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Hybrid rice produces a higher yield and emits less methane

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Abstract: Hybrid rice has a higher yield potential than inbred rice, but the difference in CH_4 emissions between the two groups is still unclear, particularly regarding straw incorporation. In the present study, a pot experiment was conducted to examine the difference in CH_4 emissions between inbred (Huanghuazhan) (IR) and hybrid (Rongyou-huazhan) (HR) rice cultivars, both with or without straw incorporation in subtropical China. The results showed that HR produced both greater grain yield and biomass than IR. In contrast, when compared with IR, HR reduced the cumulative CH_4 emissions by an average of 18.6%. No significant interactions between rice cultivars and straw management on yield or CH_4 emissions were found. HR significantly increased the abundance of methanogens and methanotrophs by 38.9% and 93.4% relative to IR, respectively, thereby reducing CH_4 concentrations in the soil pore water. Therefore, we suggest that cultivar rice can produce a higher yield and better mitigate CH_4 emissions when compared to inbred rice, regardless of the use of straw incorporation.

Keywords: CH_4 oxidation; greenhouse gas; global warming; *Oryza sativa* L.; methanogenesis

Methane (CH_4) is an important greenhouse gas with a global warming potential 34 times greater than that of the equivalent mass of carbon (C) dioxide (IPCC 2013). Rice (*Oryza sativa* L.) cultivation is one of the main contributors to anthropogenic CH_4 emissions (Carlson et al. 2017, Yuan et al. 2019). China is the largest rice producer in the world, and its rice production needs to increase further to meet the food demand of the growing population (FAOSTAT 2017). Therefore, researchers must explore new strategies that can reduce CH_4 emissions in paddies while still maintaining a high yield of rice.

Rice cultivars with a high yield potential are essential to meet the growing demand for rice (Peng et al. 2008). In China, hybrid rice cultivars have a significant yield advantage over inbred cultivars (Jiang et al. 2016a). Since the late 1970s, the total planted-area of hybrid rice has expanded rapidly and now accounts for more than half of the total rice-growing area in China (Yuan 2014). However, the effect of hybrid rice cultivars on CH_4 emissions is inconsistent in previous studies (Hussain et al. 2015). On the one hand, hybrid cultivars have larger biomass than inbred ones, and thus produce more C sources for CH_4 production (Bhattacharyya et al. 2019). On the other hand, hybrid rice cultivars with higher root volume and surface may transport greater O_2 into the soil for methanotrophs, thereby promoting more CH_4 oxidation than inbred cultivars (Ma et al. 2010, 2012). However, the net effect of hybrid rice cultivars on CH_4 emissions is not well documented and has not been fully elucidated (Jiang et al. 2017).

Straw incorporation is a widely applied practice to improve both soil fertility and rice yield (Huang et al. 2013, Liu et al. 2019). Multiple studies have shown that straw incorporation can greatly promote CH_4 emissions from rice paddies due to increased substrate availability

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(Jiang et al. 2018, 2019, Wang et al. 2019). However, previous research focused primarily on the separate effects of rice cultivars or straw incorporation on CH_4 emissions; the interactive effects of rice cultivars with straw addition on CH_4 emissions have not yet been elucidated (Gutierrez et al. 2013, Wang et al. 2019). Therefore, the objective of the present study was to investigate the effects of hybrid rice on the yield and CH_4 emissions in comparison with inbred rice and to examine whether these effects depended on straw incorporation.

MATERIAL AND METHODS

Site description. A pot experiment was conducted in a glasshouse at the experimental farm of the Jiangxi Agricultural University, Nanchang, Jiangxi province, China (115°83'E, 28°76'N), between May and September in 2017. The windows on all sides of the glasshouse were open throughout the rice growth period. Thus, the glasshouse acted as a rain shelter in this experiment. The soil was collected from a paddy field that has been cropped for rice for over 30 years. The soil was developed from Quaternary red clay and is classified as a Typic Stagnic Anthrosol (IUSS Working Group, WRB 2006). The initial soil properties and meteorological conditions during the experiment are presented in Table 1.

