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Carbonaceous Greenhouse Gases and Microbial Abundance in Paddy Soil under Combined Biochar and Rice Straw Amendment

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Abstract: Little is known about the carbonaceous greenhouse gases and soil microbial community linked to the combination of biochar (BC) and rice straw (RS) in paddy soils. The objectives of this research were to evaluate the effects of combining BC and RS on (1) CH_4 and CO_2 production from paddy soil, (2) archaeal and bacterial abundance, and (3) rice grain yield. The experiments consisted of a pot trial and an incubation trial, which had a completely randomized design. The experiments included five treatments with three replications: (a) the control (without BC, RS, and chemical fertilizer (CF)); (b) CF; (c) BC 12.50 t ha^{-1}; (d) RS 12.50 t ha^{-1}; and (e) combined BC 6.25 t ha^{-1} + RS 6.25 t ha^{-1} + CF. In the sole RS treatment, CH_4 production (0.0347 mg m^{-2} $season^{-1}$) and the archaeal and bacterial abundance (5.81×10^8 and 4.94×10^{10} copies g^{-1} soil dry weight (DW)) were higher than outcomes in the sole BC treatment (i.e., 0.0233 mg m^{-2} $season^{-1}$ for CH_4 production, and 8.51×10^7 and 1.76×10^{10} copies g^{-1} soil DW for archaeal and bacterial abundance, respectively). CH_4 production (0.0235 mg m^{-2} $season^{-1}$) decreased significantly in the combined BC + RS + CF treated soil compared to the soil treated with RS alone, indicating that BC lessened CH_4 production via CH_4 adsorption, methanogenic activity inhibition, and microbial CH_4 oxidation through bacterial methanotrophs. However, the archaeal abundance ($3.79–5.81 \times 10^8$ copies g^{-1} soil DW) and bacterial abundance ($4.94–5.82 \times 10^{10}$ copies g^{-1} soil DW) in the combined BC+ RS + CF treated soil and the RS treated soil were found to increase relative to the treatments without RS. The increase was due to the easily decomposable RS and the volatile matter (VM) constituent of the BC. Nevertheless, the resultant CO_2 production was relatively similar amongst the BC, RS, and BC + RS treated soils, which was indicative of several processes, e.g., the CO_2 production and reduction that occurred simultaneously but in different directions. Moreover, the highest yield of rice grains was obtained from a combined BC + RS + CF treated soil and it was 53.47 g pot^{-1} (8.48 t ha^{-1}). Over time, the addition of BC to RS soil enhanced the archaeal and bacterial abundance, thereby improving yields and reducing CH_4 emissions.

Keywords: global warming; archaeal 16S rRNA gene; bacterial 16S rRNA gene; rice yields; qPCR; soil amendments

1. Introduction

To maintain the soil fertility and rice yield, the incorporation of rice straw (RS) into paddy soil has been widely practiced. However, in flooded soil conditions, the decomposition of RS results in high levels of CH_4 and CO_2 emissions from its high cellulose and hemicellulose content (at more than 50%

dry weight), both of which are easily decomposable C compounds [1]. In addition, some intermediate C products include dissolved organic carbon (DOC), which comprises low molecular-weight organic compounds such as acetates, formates, methylated compounds, primary and secondary alcohols, and some gases, e.g., CO_2 and H_2. All of these compounds are substrates for the methanogenic archaea which stimulate CH_4 production [2]. Concurrently, CH_4 oxidation mediated by methanotrophic microorganisms existing in the flooded soil system also occurs. The CH_4 oxidation results in the production of CO_2, as well as a decrease in CH_4 emissions into the atmosphere.

Contrary to the easily decomposable RS, biochar (BC), which is made from woody feedstock materials, has high contents of C resistant compounds, such as lignin [1]. Therefore, it is considered to be a resistant organic material. In particular, eucalyptus wood BC contains over 70% lignin (DW), which suppresses microbially mediated C mineralization [1,3]. The addition of BC creates a low available C condition, creating unsuitable circumstances for methanogenesis by archaea [4]. Although the BC incorporated into paddy soils suppresses CH_4 emissions, it also increases nutrient availability and rice yields [3]. When RS and BC were individually applied to paddy soils, contrasting effects on CH_4 production were found. Owing to contrasting chemical compositions, RS produced enhancing effects, whereas BC produced suppressing effects given its high content of fixed C, such as lignin [3], which are unfavorable to methanogenic activity [5]. Nevertheless, it is worth studying the incorporation of combined BC with RS, as well as the biological aspects of methanogen. In field situations, when BC is applied to paddy soil, it inevitably mixes with RS residues that remain after the paddy fields have been harvested. Therefore, it is imperative to investigate the effects of combining BC and RS on greenhouse gas production. An earlier study by Liu et al. [5] showed that when medium to high amounts of rice straw derived BC were mixed with RS, the CH_4 emissions from the incubated paddy soils declined by 21–35% compared to emissions without BC, citing the inhibiting effect of BC on methanogenic activity. However, the study did not investigate the microbial abundance. Findings on methanogen stimulation by BC were later reported by Feng et al. [6], who employed microbial gene abundance as the main indicator of microbial influence on CH_4 emissions in soils treated solely with BC. The research showed that corn stalk BC stimulated both the methanogenic archaea and the methanotrophic bacteria, as determined by their gene abundances. However, the CH_4 produced by the archaea could not meet the requirements of the bacteria. To our knowledge, there is no known work which has combined easily decomposable RS and resistant eucalyptus BC to test the effects of this mixture on the production of CH_4 and other carbonaceous greenhouse gases, using microbial gene abundance as a major indicator.

