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Temporal Effects of the Combined Use of Cricket Frass and Eucalyptus Biochar on the Yield and Tissue Nitrate Content in Chinese Kale

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ABSTRACT

A greenhouse experiment was conducted to estimate the influence of various application rates of eucalyptus-derived biochar combined with cricket frass on the soil properties and soil N transformation, and, in turn, affecting both shoot biomass yield and nitrate (NO_3^-) contents of Chinese kale (*Brassica oleracea*). Two consecutive kale crops were grown to investigate the temporal effect of the combined amendments of cricket frass and biochar. Six rates of biochar, 0%, 0.125%, 0.25%, 0.5%, 1%, and 2% w/w in combination with 0.55% w/w of cricket frass, were applied only once at the start of the experiment in sandy loam soil. Shoot biomass significantly increased under treatments of 0.125% to 0.5% w/w in the first kale crop and 0.125% to 0.25% w/w in the second crop compared to the cricket frass alone. However, the higher rates of 0.25% and 0.5% w/w within the first and second crops decreased shoot biomass relative to their lower rates in each crop. Tissue NO_3^- concentrations of the first kale crop significantly decreased under all biochar rates, whereas the opposite effect was observed in the second crop. These contrasting effects of biochar on tissue NO_3^- concentrations were attributed to nitrification inhibition in the first crop and nitrification stimulation in the second crop. The 0.125% w/w rate of eucalyptus-derived biochar was, therefore, recommended to be combined with cricket frass to improve yield and reduce tissue NO_3^- content in the production of Chinese kale.

Keywords: Cricket faeces, *Eucalyptus* branch-derived charcoal, nitrification inhibition, nitrogen transformation, vegetable nitrate

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INTRODUCTION

Cricket keeping has risen in many countries due to a change in consumer habits. Their popularity has since moved into more mainstream urban communities where crickets were once consumed only in poor countryside locales (Hanboonsong et al., 2013). Cricket farms are distributed throughout Southeast Asia, including Thailand, Laos People's Democratic Republic, Cambodia, South Africa, the Democratic Republic of the Congo, and Kenya (Halloran et al., 2018). Cricket-keeping systems in Thailand have been reported to be the world's most advanced (Halloran et al., 2017), numbering over 20,000 farms (Hanboonsong et al., 2013). As a result, massive waste, particularly cricket excrement, termed cricket frass, is released from the farms at an average of 44 Mg/farm/year (Halloran et al., 2017). Mismanagement of these wastes creates the potential for both environmental pollution and human health risks.

Cricket frass is rich in plant nutrients, particularly nitrogen (N), reported at 2.3 - 2.6% (Darby et al., 2017; Halloran et al., 2017) significantly higher than that of poultry manure, reported at 1.7% N (Halloran et al., 2017). The cricket frass-derived organic N mineralizes quickly into NO_3^- due to its N-rich material content. Nitrate leaks into the ground and surface water resources via improper waste management practices. Consumption of water contaminated with NO_3^- may lead to severe human health risks, including cancer, methemoglobinemia, hyperthyroidism, diabetes, and blue baby syndrome (Santamaria, 2006). Recycling cricket frass to a soil amendment will mitigate environmental pollution and human health risks and save chemical fertilizer costs. While peasant farmers in Northeast Thailand have claimed that cricket frass improves rice yield (Halloran et al., 2017), experimental research into the agronomic benefits of cricket frass is required. Very few studies exist to date relating to the employment of cricket frass as a soil amendment. Treelokes (2013) determined that 15.6 Mg crick frass/ha adding into a Ultisol rendered increased yields of morning glory (*Ipomoea aquatica*), kale (*Brassica alboglabra*), and coriander (*Coriandrum sativum*); over organic fertilizers, like cattle manure and urban composts. Furthermore, Darby et al. (2017) demonstrated that 3.16 and 3.83 Mg cricket frass/ha increased sweet corn yields to 21.2 and 25.4 Mg/ha, respectively, while unamended soil produced only 16.3 Mg/ha.

