1 Win-win: application of sawdust-derived hydrochar in low fertility soil improves rice yield and
2 reduces greenhouse gas emissions from agricultural ecosystems

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Abstract

As a good soil synergist, biochar has a wide prospect in improving soil fertility and crop production. Although hydrochar, produced by hydrothermal carbonization process has attracted attention due to production advantages, hydrochar application in low fertility soils as well as its impact to the associated greenhouse gas (GHG) emissions in farmlands is rarely reported. To advance our understanding on the effect of hydrochar addition on grain yield from low fertility soils and the corresponding CH₄ and N₂O emissions, a soil-column experiment, with two hydrochar types (sawdust-derived hydrochar (SDH), microbial-aged hydrochar (A-SDH)) at two application rates (5‰, 15‰; (w/w)), was conducted. The results showed that hydrochar addition evidently increased rice yield. The N₂O emissions were mainly related to the substrate supply of the hydrochar itself and less affected by the denitrifiers (functional genes) present. Hydrochar amendment at low application rate (5‰; SDH05, A-SDH05) significantly decreased the cumulative N₂O emissions by 26.32%~36.84%. Additionally, hydrochar amendment could not increase the CH₄ emissions due to the substrate limitation; the cumulative emissions were similar with those from the control, ranging between 11.1 g m⁻² - 12.8 g m⁻². Regarding grain yield and global warming potential, greenhouse gas intensity from the soils subjected to hydrochar (SDH05, A-SDH05, A-SDH15) were significantly lower than that of the control, observation attributed to the high yield and low N₂O emissions. Overall, hydrochar addition is an effective strategy to ensure grain yield in low fertility soils with relatively low/controlled GHG emissions, especially when the amendment is applied at low application rate.

Keywords: biowaste; hydrothermal carbonization; low fertility soil; crop production; greenhouse

gas emission; microbial functional genes

1. Introduction

Global food supply has to be re-visited due to the stable growth of the human population (Prosekov and Ivanova, 2018) that risks adequate nutrition for all. Increase of grain production per unit of land could promote global food security (Arora, 2018). However, soils of low fertility, such as degraded sites, newly-constructed cultivated lands etc., lead to limited food supply and is a common problem in many regions worldwide (FAO, 2011). It is known that soil fertility is a key factor that dictates not only crop productivity in agricultural systems, but also affects the retention of fertilization nutrients as well as the soil amendments (when applicable) (Haefele *et al.*, 2014). Hence, increasing crop productivity in low fertility soils has always been a major task to ensure food security for the growth of human civilization and not only.

Integrating inorganic fertilizers with organic amendments, such as crop residue, organic fertilizer, and biochar etc. is a common practice to improve soil quality as well as crop productivity, especially for low fertility soils (Liu *et al.*, 2014a; Liu *et al.*, 2014b; Mi *et al.*, 2018). Biochar, as an additive in soil is considered a good synergist because of its potential in carbon sequestration, soil remediation, as well as in improving soil quality and crop productivity (Bi *et al.*, 2019; El-Naggar *et al.*, 2019b; Purakayastha *et al.*, 2019). Numerous biochar-based amendments typically derive from different waste streams (as by-products). Biochar addition inevitably changes the soil nutrient release and soil C/N cycling, and subsequently affects greenhouse gas emissions from farmlands (El-Naggar *et al.*, 2019a). It is worth noting that the greenhouse gas emissions often vary due to the variability in biochar type, cropping system and soil properties (Liu *et al.*, 2019b; Ma *et al.*, 2019; Meschewski *et al.*, 2019; Qi *et al.*, 2020; Xu *et al.*, 2020). This

is very important considering that immediately behind the energy sector, agricultural ecosystems is the second largest source of global anthropogenic greenhouse gas emissions, accounting for 56% of the total non-CO₂ emissions (EP, 2015) (Methane (CH₄) and nitrous oxide (N₂O), with 25 and 298 times more global warming potential (GWP) than carbon dioxide (CO₂) based on a 100-year timescale (IPCC, 2013)).

Recently, hydrochar has attracted attention due to its advantages over the conventional pyrolytic biochar (pyrochar) (i.e. easier production process - higher product quantity over time, reduced processing cost and lower environmental pollution) (Feng *et al.*, 2019). Due to the differences in processing and manufacturing between pyrochar and hydrochar, the latter often has more soluble organic ingredients and abundant oxygen-containing functional groups (Gai *et al.*, 2016; Reißmann *et al.*, 2018; Zhang *et al.*, 2019). Thus, the hydrochar exhibits numerous advantages than pyrochar, such as a wider prospect in improving soil fertility by increasing soil nutrients fixation and providing with more available nutrients (Malghani *et al.*, 2015). Additionally, Sun *et al.* (2020) reported that hydrochar application could also decrease the labile soil organic carbon fraction and improve the stable soil organic carbon fraction.

