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Original Article

Response of methane emissions, redox potential, and pH to eucalyptus biochar and rice straw addition in a paddy soil

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Abstract

This study aims to comprehend the links between soil Eh and pH changes in eucalyptus biochar (BC) and rice straw (RS) amended soils and CH_4 emissions. Increased CH_4 emission rates and high total CH_4 emissions (TCH_4) were found in RS soils. In contrast, higher concentrations of refractory lignin, fixed C and volatile matter in BC suppressed C mineralization and terminal methanogenesis, resulting in low TCH_4. Eh in RS soils decreased more rapidly than in BC soils during the first phase as a single exponential function. This indicated that RS is a fast electron donor for an instant electron acceptor reduction and methanogenesis. During the second phase, Eh in BC soils decreased to very low values, probably because of the higher concentrations of electron donating phenolic compounds coupled with terminal methanogenesis. Meanwhile, hydrogen is consumed via electron acceptor reduction and methanogenesis simultaneously produce OH^- corresponded with a rise of pH, a characteristic of reverse single exponential function.

Keywords: organic residue quality, organic decomposition, eucalyptus wood biochar, greenhouse gas emission, carbon mineralization

1. Introduction

Rice straw (RS) is usually incorporated into soil to improve productivity. RS consists of high contents of labiled, notably, cellulose and hemicellulose. It also is an easily decomposable organic residue providing active labile organic carbon (C) pool, e.g., H_2+CO_2, organic acids, alcohols and amines, major substrates for methanogens and thus contributing to carbon dioxide (CO_2) and methane (CH_4) production under anaerobic condition (Le Mer and Roger, 2001; Khosa *et al*., 2011; Moterle *et al*., 2013). Biochar (BC), a stable fixed carbon (C) rich form of charcoal, mainly consists of resistant compounds, i.e., graphite-like carbon compounds, lignin, volatile matter, polyphenol and aromatic compounds. BC also can be applied to agricultural land as a soil amendment to improve not only soil productivity, but also reduce greenhouse gas emissions from paddy fields (Lehmann *et al*., 2006; Feng *et al*., 2012). Chemically resistant property of BC causes labile organic substrate deficiency for methanogenesis. Application of BC gave an increase in soil pH, which may affect methanogenic activity (Wang *et al*., 1993); meanwhile methanotrophs are more tolerant to alkaline pH than methanogens (Le Mer and Roger, 2001) leading to methanotrophic abundance and a decrease in ratio of methanogen to methanotroph (Feng *et al*., 2012). All of these mechanisms cause reduction of CH_4 emissions by incorporation of BC into soil.

The chemical composition and quantity of organic residues take part in controlling the decomposition process (Johnson *et al*., 2006; Samahadthai *et al*., 2010; Sutton-Grier *et al*., 2011) and therefore play an important role in determining C mineralization, availability of C substrate, methanogenesis and greenhouse gas emissions. Cross and Sohi (2011) demonstrated that BC has low labile organic C, 0.22 to 1.8%. However, Yun *et al*. (2001) found high labile extractable C in RS was 9.8%. RS has higher contents of labile fraction

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such as cellulose, hemicellulose, but lower lignin and polyphenol contents than BC (Rashad and Hussien, 2013). These contrasting chemical characteristics between BC and RS result in different decomposition rates. Therefore, given the chemical characteristics of BC, application of BC may result in greenhouse C gas mitigation. However, for the moment, no comparative study on the contrasting impacts of BC and RS on organic C mineralization and CH_4 emissions in tropical soils has been published.