Experimental design. The pot experiment consisted of 24 pots (height 24 cm; diameter 22 cm) and was established following a completely randomized design. The pots were randomly arranged and were re-randomized every five days during the experiment. Our experiment included two cultivars of rice, two straw management practices, and contained six replicated pots for each treatment combination. The inbred rice cv. Huanghuazhan (IR) and hybrid rice cv. Rongyouhuazhan (HR) were used, while straw treatments consisted of no straw incorporation (–S) and straw incorporation (+S). Both cultivars are widely cropped in the Jiangxi province with a growth period of approximately 130 days. Among the two rice cultivars, Rongyouhuazhan showed a greater tillering capacity than Huanghuazhan. Rice straw was chopped into 10-mm pieces and then incorporated into the soil in the +S treatments at a rate of 20 g/pot. Each pot was filled with 5.0 kg of air-dried and 2-mm-sieved soil. Three pots in each treatment were used to measure CH_4 emissions, CH_4 concentrations in soil pore water, and the abundance of soil methanogens and methanotrophs, while the other three were kept intact for determining grain yield and biomass. Seeds were pre-germinated under 25°C for 24 h and then sown into a seedbed on 13 May 2017. Two rice seedlings were transplanted into each pot on 7 June 2017. The rates of inorganic N, P, and K fertilizers were 0.47 g/pot, 0.10 g/pot, and 0.28 g/pot, respectively. Nitrogen fertilizer was applied three times: 50% as basal fertilizer before planting, 20% at tillering, and 30% at panicle initiation. The entirety of the P fertilizer was applied as basal, while half of the K fertilizer was applied as basal and the other half was applied at panicle initiation. Urea, calcium magnesium phosphate and potassium chloride were used for the inorganic N, P, and K fertilizers, respectively. A line was drawn on the wall of each pot and water was added every day to maintain a 3-cm water layer in the pots during the entire rice growth period.

Table 1. Initial soil properties and meteorological conditions at the rice site

Soil property	Unit	Value
pH (1:2.5 H_2O)		5.8
Organic carbon	(g/kg)	14.2
Total nitrogen		1.2
Total phosphorus		0.4
Total potassium		4.6
Alkaline hydrolyzable-N	(mg/kg)	113
P_{Olsen}		7.9
Available K		25.1
Mean temperature	(°C)	28.5

P_{Olsen} – measured as Olsen-extractable phosphorus

Sampling and measurement. Methane emissions were measured using the static closed chamber technique every 5 days after rice transplantation (Jiang et al. 2017). On each sampling occasion, the pots were transferred to a large container filled with water to a depth of 2 cm that was used as a seal to close the static chamber (length 30 cm; width 30 cm; height 130 cm). After chamber closure, gas samples were collected between the hours of 9:00 AM to 11:00 AM using a 50 mL syringe at 5-min intervals over a 20 min period. Methane concentrations were measured using a gas chromatograph (Agilent 7890A, Agilent Technologies, California, USA) with a flame ionization detector (stainless steel column, length 6 ft, 1/8 in, paropak Q packing, California, USA). The CH_4 fluxes and cumulative CH_4 emissions were calculated following the procedure described by Wang et al. (2019).

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Fresh soil was collected to measure soil methanogenic and methanotrophic abundances on day 25 after rice transplantation when CH_4 fluxes were high. We observed that the difference in CH_4 fluxes was significantly different among treatments. Fresh soil samples were collected using a small auger (0.9 cm in diameter). Roots and crop residues were removed, and the soil samples were subsequently stored at –80°C. Soil DNA was extracted from 0.25 g soil using a Power Soil DNA Isolation Kit (MoBio, Carlsbad, USA). The copy numbers of *mcr*A genes, which represent the abundance of methanogenic archaea in soil, were quantified using the primer pair *mcr*Af/*mcr*Ar (Luton et al. 2002). Two forward primers of MB10γ and MB 9α, along with their common reverse primer 533r, were used to quantify the 16S rRNA gene copy numbers of type I and type II methanotrophs, respectively (Henckel et al. 1999). Quantitative real-time PCR was performed using a Mastercycler ep realplex instrument (Eppendorf, Hamburg, Germany).