These results indicate that the N-rich properties of cricket frass are suitable as soil amendments for the production of organic vegetables that are commonly subject to N deficiency (Hartz & Johnstone, 2006). However, the rapid nitrification rate of N-rich organic material, *viz* cricket frass, results in a high concentration of soil NO_3^-, producing high NO_3^- accumulation in vegetables (Umar & Iqbal, 2007). Therefore, lowering the nitrification rate is of critical interest in alleviating the resulting NO_3^- content in vegetables.

Biochar is a pyrolyzed organic material used to improve soil and plant growth, particularly in organic vegetables (Suksawang, 2016). Most attractive to scientific study are the consequential biochar benefits in soil and plant improvement (Butnan et al., 2015; Deenik et al., 2011). Such biochar constituents include fixed carbon (C), ash, and volatile matter (Butnan et al., 2015; Deenik et al., 2011). Volatile matter-derived molecules, such as ethylene, acetylene (Spokas et al., 2010), polycyclic aromatic hydrocarbons (Borchard et al., 2014), and α-pinene (Clough et al., 2010), have been shown to play a critical role in the inhibitory effect on nitrifying microorganisms (Dempster et al., 2012; Spokas et al., 2010, 2011). Furthermore, the chemical characteristics of biochar-derived-organic molecules change chronologically once incorporated into the soil (Spokas, 2013). While these chronological changes of biochar promise to affect the NO_3^- accumulation in plants, the temporal effects of biochar as a nitrification inhibitor have not yet been reported.

It was hypothesized that (i) cricket frass would enhance plant growth and yield, (ii) increases in biochar rates would decrease nitrification rates and ameliorate plant tissue NO_3^- content, and (iii) biochar effects on nitrification inhibition and alleviation of plant NO_3^- accumulation would fluctuate with each application. Therefore, the objective of the current study was to evaluate the temporal effects of cricket frass with different biochar rates on vegetable yield and tissue NO_3^- content in two consecutive crops.

MATERIALS AND METHODS

Soil, Cricket Frass, and Biochar

The Roi-et soil series (isohyperthermic Aeric Kandiaquults) was collected at 0–15 cm depth from the Field Research Facilities of the Plant Science Section, Sakon Nakhon Rajabhat University, Sakon Nakhon, Thailand (17°11' 10.1" N, 104° 05' 17.3"E). The soil was air-dried and sieved through a 2 mm sieve for further use in the pot experiments. Soil properties before the start of the experiment are shown in Table 1.

Cricket frass was obtained from a cricket farm in Thailand's Sakon Nakhon province. Contaminant matters, such as dead crickets and body parts, were removed, as well as the remaining feeds. Biochar was produced from eucalyptus (*Eucalyptus camaldulensis*) because of its high content of volatile matter (Antal et al., 2000), a nitrification inhibitor source. The eucalyptus branches were pyrolyzed in a kiln modified from a 300-litre metal tank at approximately 450 °C for two hours and left to cool down for about six hours. The cricket frass and eucalyptus biochar were sieved through a 2 mm mesh. Characteristics of the cricket frass and eucalyptus biochar are presented in Table 1.

Greenhouse Experiments

The pot bioassays of two consecutive crops were conducted in greenhouse conditions from January to April 2021. The average air temperature throughout the experiment was 33.9 °C. The pot experiments were arranged in a randomized complete block design and

Table 1
Properties of soil, cricket manure, and biochar

Material	Proximate analysis			Soil particle distribution			Soil texture	BD	pH	EC	TC	TN	NH_4^+-N	NO_3^--N
	VM †	Ash	fC	Sand	Silt	Clay								
	-------- (%) ------			---------- (%) ----------				(g/cm^3)	(1:10)	(mS/cm)	----- (g/kg) -----		----- (mg/kg) -----	
Soil	–	–	–	71.91	6.29	21.8	Sandy Loam	1.51	5.79	0.070	0.41	0.047	11.6	9.22
Cricket frass	–	–	–	–	–	–	–	0.32	7.92	15.85	376.0	39.1	1,099	147.0
Eucalyptus biochar	19.9	9.5	70.6	–	–	–	–	0.24	9.42	1.36	651.4	5.84	5.55	4.23

