/w, 2% w/w and 4% w/w). The experiment was arranged in 2 × 2 × 4 factorial in a randomized complete block design with three replications. Each pot (diameter = 0.16 m and volume = 3016 cm^3, with four drainage holes at the bottom) was placed on a tray and filled with 2 kg of air-dried soil mixed thoroughly with the desired biochar. All together, three corn (*Zea mays*) crops were consecutively grown in the pots. Details of the specific characteristics of corn studied can be found in Butnan et al. (2015). Each corn crop lasted 39 d from planting to harvest. The interval between harvesting the first crop to seeding the second crop was 4 d, while that between the second and third crop was 23 d. Biochar was applied only once on the same day just before the first corn crop planting. All together, the experimental period was 144 d from biochar application to harvesting of the third crop. Planting involved seeding of six seeds, which after 10 d were thinned down to two plants per pot. The soil moisture content was kept constant at 70% of water holding capacity (20.5% w/w for the Khorat soil and 63.4% w/w for the Wahiawa soil) by weighing a whole pot once a day and adjusting the weight with deionized (DI) water to a predetermined weight. Fertilizers were applied twice per crop at 1 and 25 d after biochar incorporation (DAI). Details of fertilizer characteristics are shown in Butnan et al. (2015). At each harvest, once the corn roots had been extracted, the soils were sieved (using a 2 mm mesh) and any remaining roots removed, while biochar pieces attached to the roots were picked out and returned together with the sieved soils to the pots. Soil samples used for evaluation of biochar-affected SOC stability were collected from the biochar-amended soils after the harvest of the third corn crop (144 DAI).

Before the third corn crop harvest, soil samples to measure bulk density (BD) were collected using a micro-auger (diameter = 1.6 cm, volume = 10.05 cm^3). Soil samples for chemical analyses were collected after the third corn crop harvest (144 DAI), except for saturated paste Mn which was determined in soil samples at the beginning of the third crop (82 DAI). Soil samples were air dried and sieved to pass through a 2 mm sieve for further use in chemical analyses. Biological analysis, based on microbial activity was done using fresh soil samples.

Laboratory analyses

Determination methods for various biochar properties, including proximate and ultimate analyses and elemental contents in ash are provided in Butnan et al. (2015).

Total organic C (TOC) (Nelson and Sommers, 1982) and total N (Rutherford et al., 2008) were determined using a dry combustion method on a Shimadzu TOC-VCSH high-sensitivity combustion TOC analyzer (Shimadzu Corp.; Kyoto, Japan). Total C of the biochars was determined using the ultimate analysis method by Hazen Research, Inc., Golden, CO, USA. The pH values of the biochars, initial soils and biochar-amended soils were determined in a ratio of one part of the component to five parts DI water. The soil cation exchange capacity (CEC) was determined using the 1 M NH_4OAc saturation method at pH 7 (Pansu and Gautheyrou, 2006). The CEC was calculated based on a concentration of NH_4^+ replaced by 1 M KCl. The NH_4^+ concentration was determined colorimetrically using a flow-injection analyzer (FIAstar® 5012, FOSS Tecator; Hilleroed, Denmark). Exchangeable basic cation concentrations (K^+, Ca^{2+}, and Mg^{2+}) were extracted using 1 M NH_4 OAc at pH 7 (Pansu and Gautheyrou, 2006), and the acid cation (Al^{3+}) was extracted using 1 M KCl (Bertsch and Bloom, 1996). Soluble Mn was measured in a water saturated paste extract (Porter et al., 2004). The contents of these cations were quantified using inductive coupled plasma spectrophotometry on a Thermo Jarrell Ash Atom Scan 16 instrument (Franklin, MA, USA). Soil microbial activity was determined using the fluorescein diacetate (FDA) hydrolysis method following Green et al. (2006).