Submerged soils with rice plants provide a unique anaerobic system. This is due to chemical and biological activities that lead to changes in soil redox (Eh) status and pH. The shifts from high to low Eh and from low to high pH occur in anaerobic microbial respiration leading to the formations of reductive inorganic products, intermediate organic products and CH_4. In methanogenesis, CO_2 and acetic acid are used by methanogenic archaea as terminal electron/H^+ acceptors when CH_4 is formed (Le Mer and Roger, 2001; Feng *et al.*, 2012). Therefore soil Eh and pH are important mechanistic indicators of CH_4 production. Bossio *et al.* (1999) demonstrated that RS application to paddy soils brought about a lower soil Eh (-275 mV) than did BC application (-225 mV). In addition, there has been no hard evidence on soil Eh for mechanism related to methanogenesis with comparison between the contrastive attributes of these two important soil organic amendments, BC and RS, in flooded rice soil.

In this study, we hypothesize that the chemically resistant properties of BC, i.e., lignin, polyphenol, and aromatic compound, contribute to mechanisms underlying methanogenesis leading to a decrease in CH_4 gas emission relative to an equivalent addition of RS. Therefore, the aims of this study were to evaluate total CH_4 emissions during a rice growth period and mechanisms involving soil Eh-pH changes underlying methanogenesis as affected by application of BC and RS amendments to paddy soils.

2. Materials and Methods

A plant-pot experiment with rice plants and a microcosm experiment in rice-free soil were carried out to identify the impact and magnitude of BC and RS additions on soil Eh and pH and CH_4 production in the overlying flood water.

2.1 Soil, BC and RS characteristics

The plant-pot experiment was conducted from November 2012 to May 2013. The studied soil was classified as fine, mixed, isohyperthermic Aeric Endoaquepts (USDA, 1999), and Ratchaburi (Rb) soil series in the Thai soil classification system (LDD, 2005). Physicochemical characteristics of the studied top soil (0-15 cm) were SOC 0.71%, total N 0.08%, cation exchange capacity (CEC) 11.5 cmol kg^{-1}, pH 5.0 and bulk density 1.45 g cm^{-3}. Particle size distribution was 50.0% sand, 36.7% silt and 13.3% clay, and was classified as loamy soil texture.

The BC used was produced from eucalyptus wood (*Eucalyptus camaldulensis Dehnh.*) by a pyrolysis process in a conventional kiln at the temperature of 350°C for a total of 48 hrs. It was then crushed, ground, and passed through a 2 mm sieve. The studied BC is slightly alkaline, high fixed C, volatile matter, lignin, total organic C (TOC) and low total N. BC has very high C:N ratio of 114 and low labile organic carbon (LOC, 13.28%) (Table 1). RS used in this study was chopped into 5-10 cm lengths before being incorporated into the soil. RS displays neutral pH, high contents of cellulose and hemicellulose. TOC of RS is lower than that of BC. RS has low total N. C:N ratio of RS is 60. And it possesses high amounts of LOC 66.74% (Table 1).

2.2 Treatments

Either biochar (BC) or rice straw (RS) was applied based on their dry weights, encompassing low to high rates studied in rice field experiments by previous soil scientists (Shen *et al.*, 2014). Ten treatments were designated as follows: (1) control (no BC, RS), (2) sole chemical fertilizer (CF) (no BC, RS), (3) BC 6.25 t ha^{-1} (BCL), (4) BC 12.50 t ha^{-1} (BCML), (5) BC 18.75 t ha^{-1} (BCMH), (6) BC 25.00 t ha^{-1} (BCH), (7) RS 6.25 t ha^{-1} (RSL), (8) RS 12.50 t ha^{-1} (RSML), (9) RS 18.75 t ha^{-1} (RSMH) and (10) RS 25.00 t ha^{-1} (RSH). The whole experiment (treatments 2-10) received CF, 16-16-8 (16% N, 16% P_2O_5, 8% K_2O) as basal fertilizer at a rate of 250 kg ha^{-1} and was topped with urea (46% N) in two applications to give a total of 187.5 kg ha^{-1}. Topping with urea was not done for the experiment in rice-free soil microcosms.

2.3 Rice-planted pot experiment

The plant-pot experiment was carried out in a greenhouse in a completely randomized design (CRD). Each treatment was tested in triplicate. The cylindrical plastic

Table 1. Chemical characteristics of biochar and rice straw used in the experiments. TOC and LOC represent total organic carbon and labile organic carbon.