It is worth mentioning that hydrochar often contains large amounts of organic compounds (DOC, PAHs, and phenols), which may inhibit crop growth (Bargmann *et al.*, 2013; Busch *et al.*, 2013; Zhou *et al.*, 2018). However, this is not always the case; Hao *et al.* (2018) showed that the negative effect of DOM increases with the increase of hydrochar production temperature; on the other hand, DOM derived from low-temperature hydrochar production - shows minimal biotoxicity and hence, limited growth inhibition. Additionally, Fincheira and Quiroz (2018) pointed out that suitable volatile organic compounds (VOCs) can stimulate crop growth. Limited

studies have so far showed the benefits from the application of hydrochar, derived from low-temperature production, to soil for the improvement of crop yield; especially when following an appropriate application strategy (Yu *et al.*, 2019; Chu *et al.*, 2020). These limited studies showed the good prospects of hydrochar application in increasing soil quality as well as crop yield in agro-ecosystems. However, the impact of the application of hydrochar on low fertility soil is a concept that remains unexplored. Hydrochar addition inevitably affects soil C/N cycling, and subsequently the greenhouse gas emissions from a farmland. The questions that remain unanswered are i) how does hydrochar application affects grain yield and greenhouse gas emissions when applied in low fertility soil; ii) what are the key drivers of greenhouse gas emissions under hydrochar addition in low fertility soils? Finally, the biological modification of the hydrochar is a key method to minimize its inhibitory effect and ensure high grain yield (Yu *et al.*, 2019; Yu *et al.*, 2020). Compared with the un-modified hydrochar, the pore structure of hydrochar, after biological modification, is greatly improved with the organic compounds (acids, phenols) almost absent; the content in nutrient elements also changes (Yu *et al.*, 2020). These modifications could alter soil C and N cycles and subsequently, the associated CH₄ and N₂O emissions from the farmlands. However, there is no study yet available describing the greenhouse gas emissions from farmlands subjected to microbial-aged hydrochar application.

Rice, the indispensable food grain, is planted widely and feeds billions of people around the world (Seck *et al.*, 2012). Paddy fields, where the rice typically grows, are the largest greenhouse gas emission sources in agriculture, accounting for approximately 10% of the total agricultural greenhouse gas emissions (FAO, 2013). Hence, it is of great importance to improve grain yield, especially in low fertility soils, and decrease the greenhouse gas emissions from such paddy fields.

In this study, the objectives were to investigate the effects of sawdust-derived hydrochar and its derivative product (microbial-aging hydrochar) when applied in low fertility soil (paddy fields), and to evaluate the rice grain yield as well as the CH₄ and N₂O emissions. We expect this study to develop a rationale for more sustainable fertilization strategies, applicable for low fertility soils, to increase grain yield and limit the global warming potential.

2. Materials and methods

2.1 Materials

Two types of hydrochars, i) sawdust-derived hydrochar (SDH) and its derivative product ii) microbial-aged hydrochar, (A-SDH), were selected as the amendments in this study's column experiment. Poplar sawdust, a common biowaste, was selected as the crude material to produce the hydrochar in a high-pressure hydrothermal reactor. Due to the [previously investigated beneficial](#) effect of the hydrochar application on grain yield from high fertility soil (hydrochar derived at operating pressure and temperature of 8 Mpa and 260 °C respectively (Yu *et al.*, 2019; Chu *et al.*, 2020)), the operating pressure and temperature [for the preparation of the hydrochar in the current study was kept the same.](#)

After filtration, using non-woven fabrics, the produced hydrochar was used as the substrate for microbial-aged hydrochar production. Specifically, for the preparation the following steps were followed: 1) material ratio of 100 L (biogas slurry) / 11 kg (straw, carbon source) / 3780 g (sawdust-derived hydrochar, substrate); 2) pH of the fermentation substrate with 7.3; 3) moderate amount of nutrients; 4) fermentation time of 60 days. A detailed description has been provided by our previous study (Yu *et al.*, 2019). The physicochemical properties of the SDH and the A-SDH as well as the nutrient concentration in the water-extractable solution are shown in Table 1. The

pore characteristics, organic ingredients in the water-extractable solution, SEM images, SEM-Mapping, and XPS spectra of both hydrochars are provided in the supporting information (Table S1-S2 and Figure S1-S2).

Table 1

Physicochemical properties of the experimental hydrochars and the nutrient concentration in water-extractable solution.

Hydrochar type	Physicochemical properties							nutrient concentration in water-extractable solution			
	pH	CEC cmol kg ⁻¹	C %	H %	O %	N %	C/N	TOC mg L ⁻¹	TN mg L ⁻¹	NH ₄ ⁺ -N mg L ⁻¹	NO ₃ ⁻ -N mg L ⁻¹
SDH	3.7	24.4	60.8	3.3	34.0	1.5	39.5	432.3	35.2	20.2	11.32
A-SDH	7.0	24.4	69.0	3.4	25.2	2.3	30.3	157.7	252	161.6	1.72

A low fertility soil, Gleyi-Stagnic Anthrosol (CRGCST 2001) was employed for this study's soil-column experiment. The characteristics of the experimental soil were: coarse sand content (0.2mm<D≤2mm), 1.0%; fine sand content (0.02mm<D≤0.2mm), 22.5%; powder sand content (0.002mm<D≤0.02mm), 20.7%; clay content (D<0.002mm), 56.7%; soil pH, 7.0; soil organic matter, 9.0 g kg⁻¹; total nitrogen, 0.7 g kg⁻¹; Olsen-P, 4.7 mg kg⁻¹; and available potassium, 147.5 mg kg⁻¹. It has been proved that soil C and N are the dominant elements for soil fertility (Milne *et al.*, 2015; Seyedalikhani *et al.*, 2019). The experimental soil C and N concentrations of the soil were relatively low and classified as the fifth grading (nutrient deficiency) according to the second national soil survey data of China (Pan *et al.*, 2015). The soil Olsen-P content was also found limited (fifth grading).