We also measured CH_4 concentrations in soil pore water on day 25 after rice transplantation. All samples were collected from 9:00 AM to 11:00 AM. A Rhizon sampler (SMS, Eijkelkamp, Holland) was vertically inserted into the soil to a depth of 5 cm in the center of each pot following transplantation (Ma et al. 2010, Jiang et al. 2016b). When sampling, a 20-mL soil solution was drawn and then brought to the lab immediately. All the sampling vials were first equilibrated by filling them with pure N_2 gas, and then about 5-mL of gas in the headspace was collected to analyze CH_4 concentrations using a gas chromatograph. CH_4 concentrations in soil pore water were calculated following the procedure of Jiang et al. (2013).

At maturity, all the soil in the pot was transferred to a large container and washed with tap water for root sampling. Plant biomass was determined after oven-drying at 70°C until a constant weight was observed. Grain yield was determined and adjusted to a moisture content of 0.14 g H_2O/g fresh weight.

Statistical analyses. All analyses were performed with the statistical package SPSS 18.0 (SPSS Inc., Chicago, USA). We analyzed CH_4 emissions, biomass, soil methanogenic and methanotrophic abundances, and CH_4 concentrations in soil pore water using two-way analyses of variance (ANOVA). A plot of the standardized residual was used for checking the normality assumption. Where necessary, the data were $\log_{10}$-transformed to meet the homogeneity of variance and normality assumptions of the ANOVAs. Means were tested using Fisher's least significant difference (*LSD*) with $P < 0.05$ indicating significance. For parameters for which the interaction is not significant, we presented the means of the main effects (i.e., straw management and rice cultivar). For parameters for which the interaction between the two factors is significant, the mean of each treatment combination was presented. In the present study, only CH_4 concentrations in soil pore water were significantly affected by the interactions between straw management techniques and rice cultivars.

RESULTS

Grain yield and biomass. Hybrid rice produced a higher grain yield than IR, and straw incorporation significantly increased rice yield when compared to no straw incorporation (Figure 1a,b). Above-ground biomass was significantly increased by straw incorporation and was marginally greater for HR than IR (P = 0.052) (Figure 1c,d). Root biomass was significantly higher for HR than IR but was not affected by straw incorporation (Figure 1e,f).

CH_4 emissions. Methane fluxes increased rapidly the following transplantation, and peaks were observed at the tillering stage in straw-applied treatments (Figure 2). In contrast, CH_4 fluxes increased slowly until panicle initiation in the no straw treatment groups. On average, HR significantly reduced cumulative CH_4 emissions by 18.6% when compared with IR, while straw incorporation increased CH_4 emissions (2.2 times greater) when compared with the no straw incorporation groups (Figure 3).

The abundance of methanogens and methanotrophs. Both HR and straw incorporation significantly enhanced methanogenic and methanotrophic abundances (Figure 4). On average, the abundance of methanogens and methanotrophs was increased by 38.9% and 93.4% in the HR group when compared with IR (Figure 4a,c), and by 2.2 times and 1.1 times in the straw incorporation groups relative to groups without straw incorporation, respectively (Figure 4b,d).

CH_4 in soil pore water. Hybrid rice significantly reduced the CH_4 concentration in soil pore water when compared with IR, which, however, were significantly increased by straw incorporation when compared to groups without straw incorporation (Figure 5). Compared with IR, HR reduced the concentration of CH_4 in soil pore water by 84.2% and 65.0% without and with straw incorporation, respectively.