Calculation of soil organic carbon stability and statistical analysis

The SOC stability was indicated by the relative C remaining which was computed by using the following equation: relative C remaining (%) = [final total SOC content/(initial native total SOC content + biochar C)] × 100, where the final total SOC content was obtained at the time after the third corn harvest (144 DAI).

Three-way analysis of variance under a randomized complete block design was used to evaluate the effects of biochar type, biochar rate, soil type and their interactions on biochar-derived soil properties and the relative C remaining. Treatment means were compared using Tukey's Studentized Range Test. Linear relationships among biochar constituents, biochar-derived soil properties and relative C remaining were assessed using Pearson's correlation, while those demonstrating non-linear relationships were further tested using Spearman's rank correlation. All statistical analyses were conducted using the SAS software version 9.1 (SAS Institute; Cary, NC, USA). In all analyses, significant differences were considered at $p \leq 0.05$.

Results

Soil properties as affected by biochar amendment

Interactions between soil type, biochar type and biochar rate significantly influenced some physical, chemical and biological soil properties (Table 1). In the Khorat soil, the BD generally decreased with increased rates of biochar. Significant decreases were observed at the higher biochar rates of TK (4%) and FC (2%, 4%) over the control (Table 2). However, no significant difference was found in the Wahiawa soil. Soil pH tended to increase with increasing rates of both biochars in both soils. In the Khorat soil, significant increases in pH were observed under the highest FC rate (4%), while in the Wahiawa soil, thus occurred at the highest TK rate and all FC rates, compared to the control soils. Significant differences among treatments were not found for the soil CEC in both soils.

Regarding the extractable cations (Table 2), no significant changes were observed in extractable K concentrations with both biochars in both the Khorat and Wahiawa soils. Extractable Ca concentrations were higher at the 4% rate of FC than for other biochar rates in both soils. Extractable Mg concentrations were mostly not significantly different among treatments in both soils. Extractable Al concentrations in the Khorat soil showed decreasing trends with greater rates of both biochars, and were significantly lower than the control at the 2% and 4% rates of FC biochar. In contrast to Al in the Khorat soil, saturated paste Mn concentrations in the Wahiawa soil significantly increased with increasing rates of both biochars.

Fluorescein concentrations generally decreased with the application of both biochars in both soils. Significant decreases were observed at the higher rates (2% and 4%) of both biochars in the Khorat soil, and at all rates of both biochars in the Wahiawa soil compared to their respective control soils.

Soil organic carbon remaining in soils with different texture and mineralogy as affected by biochar amendment

Three-way interactions of soil type × biochar type × biochar rate appeared to significantly affect absolute TOC contents ($p \leq 0.001$) and relative C remaining [% of initial C (native soil C + biochar C)] ($p \leq 0.05$) (Table 1). The absolute TOC contents significantly increased with increasing rates of both biochars in both soils (Table 2). FC biochar had significantly higher absolute TOC contents than TK biochar at the 2% and 4% rates in the Khorat soil, and at 4% in the Wahiawa soil.

In general, in the Khorat soil, relative C remaining significantly increased with greater application rates of both biochars up to the 2% rate. At the 4% rate, it showed a decreasing trend relative to the lower rates which was significant for the TK biochar. In addition, at this highest rate, significantly higher relative C remaining in FC than TK biochars was observed (Fig. 1A). In contrast to the Khorat soil, relative C remaining in the Wahiawa soil generally decreased with higher rates of both biochars. TK biochar had a higher relative C remaining than did FC biochar with the exception at the highest (4%) rate at which the former led to lower C remaining than the latter (Fig. 1B).

Relationships between biochar-derived soil properties and biochar constituents

In the Khorat soil, relative C remaining had a significant positive correlation with the contents of added biochar C, absolute TOC and extractable Ca as well as with pH (Table 3). However, relative C

Table 1
Analysis of variance of the effects of soil types, biochar types, biochar application rates and their interactions on physical, chemical and biological properties of biochar amended soils.