Note. †VM = Volatile matter; fC = Fixed C; BD = Bulk density; EC = Electrical conductivity; TC = Total C; TN = Total N

replicated three times. Seven treatments were evaluated herein: the unamended; and cricket frass in combination with biochar at 0%, 0.125%, 0.25%, 0.5%, 1%, and 2% w/w. Chinese kale (*Brassica oleracea*) was used as the test plant, as it is typically high in NO_3^-.

In the first crop, pots (d = 20.3 cm, h = 30.5 cm, v = 4,936 cm^3) were filled with 5 kg of dry soil. Afterward, 12.5 Mg/ha or 0.55% w/w (27.6 g/pot) of cricket frass, a recommended organic fertilizer for Chinese kale (Chakatrakarn & Jala, 2015), was applied to each amended pot. Biochar at the rates of 0% (0 g/pot), 0.125% (6.25 g/pot), 0.25% (12.5 g/pot), 0.5% (25 g/pot), 1% (50 g/pot), and 2% w/w (100 g/pot) was added accordingly. The cricket frass and biochar applied to each pot were computed using the soil weight basis with an initial soil bulk density of 1.51 g/cm^3. The cricket frass and biochar were applied only once at the beginning of the first kale crop. The mixtures were incubated at a 65% moisture level of the soil water holding capacity (WHC) or 19.04% w/w (0.93 L/pot) for 15 days before the Chinese kale was transplanted. A commercial variety of Chinese kale was seeded and nursed in a plug tray for 15 days. A single 15-day-old seedling, selected for its homogeneity and health, was transplanted to each pot. Each pot was weighed daily, and the soil moisture content was maintained at 65% WHC throughout the experiment.

The above-ground kale was cut at 50 days after planting and then oven-dried at 65 °C until the weights were constant and dry shoot biomass was achieved. On the same day, the soil bulk density was measured. Fresh soil was immediately sampled for mineral N content. The soil in each pot was left to air dry. The air-dry soil was sampled and sieved through a 2 mm mesh for laboratory analyses. Subsequently, all pots were managed to achieve equivalent soil weight (4 kg/pot) to grow the second kale crop. The management of the second kale crop was consistent with the first crop.

Laboratory Analyses

Volatile matter, ash, and fixed C of biochar were analyzed following ASTM D7582-15 (American Standard of Testing Material [ASTM], 2012). In addition, biochar total C (TC) and N (TN) were determined on a TN analyzer (multi-N/C® 2100S, Analytik Jena, Germany).

Particle size distribution and soil texture were determined using the pipette method, and soil bulk density was assessed via the core method (Pansu & Gautheyrou, 2006). The pH and electrical conductivity of soil, cricket frass, and biochar were determined in the ratio (of these materials) to water at 1:10. Total C of both the soil and cricket frass was analyzed using the Walkley and Black method (Nelson & Sommers, 1983), and TN concentrations were measured using the micro-Kjeldahl method (Bremner & Mulvaney, 1983). Ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) extraction of soil and the frass was performed via the 2 M potassium chloride (KCl) solution and determined through the distillation method (Stevenson, 1983). Tissue NO_3^--N concentration of the Chinese kale was determined following the salicylic acid assay of Cataldo et al. (1975).

Data Calculations and Statistical Analyses

Calculations of nitrification rate and nitrification inhibition were modified from Sahrawat (1980) through the following equation: nitrification rate (%) = [NO_3^--N/(NH_4^+-N+NO_3^--N] × 100; and nitrification inhibition (%) = [(nitrification rate of cricket frass without biochar – nitrification rate of cricket frass with biochar)/nitrification rate of cricket frass without biochar] ×100.