Properties	Biochar	Rice straw
pH (1:5)	7.98	7.01
EC (1:5) (dS m^{-1})	0.94	5.19
Total N (%)	0.54	0.65
TOC (%)	61.43	39.29
C:N ratio	114	60.00
LOC (g kg^{-1})	13.28	66.74
Cellulose (%)	6.25	50.84
Hemicellulose (%)	1.00	22.19
Lignin (%)	75.69	3.33
Volatile matter (%)	22.86	-
Ash (%)	2.99	-
Fixed C (%)	69.56	-

plant-pot used in the experiment was 19 cm in diameter and 24 cm in height. Three kilograms of soil was mixed thoroughly with an equivalent weight of BC and RS according to respective treatments 20 days before transplanting the rice (-20 DAT; day after planting). Five, twenty-five day old rice seedlings, non-photosensitive Pathum Thani 1, were transplanted per hill per plant-pot. Water-logging was maintained to give a water depth of 5-7 cm above the soil surface until 27 DAT. Thereafter, shallow flood water (0-7 cm) was controlled throughout the growth period. Platinum (Pt) electrodes for soil Eh measurement were installed at 4 cm distance from the rice hill at 7 cm depth from the soil surface. Duplicate measurements for each treatment were performed. CH_4 emission rate, soil Eh and pH in the flood water were measured throughout the rice growing season.

2.4 Non-rice microcosm

A laboratory based, microcosm experiment with bare soils (no rice) was conducted to evaluate the impact of BC and RS amendments without interference of rhizodeposition and oxidation from rice roots on Eh and pH in the flood water of the submerged soils. BC and RS was ground and sieved (2 mm) before use. Ten treatments, similar to the plant-pot experiment, were prepared with 500 g of air-dried soil in polyethylene plastic jars of 6 cm diameter and 18 cm height. Flood water was maintained at a depth of 5 cm above the soil surface. A Pt electrode was inserted into the soil to 7 cm depth. All treatments were arranged in a completely randomized design (CRD) with two replications. Soil Eh and pH in the flood water were measured immediately after flooding, every 6 h for 4 days and subsequently every 12 hrs for 5-15 days.

2.5 Analysis of soil, BC, and RS

Organic carbon in soil (SOC), BC, and RS (TOC) were determined with an Elemental CNS Analyzer (Thermo Fisher Scientific, Flash 2000, England). Labile organic carbon (LOC) in soil, BC and RS were determined by the $KMnO_4$ (33 mM) oxidation method (Moody and Cong, 2008). Cation exchange capacity (CEC) was determined by distillation and titration (Sumner and Miller, 1996). The pH and electrical conductivity (EC) of soil, BC and RS were measured by using standard method in a specimen: water ratio of 1:5 (w/v) suspension with a pH meter (Seven Easy Mettler Toledo, China) and EC meter (Seven Easy Mettler Toledo, China). Total N was determined by the Micro-Kjeldahl method (Bremner and Mulvaney, 1982).

The approximate BC properties representing ash content, volatile matter and fixed C were obtained following standard ASTM (2007) methods. Cellulose, hemicellulose, and lignin content in RS and BC were analyzed following the methods of Aravantinos-Zaris *et al.* (1994).

Prior to use, the Pt electrodes were soaked in Regia reagent for 24 hrs. They were checked against known oxidation-reduction potential (ORP) solutions. The measured Eh value was stable in the range of 450 to 500 mV. Pt electrodes (EP-201), reference electrode (4400), Eh meter (PRN-41) and ORP standard solution were manufactured by DKK Corporation, Japan.

2.6 CH_4 gas sampling and analysis for plant-pot experiments

CH_4 gas was measured during the rice growing period. The closed chamber method was used to collect gases from plant-potted soils with a gas chamber of 0.21 m × 0.21 m × 1 m. Gas samplings were performed weekly throughout the rice growing season (Saenjan *et al.*, 2002; Ro *et al.*, 2011).