2.2 Experimental design

The column experiment was conducted at the experimental farm of the Jiangsu Academy of

Agricultural Sciences, Nanjing City, Jiangsu Province, China, in 2019. The diameter and height of the soil-column was 30 cm and 50 cm respectively, described in detail by Feng *et al.* (2017); the details of the installation diagram of the experimental column are shown in Fig. S3. The rice seedlings (Nanjing 46, a conventional *japonica* rice variety) were transplanted and harvested on the 10th of June and on the 10th of October 2019. The transplanting density included 3 holes per container with 3 plants per hole. A floodwater layer at a depth of 3-5 cm was maintained in the container during the rice growing season, exceptions were the midseason aerations (critical leaf-age of productive tillers) and the final drainage prior harvest periods.

Two hydrochar types (SDH, A-SDH) at two application rates (5g hydrochar /kg soil (5‰), 15g hydrochar /kg soil (15‰)) were selected. The treatments are marked as SDH05 (sawdust-derived hydrochar with 5‰ (w/w) application rate), SDH15 (sawdust-derived hydrochar with 15‰ (w/w) application rate), A-SDH05 (microbial-aged hydrochar with 5‰ (w/w) application rate), and A-SDH15 (microbial-aged hydrochar with 15‰ (w/w) application rate). Conventional split fertilization without hydrochar addition (CKU) was selected as the control. Each treatment was assessed in triplicates. For hydrochar application treatments, the two hydrochar types were mixed with the experimental soil according to the design proportion (5‰/15‰, w/w), and then transferred to the pots as topsoil. For all treatments the amounts of N, P₂O₅ and K₂O were kept the same, 240 kg ha⁻¹ (N), 96 kg ha⁻¹ (P₂O₅), and 192 kg ha⁻¹ (K₂O). Thirty percent of the N (urea), 100% of the P₂O₅ (calcium superphosphate) and 100% of the K₂O (potassium chloride) were applied on the 11th of June (1st day after transplanting). Thirty percent of the N (urea) was spread over the column at the tillering stage (14th days after transplanting), the remaining of the additives (40% of the urea) were applied at the panicle initiation stage (51 days after transplanting).

2.3 Sample and data collection

2.3.1 Grain yield

The entire rice plants per column were harvested at maturity stage. The rice yield was calculated after air drying and adjusted to a standard moisture content of 13.5%.

2.3.2 CH₄ and N₂O emissions from paddy fields

The emission rates of CH₄ and N₂O were determined by using a static chamber gas chromatography (GC) method. Gas samples were taken every 2 days during fertilization and drainage stages and at 2-weeks cycle for the other stages (using a cylindrical gas sampling chamber (*Height*: 100 cm, *Diameter*: 35 cm)). Samples' CH₄ and N₂O concentrations were determined using a gas chromatograph (Agilent 7890A, Santa Clara, CA, USA). Emission rates of CH₄ and N₂O were calculated by the slopes of the samples' concentration, which were collected at 0, 15, 30, and 45 minutes, respectively.

The cumulative seasonal emissions of CH₄ or N₂O were analyzed from the emission rates between every two adjacent measurements' intervals over the rice growing season (Zhou *et al.*, 2018). Global warming potential (GWP) is a widely used indicator to reflect the potential impact of an activity's generated greenhouse gases on the climate (converting all emissions of CH₄ and N₂O into "CO₂-equivalents") (IPCC, 2013). Hence, the GWP was selected to understand the overall GHG impact of the hydrochar addition on low fertility soil. Here the global warming potential of CH₄ and N₂O was converted into "CO₂-equivalents" by 25 and 298 times respectively based on a 100-year timescale (IPCC, 2013). Additionally, Green house gas intensity (GHGI) is defined as the ratio of GWP to crop yield; this is an effective indicator to relate grain yield and climatic impact (Liu *et al.*, 2019b). Hence, GHGI was also calculated via dividing the GWP by

rice grain yield.

2.3.3 Abundance of soil microbial functional genes (*mcrA*, *pmoA*, *nirK*, *nirS*, and *nosZ*)

For each treated soil, real-time quantitative PCR (*qPCR*) was used to analyze the abundance of the microbial functional genes related to CH₄ and N₂O emissions present in soil. The primers of the functional genes are shown at Table S3 according to Steinberg and Regan (2008) (*mcrA*), Holmes *et al.* (1995) and Kolb *et al.* (2003) (*pmoA*), Yan *et al.* (2003) (*nirK*), Throback *et al.* (2004) (*nirS*), and Throback *et al.* (2004) (*nosZ*). The details of the operational process of *qPCR* has been provided by Zhou *et al.* (2016).