A one-way analysis of variance (ANOVA) based on a randomized complete block design was used to evaluate the effects of each combination of cricket frass and the corresponding biochar rates on the selected soil properties, soil N transformation, and shoot biomass and tissue NO_3^--N concentrations of the Chinese kale. ANOVA was performed using PROC ANOVA with SAS software 9.1 (SAS Institute Inc., 2004). Multiple comparisons were performed employing Tukey's honest significant difference test. To identify the most important variables used to determine the tissue NO_3^--N concentrations of the kale in both crops, we employed principal component analysis (PCA) using the PROC PRINCOMP model. Significant differences in all statistical analyses were at $p \leq 0.05$.

RESULTS AND DISCUSSION

Soil Bulk Density, pH, Electrical Conductivity, and Total N

Lowered bulk density (BD) and higher porosity are distinguished properties of biochar in soil physical improvement, as evidenced in our current study, in which varied combinations of biochar with cricket frass significantly affected soil bulk density (Table 2). The 2% w/w inclusion of biochar in the first crop and 1% and 2% w/w inclusions in the second crop significantly decreased soil BD compared to cricket frass with 0% biochar (CrF+BC_0) (Table 3). Decreases in soil BD were a result of the dilution effect of biochar (Verheijen et al., 2010). Low BD of our biochar was shown at 0.24 g/cm^3 (Table 1), as similarly reported by Zhang et al. (2010); at 0.25 – 0.30 g/cm^3, while that of soil was higher (1.51 g/cm^3).

Biochar rates increased soil pH and EC in both crops compared to the cricket frass alone (Table 3). The alkalinity of ash components of biochar prompted a rise in soil pH (Yuan et al., 2011). Electrical conductivity is the measurement of the concentration of cations and anions in soil (Miller & Curtin, 2007). Therefore, increased soil EC with concomitant increases in the biochar rates was a consequence of the ash-derived ions.

Increases in pH and EC through cricket frass application, as seen in higher pH and EC in cricket frass alone than in unamended soil (Table 3), were accountable to the alkalinity and salinity properties of cricket frass (Table 1). Our results agreed with Azeez and van Averbeke's (2012) results,

Table 2

Analysis of variance of the effects of combined uses of cricket frass and eucalyptus biochar on soil properties and plant growth, yield, and tissue NO_3^- concentrations

Source of variance	df †	Soil									Plant	
		BD	pH	EC	TN	NH_4^+-N	NO_3^--N	Total mineral N	NR	NI	Dry shoot biomass	Tissue NO_3^--N
		(g/cm^3)	(soil: H_2O =1:10)	(mS/cm)	(g/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(%)	(%)	(g/plant)	(g/kg)
Amendment (A)	6	***	***	***	***	*	***	***	***	***	***	***
Time (T)	1	***	***	***	***	***	***	***	***	***	***	***
A × T	6	**	ns	*	ns	*	***	***	***	***	***	***

Note. * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$

+df = Degree of freedom; BD = Soil bulk density; EC = Soil electrical conductivity; TN = Soil total N; NR = Nitrification rate; NI = Nitrification inhibition; and Tissue NO_3^--N = Tissue NO_3^--N concentration in kale which demonstrated that animal faeces, like poultry, cattle, and goat gained pH of 6.94, 9.11, and 9.75; and EC of 93.9, 18.6, and 65.0 mS/cm, respectively. The significantly higher pH in the first crop than in the second crop (Tables 2 and 3) may result from the effects of the solubility of ash constituents, particularly calcium compounds. Butnan et al. (2015) stated that the mechanism of soil pH increases through the solubility of ash-derived minerals. They further elucidated that dissolution of ash-derived quick lime (CaO) produced immediate increases in soil pH, as observed in the first crop of this study. Meanwhile, ash derived-calcium carbonate ($CaCO_3$) rendered the extent increases in pH in the second crop.