Source of variance	Df	TOC (g/kg)	Relative C remaining[a]	BD (g/cm^3)	pH (Soil:H_2O = 1:5)	CEC ($cmol_c/kg$)	K	Ca	Mg	Al	Saturated paste Mn (mg/L)	Fluorescein released (mg/kg/3 h)
							(mg/kg)					
Soil (S)	1	***	***	***	***	***	***	***	***	–	–	***
Biochar type (Bt)	1	***	*	ns	***	*	ns	***	ns	***	***	ns
Biochar rate (Br)	3	***	***	***	***	ns	ns	***	**	***	***	***
S × Bt	1	ns	**	ns	ns	ns	ns	ns	ns	–	–	ns
S × Br	3	***	***	***	*	ns	ns	ns	**	–	–	***
Bt × Br	3	***	***	*	***	ns	ns	**	ns	**	***	ns
S × Bt × Br	3	***	*	*	***	ns	ns	ns	ns	–	–	ns

BD = bulk density; CEC = cation exchange capacity; TOC = total organic carbon.

* = significant at $p \leq 0.05$; ** = significant at $p \leq 0.01$; *** = significant at $p \leq 0.001$); ns = non-significant at $p \leq 0.05$ (F-test).

[a] Relative C remaining (%) = [final total SOC content/(initial native total SOC content + biochar C)] × 100.

Table 2
Physical, chemical and biological properties of the Khorat and Wahiawa soils as affected by application of different rates of Thai traditional kiln (TK) and Flash Carbonization (FC)™ biochars[‡].

Treatment	BD	pH	CEC	TOC	K	Ca	Mg	Al	Saturated paste Mn	Fluorescein released
	(g/cm^3)	(Soil:H_2O = 1:5)	(cmol$_c$/kg)	(g/kg)	(mg/kg)				(mg/L)	(mg/kg/3 h)
Khorat soil										
Control	1.58^{a}	4.79^{g}	3.64^{b}	4.1^{k}	21^{a}	97^{e}	5.7^{c}	18.7^{a}	–	$406^{d\text{-}f}$
1% TK biochar	1.55^{a}	4.90^{g}	3.03^{b}	10.3^{j}	32^{a}	104^{e}	5.27^{c}	18.2^{a}	–	$380^{e\text{-}g}$
2% TK biochar	1.50^{ab}	$5.13^{e\text{-}g}$	4.99^{b}	18.1^{h}	38^{a}	124^{e}	4.74^{c}	14.0^{ab}	–	312^{h}
4% TK biochar	1.34^{c}	$5.16^{e\text{-}g}$	3.92^{b}	25.4^{e}	21^{a}	171^{e}	2.82^{c}	12.9^{ab}	–	297^{h}
1% FC biochar	1.48^{ab}	5.03^{fg}	2.44^{b}	11.4^{j}	27^{a}	133^{e}	3.74^{c}	12.0^{ab}	–	$341^{f\text{-}h}$
2% FC biochar	1.34^{c}	$5.22^{d\text{-}g}$	2.56^{b}	20.0^{g}	23^{a}	172^{e}	2.92^{c}	6.6^{bc}	–	324^{gh}
4% FC biochar	1.38^{bc}	6.07^{a}	3.21^{b}	32.7^{c}	25^{a}	349^{d}	6.4^{c}	0.1^{c}	–	280^{h}
Wahiawa soil										
Control	0.93^{d}	$5.18^{e\text{-}g}$	17.79^{a}	15.3^{i}	130^{a}	592^{bc}	157^{a}	–	1.12d	623^{a}
1% TK biochar	0.91^{d}	$5.43^{c\text{-}f}$	19.46^{a}	23.1^{f}	55^{a}	593^{bc}	139^{ab}	–	2.38b	$453^{b\text{-}d}$
2% TK biochar	0.92^{d}	$5.47^{c\text{-}e}$	24.00^{a}	28.9^{d}	50^{a}	576^{c}	123^{b}	–	2.44b	485^{bc}
4% TK biochar	0.85^{d}	$5.74^{a\text{-}c}$	18.63^{a}	37.0^{b}	102^{a}	$730^{a\text{-}c}$	144^{ab}	–	4.61a	482^{bc}
1% FC biochar	0.89^{d}	$5.63^{b\text{-}d}$	17.95^{a}	22.4^{f}	108^{a}	594^{bc}	132^{ab}	–	1.39c	$421^{c\text{-}e}$
2% FC biochar	0.91^{d}	$5.73^{a\text{-}c}$	18.82^{a}	28.8^{d}	68^{a}	734^{ab}	141^{ab}	–	1.56c	472^{bc}
4% FC biochar	0.83^{d}	5.92^{ab}	17.07^{a}	46.2^{a}	148^{a}	851^{a}	137^{ab}	–	2.37b	496^{b}
p-value	<0.0001	<0.0001	<0.0001	<0.0001	0.006	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
F-test	***	***	***	***	**	***	***	***	***	***
Coefficient of variation (%)	3.8	2.66	23.05	2.38	70.76	12.38	11.74	23.51	4.06	5.28