2.4 Statistical analysis

Statistical analysis was performed via SPSS 21.0 (SPSS Inc., Chicago, Illinois, USA). The significance difference of yield, greenhouse gas emissions and functional genes for the treatments was analyzed using the LSD method with the confidence level set at 0.05. The figures were prepared by Origin 8.0 (Originlab, Northampton, Massachusetts, USA).

3. Results

3.1 Grain yield

The effect of the sawdust-derived hydrochar (SDH) and the microbial-aged hydrochar (A-SDH) addition on the rice grain yield is shown in Fig. 1. The results indicated that there is an evident increase of rice grain yield under hydrochar addition treatments. Except for the SDH15 treatment, the grain yields achieved from SDH05, A-SDH05 and A-SDH15 treatments were significantly ($P<0.05$) higher than the yield achieved from the CKU treatment (control); specifically, the yield increased by 12.2%, 16.6%, and 19.3% respectively. The difference in grain yield between different hydrochar treatments was not significant; differences between hydrochar application

rates (5‰ and/or 15‰) also had an insignificant effect on the grain yield (Table 2). The grain yields at different treatments had the following order: A-SDH15, A-SDH05, SDH05, SDH15, CKU.

Fig. 1. Grain yield (means $\pm$ sd) as affected by hydrochar application treatment. SDH05, sawdust-derived hydrochar with 5‰ (w/w) application rate; SDH15, sawdust-derived hydrochar with 15‰ (w/w) application rate; A-SDH05, microbial-aged hydrochar with 5‰ (w/w) application rate; A-SDH15, microbial-aged hydrochar with 15‰ (w/w) application rate; CKU, conventional split fertilization without hydrochar addition. The significant difference for hydrochar application treatments was determined using the LSD method with confidence level set at 0.05. The same below.

Table 2

Analysis of variance for the variables of: grain yield, greenhouse gas emissions, GWP and GHGI at different hydrochar types and application rates.

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Source of variance		Yield	CH ₄	N ₂ O	GWP	GHGI
Type	F	2.51	0.95	1.62	0.31	0.55
	<i>P</i> -value	0.152	0.357	0.239	0.592	0.481
Rate	F	0.02	2.11	26.72	6.52	2.72
	<i>P</i> -value	0.895	0.184	0.001	0.034	0.138
Type×Rate	F	0.47	1.22	0.17	0.78	1.17
	<i>P</i> -value	0.511	0.301	0.692	0.404	0.311

231 The analysis of variance was carried out to estimate the influence of hydrochar type and
 232 application rate to the variables. Bold face numbers indicate they are significant at 5% level.

233

234 3.2 Emission rate of CH₄ and N₂O

235 As shown in Fig. 2, the variability in the fluxes of CH₄ and N₂O under all hydrochar application
 236 treatments were similar and only differed temporally, mainly in amplitude. Evidently there were
 237 two peaks in CH₄ emission rates for all treatments during the rice growing season, which occurred
 238 approximately at 18 and 71 days after rice transplanting respectively. Mid-season drainage
 239 decreased the CH₄ emission rate. In contrast to CH₄ flux, the N₂O flux peaked at the tillering stage
 240 (4 days after tillering fertilizer application) and at drainage stage respectively.

241 For the CH₄ flux, the CH₄ emission rates of the two flux peaks under SDH15 treatment were
 242 higher than those achieved from all other treatments. Still, the average CH₄ emission rates under
 243 all hydrochar application treatments (SDH05, SDH15, A-SDH05, A-SDH15) were found similar
 244 with the average CH₄ emission rate for the control (CKU) ($P>0.05$) (Table 3). As per the N₂O
 245 emission rate, the amplitudes in different gas samples between treatments was different (Fig. 2);
 246 the average emission rate for SDH05, A-SDH05 and A-SDH15 treatments were significantly
 247 lower than those from SCH15 and CKU treatments ($P<0.05$) (Table 3).

Fig. 2. Emission rate of CH₄ and N₂O (means $\pm$ sd) as affected by hydrochar application treatment.

3.3 Average emission rates of CH₄ and N₂O, cumulative emissions, GWP and GHGI

Table 3 shows the cumulative seasonal emissions of CH₄ and N₂O, GWP and GHGI among the hydrochar application treatments. The results indicated that cumulative CH₄ emissions among the treatments remained similar over the rice growing season ranging from 11.1 g m⁻² to 12.8 g m⁻². Apparently there is a significant effect of hydrochar application on the cumulative seasonal N₂O emissions from paddy fields. Specifically, the cumulative seasonal emissions of N₂O from low application rate treatments (SDH05, A-SDH05) were significantly lower than those from all other treatments (SDH15, A-SDH15, and CKU) (Table 2-3). In comparison to CKU, the cumulative emissions of N₂O from SDH05 and A-SDH05 decreased by 26.3% and 36.8% respectively. Additionally, the difference of the cumulative seasonal emissions of N₂O between CKU and the

high application rate (15‰; SDH15, A-SDH15) treatments was not statistically significant ($P>0.05$). The results indicate that the cumulative seasonal CH_4 emissions for the hydrochar application treatments were similar in relation to those of the CKU treatment; lower cumulative emissions of N_2O from the low application rate (5‰; SDH05, A-SDH05) were observed in relation to CKU ($P<0.05$).