In this research, we addressed the hypothesis that adding the combined BC and RS to a paddy soil would reduce the soil's CH_4 production, raise its archaeal and bacterial abundance, and increase the rice grain yields. Therefore, the objectives of this research were as follows: (i) To evaluate the effects of the combined BC and RS on CH_4 production, CO_2 production, and the archaeal and bacterial abundance in paddy soils and (ii) to determine the effects that these conditions would have on the rice grain yields.

2. Materials and Methods

2.1. Organic Materials and Soil

BC was produced via pyrolysis at 350 °C under oxygen limited conditions in a traditional kiln commonly used in Northeastern Thailand. The feedstock consisted of the upper parts of the branches of 5 year-old eucalyptus trees (*Eucalyptus camaldulensis* Dehnh.). Meanwhile, the RS used was taken from a paddy field. The following chemical analyses of the BC and RS were conducted: (1) pH using a pH meter (BC or RS: water = 1:5); (2) total organic carbon content using a TOC Analyzer (multi EA 4000, Analytik Jena, Jena, Germany); (3) total nitrogen using the micro-Kjeldahl method [7]; (4) the content of cellulose, hemicellulose, and lignin in the RS and BC as described by Aravantinos-Zafiris et al. [8]; (5) the content of ash, VM, and fixed C in the BC, based on the American standard test method [9]; and

the functional groups on the surface of the BC and RS were analyzed using Fourier transform infrared (FTIR) spectroscopy (TENSOR27, Bruker, Germany), at frequency ranges from 600 to 4000 cm^{-1}.

The chemical characteristics of the BC and RS are shown in Table 1

Table 1. Characteristics of the BC and RS used in the experiments.

Organic Materials [1]	pH	OC [2]	TN [3]TN	C/N [4] Ratio	Cellulose	Hemicell [5]	Lignin	Fixed C	Ash	VM [6]
	(1:5)	%								
BC	6.32	60.2	0.56	101	1.24	1.65	75.69	61.72	3.3	34.97
RS	7.47	40.9	0.43	95	46.65	22.17	7.11	-	-	-

[1] BC = biochar, RS = rice straw; [2] OC = organic carbon; [3] TN = total nitrogen; [4] C/N = carbon/nitrogen; [5] Hemicell = hemicellulose; [6] VM = volatile matter.

The BC FTIR spectra contained the following peaks (Figure 1a): 3570–3200 cm^{-1} (hydroxy group); 2921 cm^{-1} (methylene C-H asymmetric); 1928–2113 cm^{-1} (aromatic combination bands) [10]; 1641–1737 cm^{-1} (C=O of aromatic group) [11]; 1373 and 1591 cm^{-1} (carboxylate); and 1205 cm^{-1} (phenol, C-O stretch) [10]. The RS spectra (Figure 1b) contained a 3570–3200 cm^{-1} (hydroxy group); 1637 cm^{-1} (carboxylate); and 1033 cm^{-1} (aromatic C-H in plane bend) [10].