Both crops significantly increased TN under 2% w/w of biochar relative to cricket frass alone (Table 3). Furthermore, while N was volatilized via hydrogen cyanide (HCN), ammonia (NH_3), and dinitrogen (N_2) in the pyrolysis process (Wu et al., 2011); some N was deformed into a complex structure that could be resistant to loss (Usman et al., 2015), as can also be seen in the current study that TN of biochar was shown at 5.84 g/kg (Table 1).

Chinese Kale Biomass

There was a significant interaction of soil amendment × time on the shoot biomass of kale (Table 2). In the first and second crops, combined uses of cricket frass with biochar rates of 0.125% to 0.5% w/w and 0.125% to 0.25% w/w, respectively, resulted in significant increases in dry shoot biomass of kale over cricket frass alone (Figure 1). The respective increases in kale shoot biomass under these lower biochar rates may be attributed to increased soil pH (Table 3) and enhanced nutrient availability (Mengel & Kirkby, 2001). The proper soil pH for the growth of leafy vegetables is 6.0–6.8 (Ebesu, 2004). In the current study, soil pH amended with 0.125%–0.5% w/w in the first crop was 6.91–7.05, and that of 0.125% and 0.25% in the second crop was 6.96–7.01. Furthermore, the improved biomass achieved through the lower rates of biochar may be due to the contribution of essential plant elements in biochar ash (Butnan et al., 2015; Deenik et al., 2011). In the first crop, 0.25% w/w trended to decreased shoot biomass compared to 0.125% w/w. Meanwhile, in the second crop, 0.5% w/w significantly decreased shoot biomass relative to 0.25% w/w (Figure 1). This decline in shoot biomass might be due to excessive increases in soil pH (Table 3), leading to the diminution of micronutrient availability. Weil and Brady (2016) demonstrated that micronutrient cations, e.g., iron, manganese, zinc, and copper, are transformed into insoluble hydroxides and oxides under alkaline conditions, rendering plants' deficiency of these nutrients. In addition, the antagonistic effects of ash-derived cations resulting from excess biochar inputs could bring about the depression of kale shoot biomass (Butnan et al., 2015).

Table 3

Soil bulk density, pH, electrical conductivity, and total N in crops 1 and 2 as affected by the combined uses of cricket frass with different rates of biochar

Amendment †	BD (g/cm^3)		pH (soil: H_2O = 1:10)		EC (mS/cm)		TN (g/kg)	
	Crop 1	Crop 2	Crop 1	Crop 2	Crop 1	Crop 2	Crop 1	Crop 2
Un	1.40 a ‡	1.66 a	6.57 f	6.74 e	0.138 d	0.143 d	0.45 c	0.46 c
CrF+BC_0	1.38 ab	1.55 b	6.82 e	6.89 d	0.364 c	0.296 c	0.59 b	0.51 bc
CrF+$BC_{0.125}$	1.42 a	1.54 b	6.91 d	6.96 d	0.379 c	0.280 c	0.64 ab	0.56 bc
CrF+$BC_{0.25}$	1.42 a	1.49 b	6.98 cd	7.01 cd	0.383 c	0.283 c	0.65 ab	0.56 bc
CrF+$BC_{0.5}$	1.34 ab	1.50 b	7.05 c	7.12 c	0.421 bc	0.332 bc	0.65 ab	0.57 bc
CrF+BC_1	1.27 b	1.43 c	7.20 b	7.31 b	0.476 b	0.385 b	0.69 ab	0.63 ab
CrF+BC_2	1.13 c	1.28 d	7.47 a	7.55 a	0.613 a	0.468 a	0.76 a	0.69 a
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
F test	***	***	***	***	***	***	***	***
CV (%)	3.25	1.37	0.43	0.61	5.87	7.47	7.83	7.41