Regarding the global warming potential (GWP), there is a decreasing tendency in emissions at low application treatments (SDH05, A-SDH05) related to the lower cumulative N_2O seasonal emissions (Table 2) (decreased by 9.6%~15.2% compared to the control (Table 3)). On the other hand, benefited from the higher rice grain yields (Fig. 1), hydrochar added treatments could obviously decrease the greenhouse gas intensity (GHGI) (Table 3). Apart from the SDH15 treatment, the GHGIs of hydrochar addition treatments (SDH05, A-SDH05, and A-SDH15) were significantly lower than that for the CKU treatment, decreased by 24.2%, 22.1% and 18.7% respectively. The order of GHGI among the different treatments was CKU, SDH15, A-SDH15, A-SDH05, SDH05.

Table 3

Average emission rate of CH_4 and N_2O , cumulative seasonal emissions, GWP and GHGI (means $\pm$ sd) as affected by hydrochar application treatment.

Treatment	Average emission rate		Cumulative seasonal emissions		GWP (g m ⁻²)	GHGI (g kg ⁻¹)
	(mg m ⁻² h ⁻¹)		(g m ⁻²)			
	CH ₄	N ₂ O	CH ₄	N ₂ O		
SDH05	3.33±0.51a	0.058±0.02b	11.10±1.15a	0.14±0.04b	317.96±39.01a	243.66±33.09b
SDH15	3.67±0.36a	0.102±0.03a	12.42±0.85a	0.19±0.01a	367.80±22.35a	296.65±53.91ab
A-SDH05	3.64±0.73a	0.046±0.01b	12.17±0.74a	0.12±0.01b	338.85±15.43a	250.31±20.91b
A-SDH15	3.62±0.53a	0.064±0.01b	12.35±0.78a	0.18±0.01a	363.15±16.35a	261.31±8.69b
CKU	3.41±1.21a	0.093±0.01a	12.78±2.04a	0.19±0.00a	374.79±50.63a	321.43±37.16a

GWP, global warming potential; GHGI, greenhouse gas intensity. The significant difference for

hydrochar application treatments was determined using the LSD method with confidence level set at 0.05.

3.4 *mcrA* gene and *pmoA* gene

The results showed that hydrochar addition significantly affects the abundance of soil *mcrA* and *pmoA* genes (Fig. 3). Specifically, except for the A-SDH15 treatment, the abundance of *mcrA* genes on hydrochar application treatments (SDH05, SDH15, A-SDH05) were significantly higher than those subjected to CKU treatment (control). The *mcrA* gene exhibited the following sequence in abundance between treatments: SDH05, A-SDH05, SDH15, A-SDH15, CKU. As per *pmoA* gene, SDH05 treatment achieved the highest abundance of *pmoA* gene, significantly higher than that from the SDH15, A-SDH15 and CKU treatments. Additionally, the difference in the copy numbers of *pmoA* gene between SDH15, A-SDH05, A-SDH15 and CKU treatments was not significant. Furthermore, the abundances of soil *mcrA* gene and *pmoA* gene at low application rate were dramatically higher than those from the high application rate treatments.

Fig. 3. Abundances of soil *mcrA* gene and *pmoA* gene (means $\pm$ sd) as affected by hydrochar application treatment.

3.5 Denitrifying functional genes

Hydrochar application exhibited a discernable influence on the copy numbers of soil denitrification functional genes (*nirK*, *nirS*, and *nosZ*) (Fig. 4). When compared with the control (CKU), SDH application (SDH05, SDH15) significantly increased the copy numbers of *nirK* and *nirS* genes, whilst the abundance of *nosZ* gene remained unchanged. On the other hand, the difference of the copy numbers of the three denitrification functional genes between A-SDH application treatments (A-SDH05, A-SDH15) and the CKU was not significant. For the application rates, the copy numbers of *nirK* gene and *nirS* gene of SDH05 were significantly higher than those subjected to the SDH15 treatment; A-SDH15 treatment significantly increased the copy numbers of *nirS* gene in comparison to A-SDH05 treatment. The copy numbers of denitrification functional genes for different treatments were shown as: SDH05, SDH15, A-SDH05, CKU, A-SDH15 (*nirK*), SDH05, SDH15, A-SDH15, CKU, A-SDH05 (*nirS*), and SDH05, SDH15, A-SDH05, CKU, A-SDH15 (*nosZ*).

Fig. 4. Copy numbers of soil denitrification functional genes (*nirK*, *nirS*, and *nosZ*) (means $\pm$ sd) as affected by hydrochar application treatment.