CH_4 concentration was analyzed using a gas chromatograph (Shimadzu GC-14B, Japan) equipped with FID and a stainless steel column packed with porapak N. Column and detector were heated to 60°C and 100°C, respectively. High purity N_2 served as a carrier gas and the retention time of CH_4 was 0.33 min. CH_4 emission rates were calculated from the increases in concentration with time using the volume of the gas chamber, corrected for temperature inside the chamber and the space height above the water level. Total CH_4 emissions (TCH_4) were calculated by summing up the emission quantities between each two adjacent intervals of measurement (Naser *et al.*, 2007).

2.7 Statistical analysis

TCH_4 was analyzed for statistically significant differences using an analysis of variance (ANOVA) indicated by the P-value ($P<0.05$) of the Duncan's Multiple-Range Test (DMRT) by using SAS version 9.1 (SAS Institute, Inc., Cary, NC, USA). Standard errors of the difference are presented on the plotted graphs. Exponential regressions were used to describe for temporal change in soil Eh and pH in flooded water by using Sigma Plot for Windows 11.0 (Systat Software, Inc., Germany).

3. Results

3.1 CH_4 emissions in plant-potted soil

From -9 DAT to 56 DAT (Figure 1a), CH_4 gas emission was low (<160.68 mg CH_4 m^{-2} d^{-1}) under BC application (Figure 1a). However, during the late experimental period, 63 DAT to 84 DAT, CH_4 emission rates in the BC treatments increased from 1276.60 to 3749.90 mg CH_4 m^{-2} d^{-1}. In contrast, RS incorporation (Figure 1b) led to high CH_4 emission rates right from the early growing period onwards, 3030.40 to 3419.60 mg CH_4 m^{-2} d^{-1}. Moreover, CH_4 emission rates from RS application remained high during the latter part of the experiment (Figure 1a, b). TCH_4 was not significantly different between BC levels, CF and the control (0.47 to 0.59 t CH_4 ha^{-1}) (Figure 1a), while TCH_4 emission rates in the RS additions increased with increasing RS application rates (0.47 to 1.45 t CH_4 ha^{-1}) (Figure 1b).

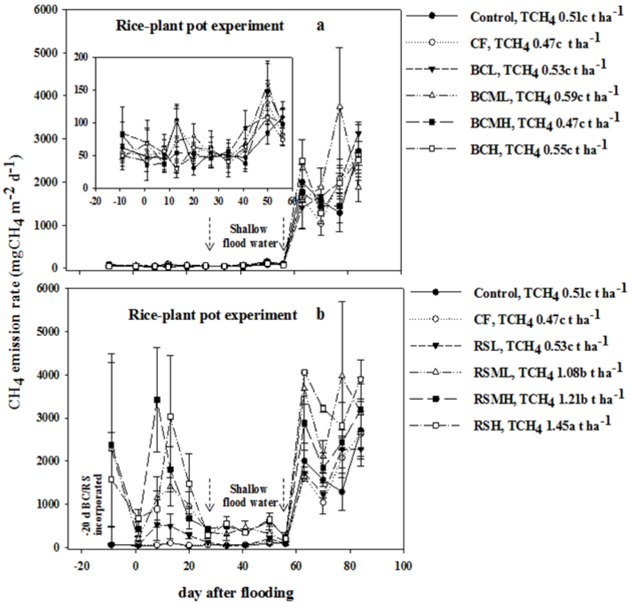

Figure 1. CH_4 emission rates and TCH_4 from rice-planted pot soil with BC (a) and RS (b). The box inserted in the upper plot (a) is CH_4 emission rates from -9 to 56 day after flooding. Bars indicate standard errors of the means. Different lowercase letters accompanying TCH_4 indicate significant difference among treatments P<0.05, n = 3.