Note. *** = $p \leq 0.001$

† Un = Unamended; CrF = Cricket frass; and BC_0, $BC_{0.125}$, $BC_{0.25}$, $BC_{0.5}$, BC_1, and BC_2 = Eucalyptus biochar rates of 0%, 0.125%, 0.25%, 0.5%, 1%, and 2% w/w, respectively

‡ Means within the same column followed by the same letter are not significantly different at $p \leq 0.05$ (Tukey's honest significant difference test)

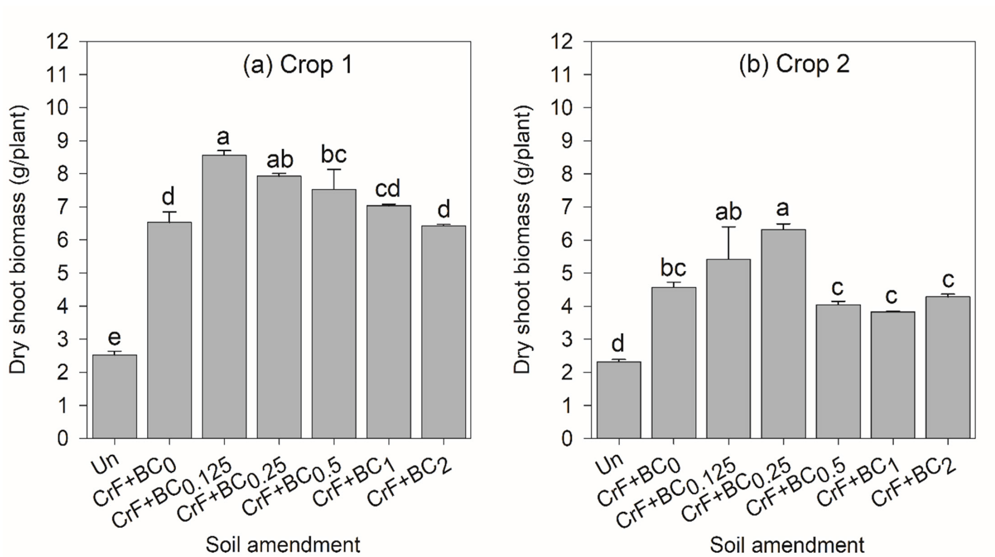

Figure 1. Dry shoot biomass of Chinese kale in the first crop (a) and second crop (b) as affected by the combination of cricket frass (CrF) with eucalyptus biochar at the application rates of 0% (BC_0), 0.125% ($BC_{0.125}$), 0.25% ($BC_{0.25}$), 0.5% ($BC_{0.5}$), 1% (BC_1), and 2% w/w (BC_2) in comparison with the unamended soil (Un); where bars with a similar letter indicate not statistically different ($p \leq 0.05$; Tukey's honest significant difference test), and error bars represent standard deviation (SD)

Soil N Transformation and Kale Tissue NO_3^- Contents

The biochar herein, derived from eucalyptus branches, acted as a nitrification inhibitor, alleviating tissue NO_3^--N concentrations of kale plants in the first crop and *vice versa* in the second crop. These contrasting effects are supported by the significant soil amendment × time interactions on soil NH_4^+-N and NO_3^--N concentrations, nitrification rates, nitrification inhibitions, and tissue NO_3^--N concentrations (Table 2).

All biochar treatments in the first kale crop significantly decreased soil NO_3^--N concentrations, bringing about significant decreases in tissue NO_3^--N concentrations (Table 4). Reductions in soil NO_3^--N concentrations in this cropping cycle resulted from the nitrification inhibitory property of biochar, as shown in the PCA (Figure 2). Soil NO_3^--N concentrations and nitrification rates were positively correlated with tissue NO_3^--N concentrations but negatively associated with nitrification inhibition (Figure 2a). An inhibitory effect of eucalyptus-derived biochar on nitrification in the first kale crop might be functioned by organic compounds constituted in the volatile matter of biochar (Dempster et al., 2012; Spokas et al., 2011). There were several volatile-derived compounds reported as nitrification inhibitors; for example, ethylene, acetylene (Spokas et al., 2010), polycyclic aromatic hydrocarbons (Borchard et al., 2014), and α-pinene (Clough et al., 2010).