4. Discussion

4.1 Grain yield

Due to the stable growth of the human population (Prosekov and Ivanova, 2018), it is of great importance to increase grain production per unit of land (Arora, 2018). To date, the beneficial effect of the conventional pyrolytic biochar (pyrochar) application on grain yield has been widely investigated (Alvarez-Campos *et al.*, 2018; Bi *et al.*, 2019; Oladele *et al.*, 2019). Based on meta-analysis, Purakayastha *et al.* (2019) pointed out that 78% of major literature shows that conventional pyrochar application increases crop production. Recently, hydrochar attracted attention over the conventional pyrochar due to certain benefits (i.e. larger product quantities, reduced manufacturing/processing cost and lower environmental pollution) (Feng *et al.*, 2019).

Thus, hydrochar application on farmlands has a wider prospect due to these advantages. Notably, soil fertility is a key factor for crop productivity in agricultural systems mainly due to the retention of the applied fertilizers' nutrients (Haefele *et al.*, 2014). However, so far, studies related to the effect of hydrochar when applied in low fertility soils are limited, especially when focusing on crop yield.

In the present study, we found that regardless the application rate (5‰, 15‰), there is an evidently increasing trend towards rice grain yield from fields subjected to hydrochar (SDH, A-SDH). It is well known that hydrochar typically bears good adsorption properties due to its Daedalus, extensive porosity (Table S1), retaining multiple abundancies of oxygen-containing functional groups (Fig. S1-S2), as well as bearing high cation exchange capacity (CEC) (Table 1) (Gai *et al.*, 2016; Gasco *et al.*, 2018; Feng *et al.*, 2019). The soil's physicochemical properties as well as the microbial activity at the root zone can be also improved via hydrochar addition. This is due to the input of its soluble organic amendments combined with the additional habitat niches for soil microorganisms colonization (due to the fine pore characteristics of the hydrochar itself) (Egamberdieva *et al.*, 2016).

It has been proved that hydrochar often carries large amounts of dissolved organic matter (DOM), such as phenols, organic acids, etc. on the hydrochar surface (Table S2), which in theory may inhibit crop growth (Bargmann *et al.*, 2013; Busch *et al.*, 2013; Zhou *et al.*, 2018). However, Fincheira and Quiroz (2018) pointed out that volatile organic compounds (VOCs), when suitable, could stimulate crop growth. Additionally, Hao *et al.* (2018) showed that the negative effect to growth, originated from DOM, often increases with the increase of production temperature, while at low-temperatures hydrochar-derived DOM shows minimal biotoxicity. The above suggests that

the relatively low temperature selected during hydrochar production combined with the low application rate (5%~15%) in this study, could more likely supply with the suitable volatile organic compounds, and subsequently promote rice growth rather than inhibit. Finally, hydrochar, rich in soluble organic compounds and nitrogen minerals, is beneficial for the crop providing with more available nutrients for uptake and growth (Yu *et al.*, 2019; Ji *et al.*, 2020). Thus, the increased yield from the hydrochar treated soils may be a combined result achieved from multiple factors as mentioned above. The beneficial effect of the hydrochar, produced under similar temperature and applied at similar rates, on grain yield from high fertility soils has been previously observed (Yu *et al.*, 2019; Chu *et al.*, 2020). The results in this study confirm that hydrochar (SDH, A-SDH) is a good soil synergist to improve grain yield at low fertility soil.

4.2 CH₄ emissions

To confirm the good synergistic property of hydrochar, it is essential to define the effect of its application on greenhouse gas emissions when this additive is applied in low fertility paddy fields. The average CH₄ emission rate over the rice growing season and the cumulative CH₄ emissions from the low fertility soils subjected to hydrochar remained similar to those estimated at the control (CKU). CH₄ emissions from paddy fields were affected neither from the hydrochar type nor from application rate. Methane emission is a composite result driven by soil environment, substrate concentration, as well as microbial presence (the functional genes (*mcrA*, *pmoA*)) (Mer and Roger, 2001; Yan *et al.*, 2005). Biochar addition on farmlands would inevitably affect the amount and composition of the soil organic carbon (SOC), and subsequently the substrate supply for the microorganisms to metabolize (Han *et al.*, 2020). In the previous study, we have found that hydrochar application decreased the labile SOC fraction in comparison to the control, whilst the

stable SOC fraction increased (Sun *et al.*, 2020).

Decomposition and/or conversion of labile SOC to stable SOC are the two possible ways to promote carbon turnover (Han *et al.*, 2020). There are two possible “conjectures” for the variation between SOC components under hydrochar addition: 1) the labile SOC of soil itself decomposes whilst the stable SOC increases mainly originated from biochar (as a source); 2) the reduced labile SOC shifts to stable SOC. The decomposition products (H_2/CO_2) of labile SOC were the precursors for hydrogenotrophic methanogenesis (Mer and Roger, 2001). Combined with the current study’s results from the hydrochar application (similar cumulative CH_4 emissions, increased *mcrA* gene), we presume that most of the reduced labile SOC shifts to stable SOC after hydrochar application. It is well known that increase of stable SOC fraction is beneficial for the steady improvement in soil fertility, and subsequently this leads to substrate limitation promoting mitigation of the climate change (Stockmann *et al.*, 2013). Thus, the substrate limitation of low fertility soil itself and soil carbon conversion can be the main reasons for the similar CH_4 emissions among treatments.