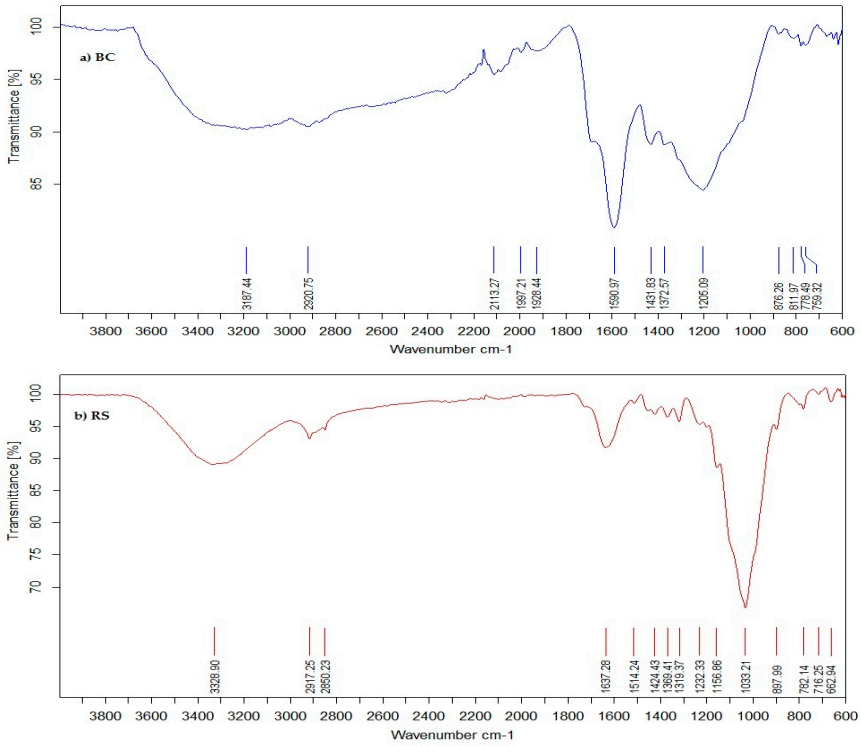

Figure 1. Fourier transform infrared (FTIR) spectra of the biochar (BC) (**a**) and rice straw (RS) (**b**) used in the experiments.

Paddy soil samples were randomly collected from the plow layer (0–15 cm) of an irrigated paddy field located in Ban Na Ngam in the Samran District of Khon Kaen, Thailand (N 16°32′45.9′′, E 102°51′15.5′′). The soil was classified as fine, mixed, and isohyperthermic Aeric Endoaquept. The soil was air-dried and then finely ground to be able to pass through a 2 mm sieve. The physical and chemical characteristics of the soil were analyzed for: (1) the soil texture using the hydrometer method [12]; (2) the soil organic carbon contents using wet digestion [13]; and (3) the total nitrogen using the micro-Kjeldalh method [7]. The soil showed the following physical-chemical properties: pH

(1:5) = 5.06; sandy loam texture with sand (65.8%); silt (21.9%); and clay (12.4%) with a soil organic carbon content of 0.83% and a nitrogen content of 0.08%.

2.2. Experiments

Two experiments (i.e., a pot and an incubation experiment) were conducted. The pot experiment was designed to evaluate the effects of the combined BC and RS on the production of carbonaceous gases, the microbial biomass, and the rice yields under non-leaching controlled conditions in the presence of rice plants. In contrast, the incubation experiment was designed to support the biological and biochemical data collected from homogeneous non-living root soils, to examine the effects of the combined BC and RS.

2.2.1. Pot Experiment

The pot experiment was performed from June to October 2015 in a greenhouse located at the Faculty of Agriculture at the Khon Kaen University in Khon Kaen, Thailand. Five treatments, performed in triplicate, were included as follows: (1) the control (without CF, BC, and RS amendments); (2) CF grade 16-16-8 [Urea (46% N), $(NH_4)_2HPO_4$ (18% N, 46%P_2O_5), KCl (60% K_2O)] at a rate of 0.188 t ha^{-1} as modified from the study by Thammasom et al. [3]; (3) BC 12.50 t ha^{-1}; (4) RS 12.50 t ha^{-1}; and (5) a mixture of BC:RS (1:1 w/w at a rate of 6.25 t ha^{-1} each) and CF. The experiment was arranged using a completely randomized design, wherein three kgs of sieved air-dried soil was placed in a pot (inner dimensions of 18 cm and a height of 23 cm, without a hole at the bottom). Based on the treatment parameters, the soil was then mixed with 2 mm sieved BC and/or RS (cut to a size of 2 cm in length). The soils in all the pots were submerged for 20 days before transplanting. This procedure was carried out to allow time for decomposition, so that the adverse effects from the toxic intermediate organic acid products of decomposition could be avoided. Then, three rice (*Oryza sativa* L.) seedlings (25 days old) of the Pitsanulok 2 (a photoperiod insensitive) varieties were transplanted to each pot. The CF was basally applied twice, that is, before transplanting and then 30 days after transplanting. Throughout the rice growing period, all the pots were maintained at a water level that was 5–7 cm above the soil surface without leaching, and the water was drained 10 days prior to the rice harvest.

2.2.2. Incubation Experiment

Treatments for the incubation trials were similar to the pot experiment treatments. Soil (2.5 g) was placed into a 60 mL glass bottle and then mixed with 2 mm BC and/or RS based on the treatments. Thereafter, 10 mL of the CF solution of the same strength as that used in the pot experiment was applied to the soil mixture. Calculations of the BC and RS weights were based on a soil bulk density of 1.39 g cm^{-3} and a soil weight of 2085 t ha^{-1}. The head space of the bottle was flushed with N_2 gas (99.99%), and then it was tightly closed using a septum and aluminum cap. The incubation of the soil was carried out at 28 °C for a period of 14 days under anaerobic conditions. The incubation period (14-days) was determined based on our previous study which found the highest CH_4 and CO_2 emissions after 14 days of incubation.