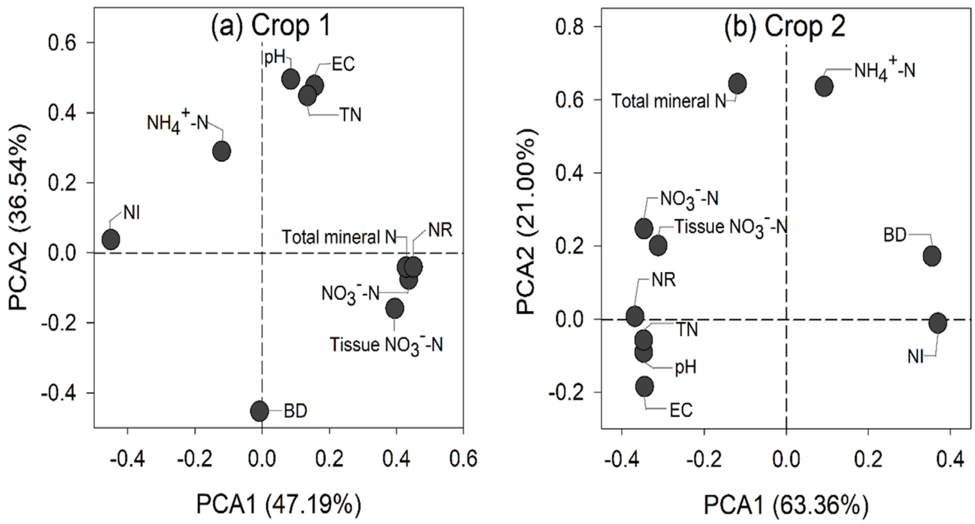

Figure 2. Eigenvectors of principal component analyses on relationships among selected soil properties, soil N transformation, and kale's tissue NO_3^--N concentration in crops 1 (a) and 2 (b)

Note. BD = Soil bulk density; EC = Soil electrical conductivity; TN = Soil total N; NR = Nitrification rate; NI = Nitrification inhibition; and Tissue NO_3^--N = Tissue NO_3^--N concentration in kale

In the second kale crop, biochar increased the nitrification rates (except for 0.5% w/w), resulting in higher NO_3^--N concentrations in the soil and plant tissues (Table 4). PCA results verified the biochar effects (Figure 2b), in which tissue NO_3^--N concentrations were positively correlated with NO_3^--N concentrations in the soil and kale tissues. In contrast, the results were negatively associated with nitrification inhibition. Therefore, biochar accelerated the nitrification rate instead of acting as an inhibitor (Table 4). Ethylene (C_2H_4), a volatile matter ingredient produced during pyrolysis and further formed after the biochar incorporation into the soil, might play a significant role in nitrification inhibition in this study. Spokas et al. (2010) determined that C_2H_4 in the volatile matter of biochar could inhibit soil nitrification. Fulton et al. (2013) further incorporated hazelnut shell-derived biochar containing 19.4% volatile matter into the soil, producing a volatile matter content similar to the levels observed herein. A large amount of volatile matter began to release from the soil on the first day of its incorporation and decreased abruptly. At 42 days after biochar incorporation, a minute amount of C_2H_4 was detected. Losses of C_2H_4 occurred within the first kale crop, bringing about no inhibitory effects of biochar on nitrification in the second crop. In addition to the loss of inhibitory effect of biochar on nitrification, increases in nitrification rates in the second kale crop might be due to the rise in nitrifier activity resulting from the elevated C and N contents in the soil mixture. These elements

Table 4

Soil mineral N concentrations and N transformation in crops 1 and 2 as affected by the combined uses of cricket frass with different rates of biochar