BD = bulk density; CEC = cation exchange capacity; TOC = total organic carbon.
** = significant at $p \leq 0.01$; *** = significant at $p \leq 0.001$ (F-test); [‡] Means within a column followed by common lowercase letters are not significantly different at $p \leq 0.05$ (Tukey's Studentized Range Test).

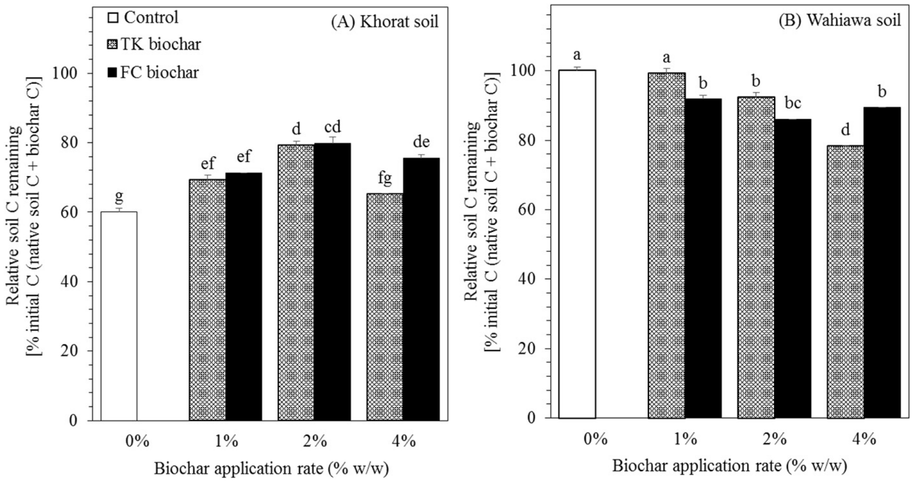

Fig. 1. Effects of Thai traditional kiln (TK) and Flash Carbonization™ (FC) biochars and their application rates on: (A) relative soil C remaining in the Khorat soil; (B) relative soil C remaining in the Wahiawa soil, where bars with a common letter across soils indicating not statistically different ($p \leq 0.05$; Tukey's Studentized Range Test) and error bars represent standard errors of the mean.

remaining was significantly negatively correlated with extractable Al, and fluorescein-released contents. In contrast to the Khorat soil, the Wahiawa soil had relative C remaining which was significantly negatively correlated with the rate of added biochar C, VM, absolute TOC, extractable Ca and saturated paste Mn contents as well as with pH. Extractable Al concentrations in the Khorat soil showed significant negative correlations with the rate of added biochar C, absolute TOC and extractable Ca contents and pH. On the contrary, extractable Al concentrations were significantly positively correlated to the BD and fluorescein concentrations. The saturated paste Mn concentrations of the Wahiawa soil were significantly positively correlated with added biochar C, VM and the absolute TOC contents.