2.3. Data Collection

2.3.1. Rice Grain and Microbial Biomass C (MBC) in the Pot Experiment

After harvesting, rice grains collected from each pot were dried in an oven at 75 °C for 48 hours, and then weighed. A fresh soil sample was taken from each pot and analyzed for the MBC using the chloroform fumigation-extraction method described in Reference [14].

2.3.2. Gas Sampling, CH_4, and CO_2 Analysis in the Pot and Incubation Experiments

In the pot experiment, gas samples were collected using the closed chamber method. We used a transparent chamber made from acrylic, that was sized 21 × 21 × 100 cm (width × length × height). Gas

sampling was performed once a week throughout the rice growing period. The process was carried out between 9.00 and 11.00 a.m., and a 1 ml insulin syringe was used to obtain the gas samples at 0, 10, and 20 min after the chamber cover had been placed over the potted soil as in Reference [15]. CH_4 and CO_2 concentrations were measured using a gas chromatograph (GC-2014, Shimadzu, Kyoto, Japan) equipped with a flame ionization detector (FID) as described in Reference [1]. The gas measurements were completed within 6 hours.

Under the incubation experiment, the 1 mL gas samples were collected 14 days after incubation from the head space of the glass bottles using a 1-mL insulin syringe. After collection, the CH_4 and CO_2 concentrations were immediately determined.

2.4. DOC Analysis and Determination of Archaeal and Bacterial Abundance in the Incubation Experiment

Extraction of DOC from the incubated soil was done by shaking the bottle for 30 min, followed by centrifuging at 4000 rpm for 15 min. The supernatant solution was filtered through a 0.45 µm syringe filter prior to the DOC analysis, using the TOC/TN_b analyzer (Multi N/C 2100s, Analytik Jena, Jena, Germany).

2.5. DNA Extraction and Quantitative Polymerase Chain Reaction (qPCR)

The total soil genomic DNA was extracted using a FastDNA™ SPIN Kit for Soil (MP Biomedicals, Santa Ana, CA, USA). The qPCR of the bacterial 16S rRNA gene and archaeal 16S rRNA gene were performed using a C1000 Touch^{TM} thermal cycler combined with a CFX96^{TM} detection module (BIO-RAD, Hercules, CA, USA). The primers and annealing conditions are listed in Table 2. The PCR mixtures (25 µL) contained 12.5 µL of EXPRESS SYBR® GreenER^{TM} (Invitrogen, Carlsbad, CA, USA), 0.4 µM primer (each; final concentration), 1 µL of DNA template (10 ng μL^{-1}), and ultrapure water for the balance. Moreover, all the samples were analyzed in triplicate. Each reaction condition included an initial denaturing step of 10 min at 95 °C, followed by 40 cycles of 30 s of denaturing at 95 °C, 30 s of primer annealing (Table 2), and then 45 s of primer extension at 72 °C. The annealing temperatures were optimized for each primer pair. The abundances of bacteria and archaea determined using qPCR were reported as DNA copy numbers of 16S rRNA genes per g of dry soil.

Table 2. The qPCR primers and conditions used in this study.

Primers	Sequences (5′ to 3′)	Annealing Temps (°C)	Targeted Groups	References
Eub338	ACCTACGGGAGGCAGCAG	55	Bacteria	[16]
Eub518	ATTACCGCGGCTGCTGG	55	Bacteria	[17]
Ar109f	ACKGCTCAGTAACACGT	57.5	Archaea	[18]
Ar912r	CTCCCCCGCCAATTCCTTTA	57.5	Archaea	[18]

2.6. Statistical Analysis

One-way analysis of variance (ANOVA) was used to assess the treatment effects on various soil microbiological and biochemical properties, carbonaceous greenhouse gas emissions, and rice yields. Mean separation was performed using least significant difference (LSD) tests. We used the Statistix 10 software to carry out the statistical tests. To determine the correlation between the abundances of archaea and bacteria, the production of CH_4 and CO_2, and the DOC content in the incubated rice soil, the SigmaPlot 12.5 software program was used.