It is worth mentioning that, in contrast to CH_4 emissions, the differentiation in hydrochar application leads to statistically significant ($P<0.05$) differences between soil microbial functional genes related to CH_4 emissions (*mcrA* gene, *pmoA* gene). Hydrochar can play the role of habitat and refuge for soil microorganisms when subjected to low application rate (Thies *et al.*, 2015); while the increased volatile compounds may inhibit microbial activity related to CH_4 emissions at high application rate (due to acidification and methanogenic inhibition) (Lehmann *et al.*, 2011; Thammasom *et al.*, 2016). Hence, the abundances of soil *mcrA* gene and *pmoA* gene at low application rate were dramatically higher than those at high application rate (Fig. 3). However, the

CH₄ emissions from the hydrochar application treatments were similar although there was significant difference in soil microbial functional genes related to CH₄ emissions. The results show that CH₄ emissions are mainly related to the substrate supply in such low fertility soils but less affected by the functional gene, confirming that hydrochar (SDH, A-SDH) is a good soil synergist for carbon sequestration without increasing CH₄ emissions in low fertility soil.

4.3 N₂O emissions

The microbial activities (nitrification, denitrification) related to farmlands' N₂O emissions greatly depend on soil temperature, soil moisture conditions, etc. Midseason drainage acts as a stimulating factor for N₂O emissions from paddy fields (Zou *et al.*, 2005). A similar phenomenon was also observed in this study. Apart from the midseason peak, another flux peak of N₂O emission rate occurred at the tillering stage (4 days after tillering fertilizer application), event also likely related to the experimental conditions. Firstly, the date of tillering fertilizer application was the period with the highest temperature over the whole season among the three fertilization events. Secondly, under greenhouse conditions, the high temperature promotes the evaporation of the soil water in the column. Hence, the N input from the fertilizer as well as the variability in soil aeration during this period could have presumably been the main reasons related to this N₂O peak.

The results indicated that the cumulative seasonal emissions of N₂O from the low fertility soils subjected to low application rate (SDH05, A-SDH05) were significantly lower than those observed from both the high application rate (SDH15, A-SDH15) and the CKU treatments ($P<0.05$); emissions remained unaffected from the hydrochar type. The same fate of N₂O emissions between two hydrochar materials with different pH (the pH of SDH and A-SDH were 3.71 and 7.04, Table 1) highlighted the importance of the application rate and revealed the

complexity of the underlying mechanisms. It is generally known that N₂O emission is a composite formation driven by several microbial processes dependent on environmental conditions (Baggs, 2008; Kuypers *et al.*, 2018). Combined with findings from previous studies, changes in N₂O emissions may be attributed to the properties of hydrochar itself (nutrient input, pH, porous property, and the adsorption property) as well as the variety of soil water-air conditions and the nitrogen cycle driven by microorganisms after hydrochar application (Liu *et al.*, 2019a; Xu *et al.*, 2020).

Carbon and nitrogen substrate supply was the main variable affecting paddy field N₂O emissions (Xu *et al.*, 2020). Nitrogen is often fixed in biochar due to the adsorption property of this material; application to soil allows its slow release rendering it a slow-release fertilizer (El-Naggar *et al.*, 2019a). Previous studies showed that slow/controlled release urea can significantly decrease the N₂O emissions from paddy fields due to the reduced NH₄⁺-N concentration in the paddy fields' surface water and soil (Guo *et al.*, 2019). Thus, the reduced NH₄⁺-N concentration via the adsorption property under the application rate of hydrochar with 5% can be the main reason for the reduced N₂O emissions (Table S1; Fig. S1). In a previous study, Xu *et al.* (2020) found that paddy field N₂O emissions were significantly affected by the substrate supply (DOC and inorganic N) from the biochar; thus, biochar with rich in DOC and inorganic N could potentially increase soil N₂O emissions. Hereby, we assumed that the application rate of hydrochar at 15%, increased C and N inputs from hydrochar itself, this may could have been tackled at a low application rate though (Table 1). Also, the N₂O reductase is typically negatively correlated to soil pH; the change of the N₂O reductase due to the variance of soil pH subjected to biochar could influence the N₂O emissions (Cayuela *et al.*, 2013; Mukumbuta *et al.*, 2018).

Generally, paddy field N₂O emissions could increase in lower pH soil by inhibiting nitrification and stimulating N₂O production (Shcherbak *et al.*, 2014; Liu *et al.*, 2018). However, the present results challenge this scenario due to the similar N₂O emissions achieved under both SDH and A-SDH (the pH of SDH and A-SDH were 3.71 and 7.04, Table 1). This shows that pH is not a determinant factor of N₂O emissions at low fertility soils subjected to hydrochar, plausibly due to substrate limitation.