Discussion

The results of this study showed that SOC stability, indicated by the relative C remaining (% initial native soil C + biochar C), in soils of contrasting textures and mineralogical types gave contrasting responses to the biochars applied. SOC stability generally increased in the coarse-textured Khorat soil (Fig. 1A), but it decreased in the fine-textured Wahiawa soil with increasing rates of biochars (Fig. 1B).

Increases in organic carbon stability in the coarse-textured Ultisol

The results revealed that the increases in SOC stability through biochar amendment in the coarse-textured Ultisol (Khorat soil) were affected by the biochar type and soil properties as seen in the significant correlations between relative C remaining and various biochar and soil properties (Table 3). Three main mechanisms are proposed based on these results that bring about enhanced SOC stability in the Khorat soil. First is the recalcitrant nature of the biochars themselves which resisted microbial decomposition as indicated by a high positive correlation between relative

Table 3

Pearson's and Spearman's correlations, or as otherwise indicated, coefficients (r) of relative soil C remaining, KCl extractable Al, saturated paste Mn and microbial activity with added contents of biochar constituents, soil physical, chemical and biological properties in the Khorat and Wahiawa soils as affected Thai traditional kiln (TK) and Flash Carbonization™ (FC) biochars.

	Added biochar C (g/kg)	Added VM (μg/kg)	TOC (g/kg)	BD (g/cm^3)	pH (Soil:H_2O = 1:5)	CEC (cmol$_c$/kg)	K	Ca	Mg	Al	Saturated paste Mn (mg/L)	Fluorescein released (mg/kg/3 h)
							(mg/kg)					
Khorat soil												
Relative C remaining[b]	0.452^{*}	0.028^{ns}	0.481^{*},[a]	-0.220^{ns}	0.473^{*},[a]	-0.074^{ns}	0.083^{ns}	0.462^{*},[a]	0.014^{ns}	-0.485^{*}	–	-0.462^{*},[a]
Al (mg/kg)	-0.701^{***}	-0.141^{ns}	-0.776^{***}	0.520^{*}	-0.906^{***}	0.208^{ns}	-0.015^{ns}	-0.878^{***}	-0.294^{ns}	–	–	0.707^{***}
Wahiawa soil												
Relative C remaining	-0.710^{***}	-0.741^{***}	-0.574^{**}	0.376^{ns}	-0.568^{**}	-0.038^{ns}	-0.017^{ns}	-0.529^{*},[a]	0.129^{ns}	–	-0.651^{***}	0.264^{ns}
Saturated paste Mn (mg/l)	0.637^{**}	0.974^{***}	0.539^{*}	-0.328^{ns}	0.308^{ns}	0.090^{ns}	-0.025^{ns}	0.240^{ns}	-0.050^{ns}	–	–	-0.132^{ns}

BD = bulk density; CEC = cation exchange capacity; TOC = total organic carbon.

* = significant at $p \leq 0.05$; ** = significant at $p \leq 0.01$; *** = significant at $p \leq 0.001$; ns = non-significant at $p \leq 0.05$.

[a] Spearman's rank-order correlation.

[b] Relative C remaining (%) = [final total SOC content/(initial native total SOC content + biochar C)] × 100.