3. Results and Discussion

3.1. Carbonaceous Greenhouse Gases and Microbial Abundance in Paddy Soil as Affected by RS

Significant increases in the production of CH_4 were observed in the soil amended with RS alone, and in both pots (0.0347 mg m^{-2} $season^{-1}$) (Table 3) and incubation experiments (1379.3 mg kg^{-1}) (Table 4) relative to the other treatments. The increases in CH_4 production were due to the high

contents of easily decomposed cellulose (46.65%) and hemicellulose (22.17%) in the RS (Table 1). When the RS was applied to the soil, it had a key role in stimulating the soil's microbial activity for C mineralization. This was indicated by a significantly higher DOC content (202.69 mg kg^{-1}), and a higher volume of archaeal abundance (5.81×10^8 copies g^{-1} soil DW) and bacterial abundance (4.94×10^{10} copies g^{-1} soil DW) in the RS compared to other treatments, with the exception of the mixed RS + BC treatment (Table 4). Dissolved organic C is a mixture of dissolved organic carbonaceous compounds with particle sizes that are smaller than 0.45 µm. It is derived from the degradation of organic materials and it contains carbohydrates, proteins, fats, hydrocarbons and their derivatives, and fractions of low molecular weight humic acids; as well as numerous simple organic compounds [19]. DOC is a crucial part of the organic labile pool which serves as substrates for soil microorganisms. Rice straw was found to generate a high content of low molecular weight DOC within two weeks after incorporation into the topsoil (0–15 cm) of a sandy soil from Northeastern Thailand [20]. During our 2-week incubation period, the soil treatments containing RS showed a higher abundance of archaea than the other treatments. Archaea was a dominant microbe that utilized the DOC, CO_2, and H_2 [21] from decomposing RS to produce CH_4. This revealed the archaea's function in methanogenesis, which involved CO_2 reduction.

Table 3. CH_4, CO_2 emissions, rice grains, and microbial biomass C (MBC) in the potted rice-soil treated with BC and RS.

Treatments [1]	CH_4 mg m^{-2}	CO_2 Season^{-1}	Rice Grains g pot^{-1}	MBC [2] mg kg^{-1}
Control	0.0298 ab	0.0018 b	41.52 b	79.81 c
CF	0.0263 b	0.0012 c	50.62 a	93.66 bc
BC 12.50 t ha^{-1}	0.0233 b	0.0013 c	35.55 b	224.08 a
RS 12.50 t ha^{-1}	0.0347 a	0.0021 a	35.43 b	129.84 bc
BC 6.25 t ha^{-1} + RS 6.25 t ha^{-1} + CF	0.0235 b	0.0012 c	53.47 a	167.94 ab
F-test	*	**	**	*
CV (%)	15.87	2.99	7.00	33.26

[1] CF = chemical fertilizer, BC = biochar, RS = rice straw, CV = coefficient of variation. [2] MBC = microbial biomass carbon. The different small letters in the columns indicate significant difference among treatments by LSD. *, ** = significant at $p \leq 0.05$ and $p \leq 0.01$, n = 3.

Table 4. The abundance of archaea and bacteria, CH_4 and CO_2 production, and DOC content in 14-day incubated soils treated with BC and RS.

Treatments [1]	Archaea Copies g^{-1}	Bacteria Soil DW	CH_4 mg kg^{-1}	CO_2 mg kg^{-1}	DOC [2] mg kg^{-1}
Control	1.21×10^8 b	1.88×10^{10} b	61.6 b	3459.7 a	98.00 b
CF	6.73×10^7 b	2.24×10^{10} b	32.6 b	1730.4 b	84.92 b
BC 12.50 t ha^{-1}	8.51×10^7 b	1.76×10^{10} b	45.7 b	229.3 c	78.88 b
RS 12.50 t ha^{-1}	5.81×10^8 a	4.94×10^{10} a	1379.3 a	507.1 c	202.69 a
BC 6.25 t ha^{-1} + RS 6.25 t ha^{-1} + CF	3.79×10^8 a	5.82×10^{10} a	4.5 b	557.5 c	186.63 a
F-test	**	*	**	**	**
CV (%)	36.16	45.70	20.15	29.96	15.75

[1] CF = chemical fertilizer, BC = biochar, RS = rice straw, CV = coefficient of variation. [2] DOC = dissolved organic carbon. The different small letters in the columns indicate significant difference among treatments by LSD. *, ** = significant at $p \leq 0.05$ and $p \leq 0.01$, n = 4.

In the soil treatments using RS alone, we observed high CH_4 production and high archaeal abundance. The DOC content was supported by the significantly high positive correlation between archaeal abundance and CH_4 production (r = 0.799 ***) and the DOC concentration (r = 0.872 ***). However, its moderately negative correlation with CO_2 production (r = −0.403) indicated that with an

increasing abundance of archaea, the CO_2 had been consumed (Figure 2a–c). Therefore, the archaea had performed a crucial role in CH_4 production.