3.2 Soil Eh and pH (rice plant-pot experiment and soil only microcosm)

In rice plant-pot experiment, soil redox potential (Eh) decreased from initial values to -106 to -218 mV in all treatments (Figure 2a) by 8 DAT. However, there was no significant effect of BC or RS application level, although there was a slight trend towards lower value of soil Eh (-258 mV) in BCH as compared to RSH.

The pH in the flood water in rice plant-pot experiment (Figure 2b) were already well above neutral (pH>7) at the first sampling (8 DAT), pH then slightly dropped and the increased towards the end of the growing season. In general, the flood water pH of the whole experiment was above neutral. At most sampling times, flood water pH values in BCMH and BCH treatments were generally higher than the rest of the treatments.

A microcosm experiment with soil but without rice plants was conducted in parallel (Figure 3a, b). Soil Eh in all treatments rapidly decreased below -100 mV 4 days after flooding (DAF) (Figure 3a). Although, no significant difference in soil Eh among treatments was found, a more rapid decrease in soil Eh occurred particularly during 1 to 4 DAF in the RSH treatment than the BC and other RS treatments, which Eh dropped at much slower rates. From 4 DAF and until the end of experiment, soil Eh became more or less stable in all of the treatments. However, soil Eh in BC treatments was visibly lower than those in RS treatments. Conversely, flood water pH measured in the corresponding submerged soil (Figure 2b, 3b) displayed higher pH values in the order of: BC treatments > RS treatments > ≈ control.

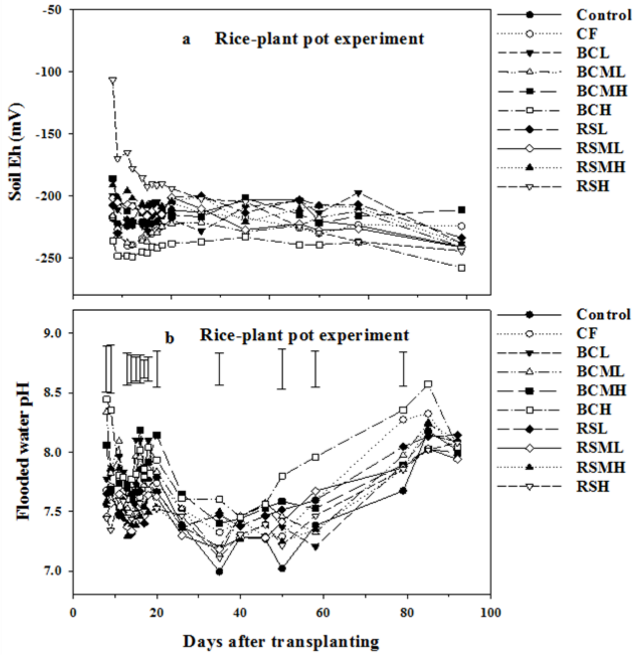

Figure 2. Soil Eh (a) and pH (b) in flood water (rice-plant pot experiment). Bars indicate standard error of the difference. Only the significant pH differences are shown (*P*<0.001), n = 3.