C remaining and added biochar C contents (Table 3). The current results agreed with another report on the recalcitrant nature of biochar (Cheng et al., 2008). The second mechanism is the physicochemical protection of native SOC by biochars and some of their constituents which leads to increased SOC stability. Various protection mechanisms include the adsorption of SOC onto biochar surfaces and its retention in biochar pores which hinder the accessibility of SOC to microbial decomposers (Herath et al., 2015). Calcium (Ca^{2+}) bridging via biochar-derived Ca^{2+} acted as a bridge consisting of a biochar/SOC-soil particle complex in aggregate formation. The Ca^{2+} bridging function has been perceived to stabilize SOC (Lützow et al., 2006). This proposed mechanism arises from the positive relationship between the relative C remaining and the extractable Ca concentrations in the coarse-textured Khorat soil. Another related protection mechanism is the biochar-induced complexation of Al with native SOC/biochar-derived C as indicated by the negative correlation between the relative C remaining and the soluble (extractable) Al concentrations (Table 3). The Al–SOC complex could decrease its microbial decomposability (Lützow et al., 2006). In addition, it has been found that the bonding strength between Al and SOC increased as the soil pH was raised (Browne and Driscoll, 1993). This was supported by the current results which showed a significant positive correlation between relative C remaining and soil pH values (Table 3). The third mechanism is the biological protection of SOC by biochar. This is related to certain biochar VM-derived substances which are toxic to microbial decomposers, as such increasing the SOC stability. These toxic substances include tannins and their derivatives (Adamczyk et al., 2013), ethylene, acetylene (Spokas et al., 2011), polychlorinated dibenzo dioxins and furans, and polycyclic aromatic hydrocarbons (Cayuela et al., 2014). Although there was no direct significant relationship between the relative C remaining and the added VM contents, the significant negative correlation between the former and microbial activity, as indicated by the fluorescein released, seemed to point to biochar-derived microbial toxic substances deterring microbial activities.

Increased biochar rates in the coarse-textured Khorat soil, however, did not increase the SOC stability throughout, as demonstrated by a significant decrease in the soil C remaining at the highest (4%) TK rate compared to its lower (2%) rate, and a leveling-off at the highest FC rate compared to its lower (2%) rate (Fig. 1A). These might have been due to the saturation of adsorption sites in the low buffered soil and those of biochars for native SOC and biochar-derived labile C. This speculation is supported by Kasozi et al. (2010), who found saturation of biochar was caused by added organic C substances. The excess SOC and biochar-derived labile C was likely to be available to microorganisms which enhanced their activity in the decomposition process.

Decreases in organic carbon stability in the fine-textured Oxisol

In contrast to the increases in the SOC stability found in the coarse-textured Khorat soil, the decrease in the SOC stability in the fine-textured Oxisol (Wahiawa soil) was rather a surprise. Correlation analysis of the relative C remaining with various soil properties (Table 3) in conjunction with the trends of responses of soil properties to applied biochars (Table 2) provides foundations to the following proposed hypotheses.

Decreases in the SOC stability in the Wahiawa soil were due mainly to soil aggregate destruction which eliminated the SOC protection and, consequently, increased microbial decomposition of SOC. Occurrence of this phenomenon was substantiated by the results of no significant response in the BD to biochars applied at various rates in the Wahiawa soil (Table 2). Aggregate destruction of the Wahiawa soil could have been a result of reductive dissolution of Mn oxides by biochar VM-derived organic molecules as seen in the increases in soluble (saturated paste) Mn concentrations with greater biochar rates (Table 2), and the significant positive correlation between added VM and saturated paste Mn (Table 3). Similar results of Mn dissolution by VM were also reported in Graber et al. (2014) and Butnan et al. (2015). More supporting evidence of aggregate destruction in this soil through dissolution of Mn oxides is provided by the current results of increased soluble Mn, and extractable Ca concentrations. De-Campos et al. (2009) found that the dissolution of Mn oxides in Mollisols and Alfisols, which are known as a potential aggregator, led to disaggregation and the release of SOC, bridging Ca^{2+} and soluble Mn into the soil solution. Another plausible factor of aggregate destruction was the increased soil pH (Goldberg and Glaubig, 1987; De-Campos et al., 2009). It has been reported that chemical bonds between Mn, Fe and Al oxides and clay (phyllosilicates) particles were weakened when pH values were higher than the zero point of charge in highly weathered variable charged soils like the Wahiawa soil of this study (Uehara and Gillman, 1981). This resulted in increases in disaggregation and consequently dispersion of soil minerals (Goldberg and Glaubig, 1987). Through biochar amendment, it was shown that the pH values of a highly-weathered, very fine Ultisol amended with a commercial Japanese charcoal/biochar and a gasified leucaena biochar at the rate of 2.5% w/w were 5.04 and 6.19, respectively, while the values of pH_0 of that soil were 4.14 and 4.17, respectively (Deenik et al., 2011). Decomposition of native SOC could be stimulated by biochar VM-derived labile C compounds. The current results showed a negative correlation between the relative C remaining and both added biochar C and added VM contents (Table 3). Several hypotheses have been put forward for the decomposition stimulation of biochar on native SOC in the Wahiawa soil: 1) biochar-derived labile organic compounds causing priming effects on the native SOC (Singh and Cowie, 2014), 2) biochar acting as *foci* (that is, locations where microorganisms inhabit and consume substrates) that enhance microbial biomass (Maestrini et al., 2014), 3) biochar enhancing microbial growth through improving soil fertility (for example, increases in nutrient availability and water holding capacity) (Singh and Cowie, 2014) and 4) biochar-surface functional groups and biochar-derived organic molecules functioning as electron shuttles promoting SOC decomposition (Briones, 2012).