Amendment †	NH_4^+-N (mg/kg)		NO_3^--N (mg/kg)		Total mineral N (mg/kg)		Nitrification rate (%)		Nitrification inhibition (%)		Tissue NO_3^--N (g/kg dry shoot biomass)	
	Crop 1	Crop 2	Crop 1	Crop 2	Crop 1	Crop 2	Crop 1	Crop 2	Crop 1	Crop 2	Crop 1	Crop 2
Un	11.8 b-d ‡	4.81 ab	9.4 e	0.51 c	21.2 d	5.32 b	44.3 d	9.6 c	–	–	0.57 c ‡	0.52 c
CrF+BC_0	11.2 cd	3.65 c	44.4 a	0.68 c	55.7 a	4.33 c	79.2 a	16.0 bc	–	–	5.83 a	2.72 b
CrF+$BC_{0.125}$	13.7 a	4.89 ab	13.7 de	1.47 a	27.4 cd	6.36 a	49.9 cd	23.0 ab	+37.0 a	-43.4 ab	2.62 b	5.02 a
CrF+$BC_{0.25}$	10.8 d	4.78 ab	21.1 b-d	1.43 ab	32.0 bc	6.21 a	66.0 b	23.0 ab	+16.6 b	-43.6 ab	3.27 b	5.04 a
CrF+$BC_{0.5}$	12.9 a-c	5.28 a	27.3 b	1.14 b	40.2 b	6.42 a	68.1 b	17.7 bc	+14.0 b	-10.5 a	2.49 b	4.37 ab
CrF+BC_1	12.7 a-d	4.46 b	23.2 bc	1.64 a	35.9 bc	6.10 a	64.7 b	27.0 a	+18.3 b	-68.2 b	3.95 b	5.01 a
CrF+BC_2	13.4 ab	3.81 c	15.9 c-e	1.49 a	29.3 cd	5.30 b	54.3 c	28.1 a	+31.4 a	-75.6 b	0.50 c	4.72 a
p value	0.041	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.004	< 0.001	< 0.001
F test	*	***	***	***	***	***	***	**	***	**	***	***
CV (%)	8.44	7.67	23.27	15.21	16.83	7.07	4.50	14.64	13.05	-30.38	20.2	15.24

Note. *= $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$

† Un = unamended; CrF = cricket frass; BC_0, $BC_{0.125}$, $BC_{0.25}$, $BC_{0.5}$, BC_1, and BC_{2} = eucalyptus biochar rates of 0%, 0.125%, 0.25%, 0.5%, 1%, and 2% w/w, respectively

‡ Means within the same column followed by the same letter are not significantly different at $p \leq 0.05$ (Tukey's honest significant difference test)

were not beneficial to the nitrifiers, possibly because the overriding effect of the C_2H_4 was abundant in the first kale crop.

Lower concentrations of soil NH_4^+-N, NO_3^--N, TN, and nitrification rate in the second crop compared to the first crop (Table 4) since the cricket frass and biochar were applied to the soil once only at the beginning of the first crop.

CONCLUSION

The results of this study showed that 0.125% to 0.5% w/w of eucalyptus branch-derived biochar in the first crop and 0.125% to 0.25% w/w in the second crop enhanced the shoot biomass of Chinese kale. Notably, increases in biochar amendments (0.25% and 0.5% w/w) in the first and second crops brought about decreases in shoot biomass.

All biochar rates reduced tissue NO_3^- concentrations of kale in the first crop but not in the second crop due to the inhibitory effects of biochar on nitrification in the first crop and, in contrast, the stimulation effect within the second crop. The most suitable rate of eucalyptus biochar in combination with 12.5 Mg/ha of cricket frass to enhance yield and alleviate tissue NO_3^- contents was 0.125% w/w or 2.831 Mg/ha. Additional applications of biochar in subsequent cropping cycles for eradicating nitrification stimulation and ameliorating tissue NO_3^- content are necessary for further investigation.

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