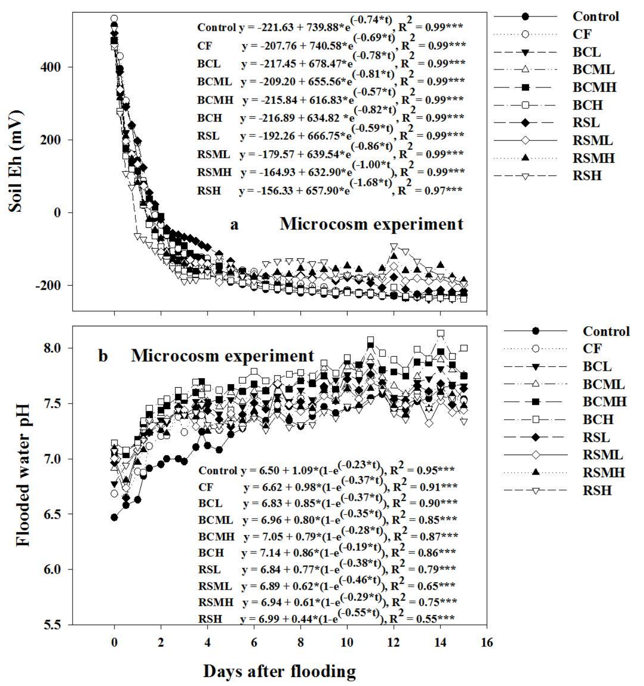

Figure 3. Soil Eh (a) and pH (b) in the flood water (non-rice microcosm experiment) with BC and RS, n = 2.

4. Discussion

4.1 Response of CH_4 emissions to contrasting effect of BC and RS

TCH_4 was low (0.47 to 0.59 t CH_4 ha^{-1}) in the plant-potted soils with added BC, CF and in the control. This is probably due to the eucalyptus wood used to make BC as it is rich in stable C (lignin 75.69% and fixed C 69.59%), but poor in labile fractions (LOC 13.28%, cellulose 6.25% and hemicellulose 1%). Though BC possesses high content of TOC, the limiting contents of mineralizable C probably explain why BC amended soils were unsuitable for methanogenesis. This is similar to the results from wheat straw biochar, which fixed C contents was as high as 60% (Wu *et al*., 2012). Dong *et al*. (2013) also reported that rice husk and wood biochar containing high fixed C ranging from 51.3 to 83.2% has no significant CH_4 emission from such amended soil, while RS application markedly increased CH_4 emission rates to 2.07 times the control. In addition, BC application to soil stimulated microbial mechanisms that decrease CH_4 emission by increasing methanotrophic abundance. This was likely due to porous structure of biochar which increased aerobic sites hence an increase in CH_4 oxidation (Feng *et al*., 2012). In addition, the alkali BC-soil pH is suitable for methanotropic proliferation (Le Mer and Roger, 2001). All of these factors led to a decreased ratio of methanogens to methanotrophs. In addition, Dempster *et al*. (2012) demonstrated that eucalyptus wood derived BC applied in coarse texture soil led to a decrease in microbial biomass C. They proposed that this decrease was caused by C limitation and the inhibition of microbial activity by volatile compounds. We propose that the increased pH in BC added soil should not negatively affect methanogen activity as pH range for metabolism of methanogens are near neutral to alkali. In contrast, we suggest that the step from C mineralization to utilizable C substrate such as LOC is the main limiting step on methanogenesis (CH_3COOH to $CO_2 + CH_4$; and $CO_2 + 4H_2$ to $CH_4 + 2H_2O$). It should therefore be investigated further.

Soil Eh in the rice plant-pot experiment (Figure 2a) under BC and RS applications ranged from -106 to -258 mV, which is suitable for methanogenesis when C substrates of sufficient quantity and quality are present. Soil Eh rapidly dropped in the first 4 DAF in no rice microcosm (Figure 3a), especially in RSH treated soil. This illustrates that RS incorporated into soil was subject to fast C mineralization. RS probably served as a substrate (CH_3COOH, for CH_4 and CO_2 production) as well as an electron donor for CH_4 formation (CO_2 reduction to CH_4), the last terminal electron acceptor in typical flooded soil. Along with the drop of Eh, pH simultaneously increased to 6.5 to 8.7 (Figure 3b) encompassing the optimal pH of 7.5 to 8.5 for methanogenesis (Kitamura *et al*., 2011). The Eh range in our plant-pot and microcosm experiments is consistent with the previously reported range of -150 to -200 mV which is one of the main controlling factors for CO_2 and CH_4 gases build up (Le Mer and Roger, 2001; Yu and Patrick, 2004; Shen *et al*., 2014).