Another plausible mechanism is that Mn, the content of which was high in the Wahiawa soil, is essential for manganese peroxidase enzyme activities in decomposing recalcitrant aromatic components of biochars (Hofrichter, 2002). Moreover, chelation of Mn with organic molecules, for example, oxalic and malonic acids, was reported to facilitate its transport resulting in the enhanced decomposition of the recalcitrant compounds (Hofrichter, 2002). Solubilization and chelation of Mn by biochar-derived organic molecules were reported in Butnan et al. (2015) and Graber et al. (2014). The aforementioned organic molecules were demonstrated to be commonly contained in biochars including eucalyptus biochar (Martius et al., 2012). Unlike the Khorat soil, some biochar VM-derived substances were rendered non-toxic by the high retention capacity of the fine-textured Wahiawa soil in adsorbing such compounds on its clay minerals.

However, the higher VM content in the TK than the FC biochars (Butnan et al., 2015) did not translate into lower C stability in the former than the latter biochar at the lower rates (1–2%) as shown in Fig. 1B. This could have been the effect of more pronounced increases in the soil pH under the FC than the TK biochar (Table 2) which induced greater aggregate destruction under the former than the latter. In contrast to the lower rates of biochar applied to the Wahiawa soil, at the highest rate (4%), higher C stability in the FC than the TK biochars was likely due to the key influencing factor of higher recalcitrant C in the former than the latter as indicated by their fixed C contents (Butnan et al., 2015).

The results of the current study confirm the primary hypothesis that there were interactions of biochar properties (notably fixed C, VM and ash contents) with texture and mineralogy under both the coarse-textured Ultisol (Khorat) and the fine-textured Oxisol (Wahiawa) affecting soil properties which in turn influenced the SOC stability. The interactions were clearly shown by the contrasting responses in the SOC stability to the increasing rates of biochars between the two soils, that is, an increase in the SOC stability occurred in the coarse-textured soil, whereas a decrease occurred in the fine-textured soil. Mechanisms were proposed to underlie the increase in SOC stability in the coarse-textured, Al-rich Ultisol: 1) the recalcitrant nature of some biochar constituents, notably those fixed-C components, 2) physicochemical protection of native SOC through adsorption on biochars and complexation with soil Al and 3) the biological protection of native SOC through toxicity to decomposer microorganisms of some organic molecules constituting biochar VM. On the contrary, the mechanisms underlying the decreased SOC stability in the fine-textured, Mn-rich Oxisol were proposed to involve soil aggregate destruction attributable to the reductive dissolution of the Mn oxides and the increased soil pH, which brought about the dispersion of clay particles. In addition, another plausible mechanism underlying the decreased SOC stability in the Oxisol was proposed to involve the stimulation of microbial activity and enzymes thereof by biochar-derived labile C and by soil native Mn.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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