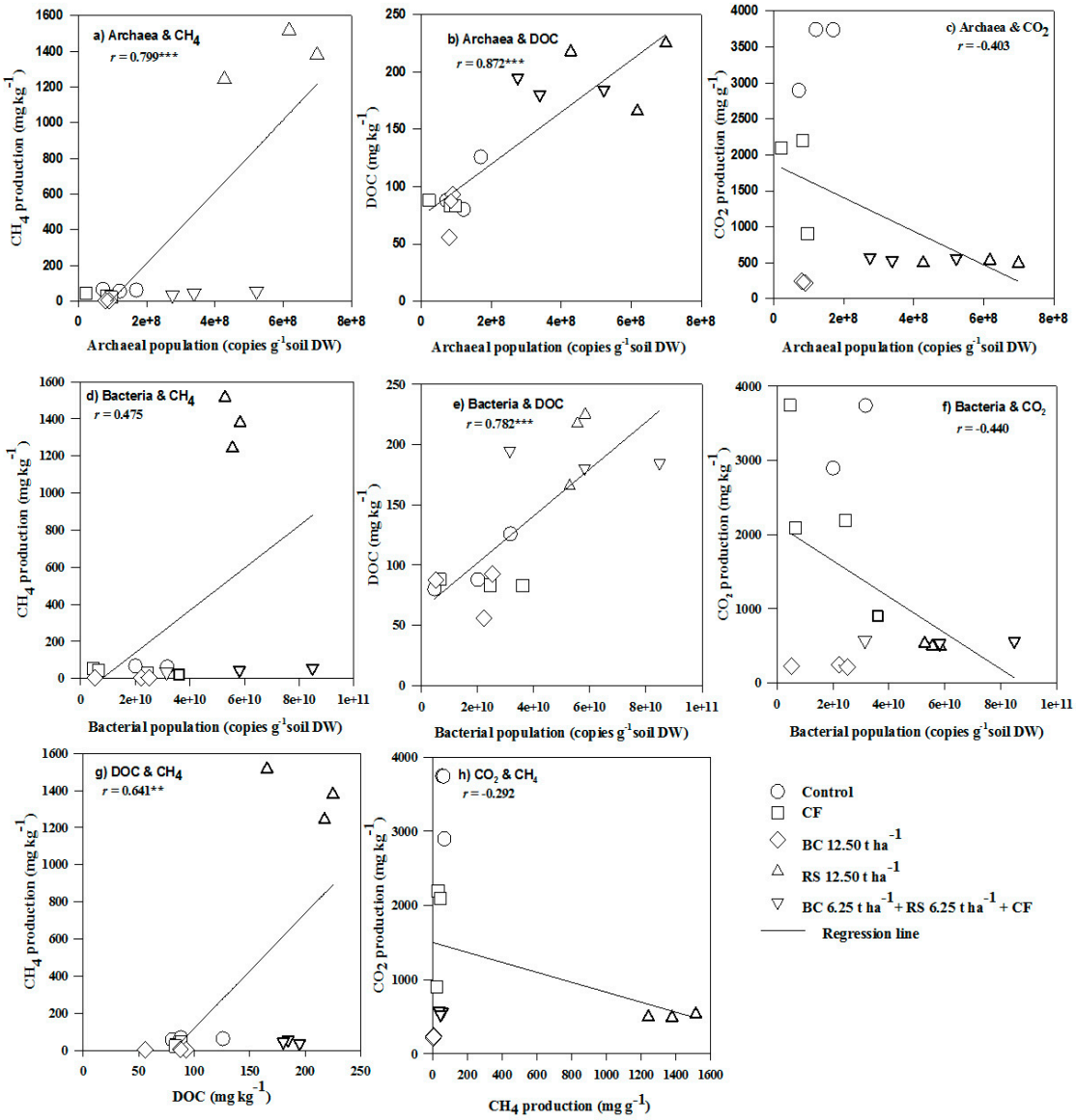

Figure 2. The correlation coefficients between Archaeal and CH_4 production (**a**), DOC content (**b**) and CO_2 production (**c**); between Bacterial abundance and CH_4 production (**d**), DOC content (**e**) and CO_2 production (**f**); between DOC content and CH_4 production (**g**); and between CH_4 and CO_2 production (**h**). ** Very significant at p-value < 0.01, *** extremely significant at p-value < 0.001, n = 15.

With respect to the bacteria, these appeared to be less effective in CH_4 production compared to the archaea. This was reflected in the moderately positive correlation between bacterial abundance and CH_4 production (r = 0.475) (Figure 2d), where the correlation between bacterial abundance and DOC concentration was significantly high (r = 0.782***) (Figure 2e). This indicated that the bacteria had played a crucial role in supplying DOC to the soil system, thereby supporting CH_4 production (r = 0.641**) (Figure 2g). However, a moderately negative correlation between the bacterial abundance and CO_2 production (r = −0.440) indicated that CO_2 had been consumed to form CH_4 with the increasing abundance of bacteria (Figure 2f). This assertion was supported by a weakly negative correlation coefficient of −0.292 between CO_2 and CH_4 production (Figure 2h).