The abundance and metabolic activity of *nirS*, *nirK* and *nosZ* genes are critical indicators of denitrification (Hu *et al.*, 2015). It has been widely proved that biochar addition can promote the denitrification functional genes abundance, which then indicates a more active biochemical reaction basis (Chen *et al.*, 2019). In this study, we also found that SDH application (SDH05, SDH15) significantly increased the copy numbers of *nirK* and *nirS* genes in comparison to the control, whilst the response of *nosZ* gene was negligible. The difference of the denitrification functional genes between A-SDH application treatments (A-SDH05, A-SDH15) and the CKU was not significant. As mentioned above, the volatile compounds of the hydrochar itself may inhibit microbial activity (Lehmann *et al.*, 2011). Compared with SDH, microbial-aged hydrochar bears reduced content of volatile compounds (Table S2). The results suggest that denitrifying microorganism are more sensitive to volatile compounds (organics act as electron donors).

Additionally, SDH with high application rate (15‰) tended to decrease the copy numbers of *nirK* and *nirS* genes, same as *nirK* and *nosZ* genes for A-SDH treatments. This may be related to the inhibiting effect caused by the lower pH at high application rate (Bakken *et al.*, 2012; Shaaban *et al.*, 2018). Although there is a distinct increase of *nirK* and *nirS* genes after hydrochar addition, especially for SDH, the N₂O emissions at low application rate for both hydrochars was relatively

low. Based on the current results, N₂O emissions under hydrochar application are less affected by the denitrification functional genes present in the low fertility soil. Nevertheless, the response of the soil functional genes in paddy soils when subjected to hydrochar is worth of further attention taking into account the autochthonous community from the microbial-aged hydrochar. This is expected to be minimal due to the low application rate, however, further investigation would be required to evaluate the exact impact in the long run. Combined with the above analysis, the results suggest that substrate availability in the low fertility soil subjected to hydrochar amendments is the crucial factor affecting N₂O emissions. It is worth to note that biochar can act as the “electronic conductor”, which can transfer the electrons towards the soil denitrifying microorganisms, promoting the conversion of N₂O to N₂ (Cayuela *et al.*, 2013; Sun *et al.*, 2017). This hypothesis is exceptionally essential for future studies aiming to define the reduction mechanism of N₂O emissions from low fertility soils when subjected to hydrochar amendments.

4.4 GWP and GHGI

In this research, we selected global warming potential (GWP) and greenhouse gas intensity (GHGI) to understand the overall GHG impact under hydrochar addition on low fertility soil (IPCC, 2013; Liu *et al.*, 2019b). The results showed that there is a decreasing trend in GWP from the soils subjected to low application rate treatments (SDH05, A-SDH05), due to the lower cumulative seasonal N₂O emissions, the difference between treatments though was not significant. As to GHGI, we found that apart from the SDH15 treatment, hydrochar application treatments (SDH05, A-SDH05, A-SDH15) significantly decreased the GHGI when compared with the control. The results suggest that hydrochar application is a suitable strategy to increase grain yield and restrict global warming from cultivation of low fertility soils.

479

480 **5. Conclusion**

481 This is one of the very few, if not the first, study that evaluates the effect of hydrochar
482 application on rice grain yield and agricultural GHG emissions from low fertility soil. Hydrochar
483 addition was found to be an effective strategy to ensure grain yield in low fertility soils with
484 relatively low/controlled GHG emissions; the low application rate of hydrochar was the optimum
485 application method. Greenhouse gas emissions under hydrochar applications were mainly related
486 to the substrate availability in these low fertility soils. Nevertheless, the response of the crop yield
487 and the greenhouse gas emissions at prolong hydrochar-application periods should be evaluated;
488 similarly, the impact of hydrochar in other crop types. These are both observations of great
489 importance for future studies considering the promising hydrochar-related findings. Additionally,
490 the internal '*modus operandi*' of the hydrochar and how it limits/regulates greenhouse gas
491 emissions is also worth of a more into detail study.

492

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498

Figure captions

Fig. 1. Grain yield (means $\pm$ sd) as affected by hydrochar application treatment. SDH05, sawdust-derived hydrochar with 5‰ (w/w) application rate; SDH15, sawdust-derived hydrochar with 15‰ (w/w) application rate; A-SDH05, microbial-aged hydrochar with 5‰ (w/w) application rate; A-SDH15, microbial-aged hydrochar with 15‰ (w/w) application rate; CKU, conventional split fertilization without hydrochar addition. The significant difference for hydrochar application treatments was determined using the LSD method with confidence level set at 0.05. The same below.

Fig. 2. Emission rate of CH₄ and N₂O (means $\pm$ sd) as affected by hydrochar application treatment.

Fig. 3. Abundances of soil *mcrA* gene and *pmoA* gene (means $\pm$ sd) as affected by hydrochar application treatment.

Fig. 4. Copy numbers of soil denitrification functional genes (*nirK*, *nirS*, and *nosZ*) (means $\pm$ sd) as affected by hydrochar application treatment.

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