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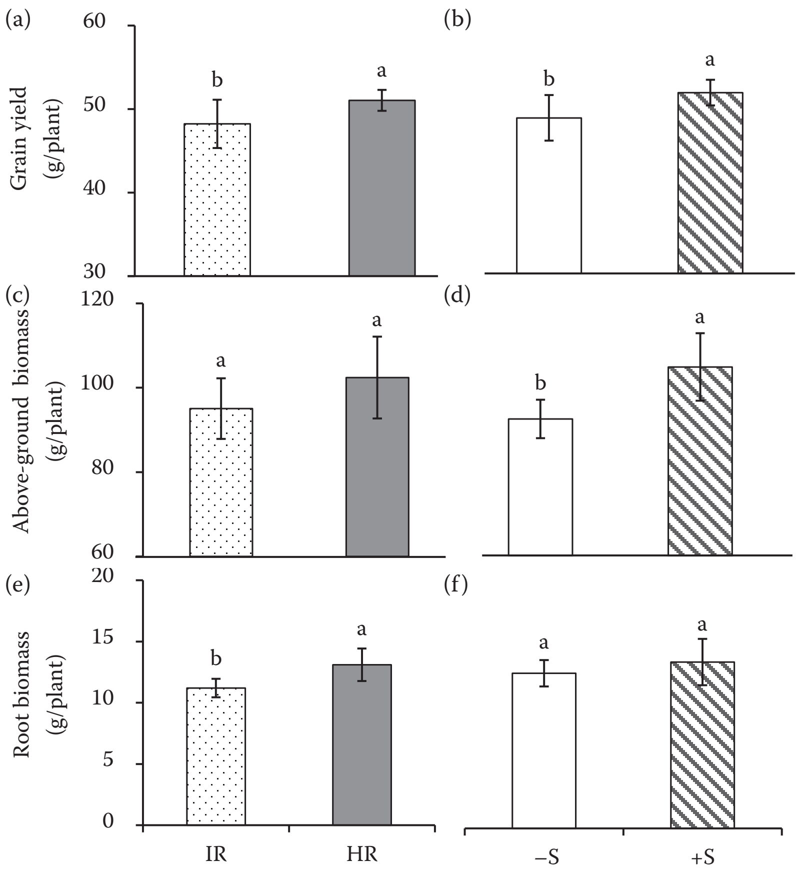

Figure 1. Grain yield (a, b); above-ground biomass (c, d), and root biomass (e, f) as affected by the main effect of rice cultivars and straw management. IR – inbred rice; HR – hybrid rice; –S – no straw incorporation; +S – straw incorporation. Error bars represent the standard deviation of the mean (n = 6). Different lowercase letters represent statistical significance among treatments at P < 0.05

DISCUSSIONS

Our findings indicate that HR significantly increase grain yield relative to IR. A large number of studies have reported that HR cultivars demonstrated a higher yield potential than IR cultivars under optimal growing conditions (Ma and Yuan 2015, Jiang et al. 2016a, Yuan et al. 2017), which is primarily attributed to high rates of photosynthesis and thus biomass production (Huang et al. 2017, 2018). Indeed, both the above-ground and root biomass of HR were higher than those of IR in the present study. Also, a larger panicle size (spikelets per panicle) of HR contributed to the higher grain yield than IR due to