With respect to archaea exerting its effects on CH_4 production, our results showed that compared to the bacteria, archaea was the more dominant microorganism. In the soil, especially in RS incorporated soil, *Methanomicrobia* was the main genus of archaea found, followed by *Methanobacteria* [22]. Moreover, in a previous pot experiment conducted with growing plants, the soil treated with RS showed a rapid decrease in soil redox potential to a range from −150 to −200 mV [1]. This was coupled with a rise in the soil pH to an optimal range of 7.5 to 8.5. This observed phenomenon resulted from electrons transferred from the RS, which had been utilized by microorganisms in the anaerobic respiration process [1]. This condition was assumed to be suitable for methanogenic archaea and bacteria. Moreover, these results were found to be concomitant with the experimental results reported by Yuan et al. [22].

3.2. Carbonaceous Greenhouse Gases and Microbial Abundance in Paddy Soil as Affected by BC

In contrast to RS, BC led to low levels of CH_4 production in soils from both the pot experiments (0.0233 mg m^{-2} $season^{-1}$) (Table 3) and the incubation experiments (45.7 mg kg^{-1}) (Table 4). This was because BC contained high levels of resistant C compounds, such as lignin (75.69%) and fixed C (61.72%) (Table 1). We discovered that the resistant constituents of BC had suppressed the soil's C mineralization. Compared to the RS treated soil, this suppression had led to a significantly lower DOC content (78.88 mg kg^{-1}), as well as lower archaeal abundance (8.51×10^7 copies g^{-1} soil DW) and bacterial abundance (1.76×10^{10} copies g^{-1} soil DW) in the BC treated soil (Table 4). This was consistent with a previous study by Liu et al, where it was found that paddy field soil amended with BC contributed to a low content of substrates [5]. In addition, the BC used in our experiments had a high content of VM (34.97%) (Table 1), which consisted of DOC, such as carboxylics and phenolics (as determined using FTIR, Figure 1), as well as aldehydes [23]. Not only could the DOC be consumed for CH_4 and CO_2 production, but it could be used by soil microorganisms for assimilation into the MBC. However, the archaeal and bacterial abundances were similar between the BC treated soil and the control soil (Table 4) because the amounts of DOC in both soil treatments were low. The archaeal and bacterial abundances in the BC treated soil (8.51×10^7 and 1.76×10^{10} copies g^{-1} soil DW, respectively) were significantly less than the abundances in RS treated soil (5.81×10^8 and 4.94×10^{10} copies g^{-1} soil DW, respectively). These results confirmed a lower CH_4 production in the BC treated soil (Table 4).

In terms of the microbial community, in the BC treated soil, a high MBC content of 224.08 mg kg^{-1} (Table 3) revealed the good biological quality of the soil containing archaea, bacteria (Table 4), and methanotrophs [6], which were involved in the CH_4 and CO_2 dynamics of such soil. Wang et al. [4] reported that soil amended with BC had significantly altered the composition of the soil's archaeal and bacterial communities. Furthermore, it was reported that the main constituents of the archaea communities included a miscellaneous Crenarchaeota group (MCG), Methanobacteria, and Thaumarchaeota archaea. In our study, the BC treatments were likely to be comprised of archaea similar to the composition reported by Wang et al. [4].

Moreover, in the BC alone and combined BC + RS + CF treated soils, the CH_4 production (0.0233 and 0.0235 mg m^{-2} $season^{-1}$, respectively) was found to be lower than in the soils treated with RS treatments (0.0347 mg m^{-2} $season^{-1}$). This may be attributed to the physical structure of BC given that it possessed several ≤ 2 mm micropores and had a large surface area [4,19], which could adsorb CH_4 gas [5] and serve as a CH_4-C substrate for the methanotrophs [6]. Biochar also supplied a habitat for the methanotrophs [6], where all these mechanisms had led to a reduction in CH_4 production in the BC amended soil (Table 3).

3.3. Carbonaceous Greenhouse Gases and Microbial Abundance in Paddy Soil as Affected by Combined BC and RS

In the incubation experiments using the combined BC + RS treatment (BC 6.25 t ha^{-1} + RS 6.25 t ha^{-1} + CF), there was an abundance of archaea and bacteria (3.79×10^8 and 5.82×10^{10} copies g^{-1} soil DW, respectively) (Table 4), which had proliferated at 6.25 t ha^{-1} RS to yield CH_4 of 4.5 mg kg^{-1} (Table 4). When the CH_4 results of the combined treatment (4.5 mg CH_4 kg^{-1}) were