4.2 Soil Eh and pH as a function of BC and RS application

In the non-rice microcosm experiment, we found that the single exponential function ($y = y0 + (ae^{-bt})$) was the best fit for soil Eh during the flooding period. Where, "b" is an exponential rate of Eh change (Figure 3a). Exponential rate b indicates a declining Eh character over time "t" (DAF). A high value of b means accelerating decline of Eh, whose shape corresponds to the first phase of soil Eh change during the first 4 DAF. For RS treatments, b values (0.59 to 1.68) were higher than those of BC (0.57 to 0.82). This implies that in RS-added-soil Eh reached strong reductive soil conditions faster than BC in the first phase. This means that more electrons were removed from RS through anaerobic microbial respiration during this phase. In other words, RS served as a faster electron donor for terminal electron donor reduction and methanogenesis via anaerobic organic C mineralization better than BC did. This is due to the high amount of easily decomposable organic compounds in RS. Furthermore "y0", the final interception on the y-axis (soil Eh values), was higher in RS treatments (y0 = -192.26 to -156.33) than in BC treatments (y0 = -209.20 to -217.45). Though soil Eh of both BC and RS treatments reflected no significant difference in reductive soil conditions, lower Eh values in BC treatments could be observed from 5 DAF until the final day. BC appeared to be able to maintain lower soil Eh in the longer term as compared to RS. This could be attributed to a slower organic mineralization rate of BC, hence a steady slow release of electrons to terminal electron acceptors. This is probably caused by the redox-active phenolic compounds in low-temperature-pyrolysis biochar similar to the finding reported by Kluepfel *et al.* (2014). However, during latter period all soil Eh values were quite stable, corresponding to the second phase of soil Eh change. The two phases of Eh change can probably be attributed to electron transferring from respiration of facultative anaerobes and methanogens in the first phase followed by sustaining electron activity by methanogens in the second phase.

In contrast to soil Eh changes, soil pH (as measured in the flood water) showed an exponential increase ($y = y0 + a(1-e^{-bt})$). This model is a reverse single exponential function and was the best fit for soil pH change during the flooding period under BC and RS application (Figure 3b). We found pH rate change in BC treatments (b = 0.19 to 0.37) were lower than in RS treatments (b = 0.29 to 0.55), these in turn provided a sharper rise of soil pH in BC treatments related to RS. In the soil system, three mechanisms are involved in increasing of floodwater pH; firstly, H^+ consumption in various reduction reactions mediated by anaerobes and methanogens accompanied by the OH^- production causing an increase in soil pH. This was observed in both pot and microcosm experiments. Secondly, CO_2 was consumed in photosynthesis by aquatic chemoautotrophs leading to production of small amount of carbonate species (HCO_3^- and CO_3^{2-}) in floodwater. This, in turn, led to a rise in floodwater pH found in both BC and RS treated soils observed in pot experiment. Thirdly, pH rise more in BC than in RS treated soils because BC-derived ash, which contains oxides and carbonate of basic cations (Joseph *et al.*, 2009). This was found in both pot and microcosm experiments. This rise in soil pH was particularly rapid at the initial change (4 DAF) in this low pH buffering capacity soil with high (50%) sand content. Empirical data from this research also showed a decrease in soil Eh values with rising pH, illustrating the inverse relationship between Eh and pH.

5. Conclusions

Application of BC substantially limited organic C mineralization and reduced total CH_4 emission. In contrast, RS addition enhanced C mineralization and total CH_4 emission. In addition, although BC provided a slight delay in the decrease of flooded soil Eh, the insufficient substrate C availability resulted in low CH_4 production. Accordingly, suppression of CH_4 emission by BC amendments is proposed to be due to the high proportion of chemically resistant compounds in BC that suppress C mineralization and the subsequent methanogenesis process. This research also highlighted the very fast soil Eh decrease (single exponential function) concomitant with an exponential increase in pH. Both pH and Eh occurred as two phases with anaerobic respiration via terminal electron acceptor reduction and methanogenesis during the first phase. In the second phase BC could maintain lower Eh than RS probably as a consequence of the slower decomposition of volatile matter, lignin and fixed C compounds which maintained methanogenesis at low rates.

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