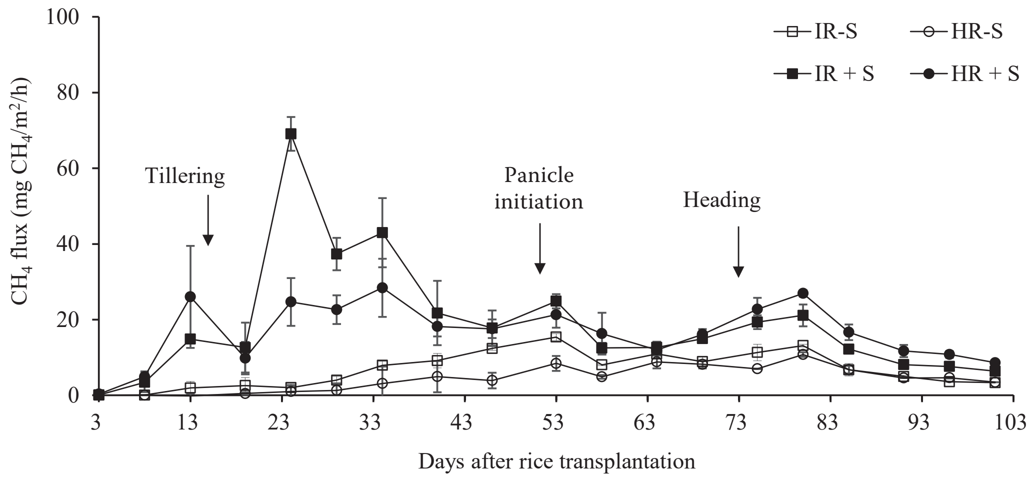

Figure 2. CH_4 fluxes as affected by rice cultivars and straw management. IR-S – inbred rice without straw incorporation; HR-S – hybrid rice without straw incorporation; IR + S – inbred rice with straw incorporation; HR + S – hybrid rice with straw incorporation. Error bars represent the standard deviation of the mean (n = 3)

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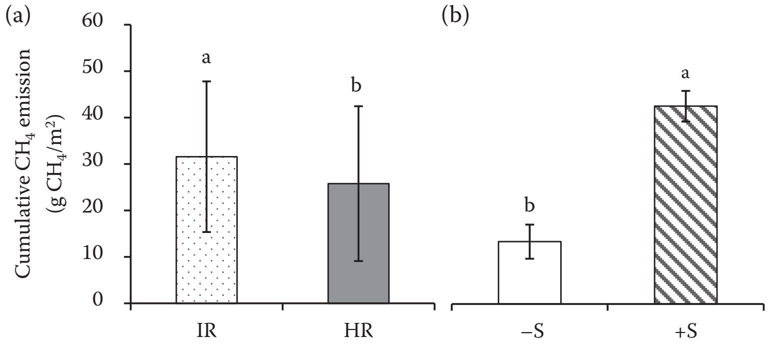

Figure 3. Cumulative CH_4 emissions (a, b) as affected by the main effect of rice cultivars and straw management during the whole growth period. IR – inbred rice; HR – hybrid rice; –S – no straw incorporation; +S – straw incorporation. Error bars represent the standard deviation of the mean (n = 6). Different lowercase letters represent statistical significance among treatments at P < 0.05

a larger sink for photosynthate accumulation (data not shown) (Jiang et al. 2016a).

Although HR produced a larger quantity of above--ground and root biomass than IR and thus increased C inputs into the soil, HR substantially reduced cumulative CH_4 emissions. The difference in CH_4 fluxes between HR and IR typically occurred before panicle initiation (Figure 2). The number of stems was higher for HR than IR during the early growth stage, though no significant difference in the number of panicles was found at maturity (data not showed). The higher stem number demonstrated that HR cultivars showed a greater tillering capacity than IR cultivars (Sun et al. 2015). HR with larger root biomass and stem number than IR may promote O_2 transport into the rhizosphere (Jiang et al. 2017, 2019). Higher oxygen concentrations can stimulate methanotrophic growth and thus promote CH_4 oxidation (Bhattacharyya et al. 2019). Indeed, the quantity of methanotrophs was larger for HR than IR (Figure 4c). Also, a recent meta-analysis discovered that rice cultivars with high biomass enhanced CH_4 emissions from lower organic C soils (≤ 8 g/kg), but significantly reduced CH_4 emissions from higher organic C soils (> 12 g/kg) (Jiang et al. 2017). The content of organic C soil was 14.2 g/kg in the present study. Thus, our results that displayed that HR produced higher biomass, but emitted lower CH_4 than IR were consistent with the conclusion of the meta-analysis.