compared to the results of the RS alone (12.50 t ha^{-1} RS) (1379.3 mg CH_4 kg^{-1}), we found that the CH_4 had been drastically reduced in the combined treatment via the countering power of the BC, probably through the BC inhibition of methanogenic activity [5] and the adsorption process of BC. This result was despite the enhancing effects that RS had on CH_4 production in such anaerobic soil through methanogenic archaea and bacteria activities (Table 4). On the contrary, the amount of CO_2 production was found to be similar amongst the treatments of BC, RS, or their combination in the incubated soils. The non-different "net" CO_2 production was the net result of several microbial processes of microbial C mineralization (CO_2 production) and CO_2 reduction (CH_4 formation) that occurred simultaneously, but to different degrees and directions in the soils treated with these studied amendments. Biochar applied alone resulted in a low C mineralization, rendering a low content of CO_2 (Table 4). In contrast, RS alone favored C mineralization to form CO_2, which was furthered reduced to CH_4 and resulted in low CO_2 in the RS treated soil. In the combined BC + RS soil, the adverse effect of BC on RS resulted in a low CO_2 (Table 4). In the combined BC + RS + CF soil, CH_4 production in the potted soil (0.0235 mg m^{-2} $season^{-1}$) was significantly lower than in the RS alone (0.0347 mg m^{-2} $season^{-1}$) (Table 3). It appeared that the results for CH_4 production from the incubation experiments and rice pot experiments behaved in the same manner. However, conditions in the potted soil planted with rice differed from the conditions in the incubated soil, i.e., in the rice pot experiment, there were C substrates derived from rhizodeposition (root exudates, sloughed root, and dead root) [24], large areas of aerobic and anaerobic interface in the rice rhizosphere soil, and an oxidizing layer on the soil surface, whereas the incubated soil was presumably completely anaerobic. The BC-enhanced microbiological oxidation process was mediated by methanotrophs, which consumed CH_4 and transformed it into CO_2 at the aerobic–anaerobic interface [25] of the rice rhizosphere and the submerged soil. Therefore, as a consequence, CO_2 was released into the atmosphere (Table 3). This process is expected to exist within the rice rhizosphere at the aerobic–anaerobic interface, and it results in decreases in CH_4 and the release of CO_2 to the soil. More than 90% of the CH_4 production in soil is oxidized to CO_2 [6]. Our findings from the rice which had been planted in potted soil, enabled us to articulate the countering effects of the BC amendments on CH_4 production in soil via two possible mechanisms. These included the adsorption of CH_4 onto the BC surfaces and the oxidation of CH_4 to CO_2 by methanotrophs which utilize CH_4 as a source of C and energy [6].

The rice grain yields obtained from the combined treatment (BC 6.25 t ha^{-1} + RS 6.25 t ha^{-1} + CF) and individual CF treatment were 53.47 and 50.62 g pot^{-1}, respectively (Table 3), being the two highest yields in our pot experiments. In contrast, BC alone and RS alone depressed rice yields. It could be deduced that the enhanced yield under the combined BC + RS + CF treatment was due to the CF effect on grain yield. With CFs favorable supply of nutrients and the high nutrient adsorption characteristics of BC, the combined BC + RS + CF treatment could supply and retain sufficient plant nutrients for rice growth. In addition, chlorosis was observed in the rice plants treated with BC or RS alone. The studied soil had a low N content (0.08%) which caused soil N deficiency and led to a low rice yield. Therefore, CF is a necessary supplement for the amendment of organic materials in the soil.

4. Conclusions

Our results proved our hypothesis, that is, the incorporation of a combination of BC and RS in paddy soil reduces the soil's CH_4 production, raises archaeal and bacterial abundances, and increases the yield of rice grains compared to unamended soil. With such a combined BC + RS soil amendment, the RS component (a cellulose and hemicellulose-rich material) was able to rapidly decompose and enhance the archaeal and bacterial abundances relative to the without-RS amendments. Concurrently, in the combined BC + RS, there was a rapid production of the intermediate products of decomposition (i.e., DOC, CO_2, and H_2) which served as substrates for microbes to produce CH_4 in the methanogenesis process. Conversely, the recalcitrant lignin-rich BC component of the combined BC + RS amendment inhibited the activity of the archaea in methanogenesis resulting in lower CH_4 production than that of the RS alone. One of the proposed mechanisms for the suppression of methanogenesis was a high

abundance of methanotrophic bacteria in the BC, which served as the bacteria's habitat. Methanotrophs performed the CH_4 oxidation process, which reduced the CH_4 content. Another mechanism was the adsorption of CH_4 onto the large and highly adsorptive surface area of the BC, which led to CH_4 reduction in the combined BC + RS treated soil. Compared to paddies receiving RS or BC applied individually, a further benefit was the high rice grain yield under the combined BC + RS treatment. The combined BC + RS material proved to be a more beneficial soil amendment than the RS or BC applied separately owing to the dual purposes of improving soil productivity and reducing greenhouse gas emissions.

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