Also consistent with previous results, straw incorporation increased rice yield, replenishing nutrients, and enhancing soil fertility (Liao et al. 2018). On the other hand, straw incorporation demonstrated a negative effect on rice tillering at the early growth stage due to microbial N immobilization resulting from the high C:N ratio of straw residues (data not shown) (Murphy et al. 2016). However, straw incorporation did not affect the number of panicles at maturity. We hypothesize that this is because the initially immobilized N was released over time and contributed to rice growth at a later stage (Liao et al. 2018). In agreement with many previous studies, straw incorporation led to higher CH_4 emissions than

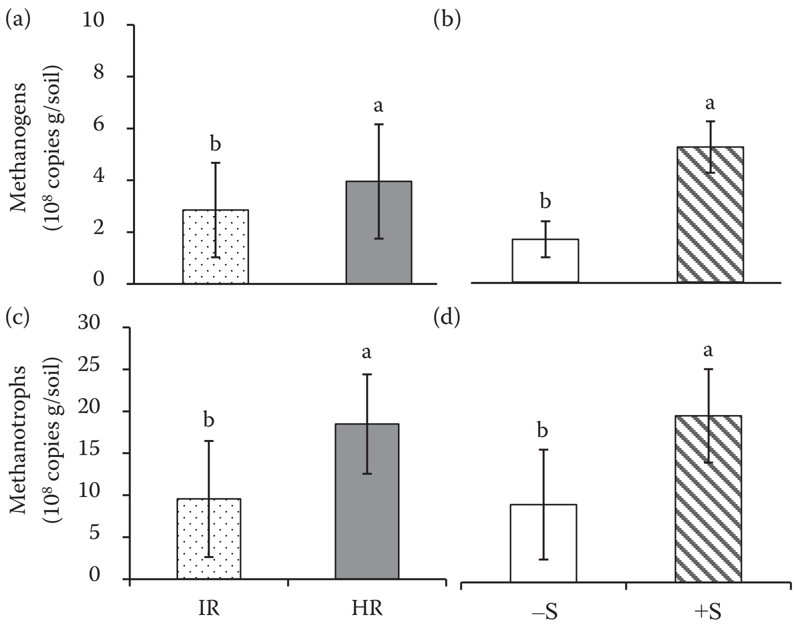

Figure 4. Abundance of methanogens (a, b) and methanotrophs (c, d) as affected by the main effect of rice cultivars and straw management on day 25 after rice transplantation. IR – inbred rice; HR – hybrid rice; –S – no straw incorporation; +S – straw incorporation. Error bars represent the standard deviation of the mean (n = 6). Different lowercase letters represent statistical significance among treatments at P < 0.05

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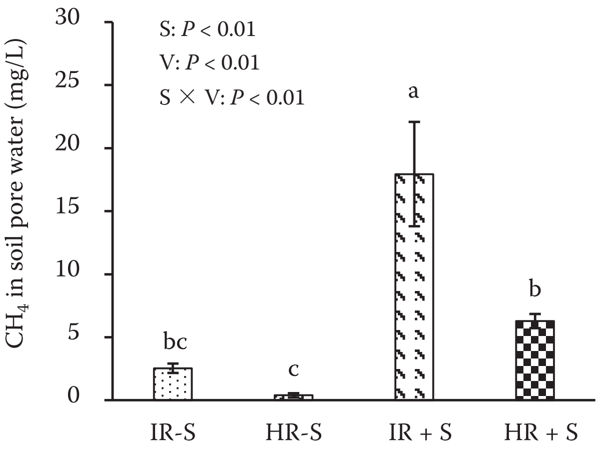

Figure 5. CH_4 concentrations in soil pore water as affected by rice cultivars (V) and straw management (S) on day 25 after rice transplantation. IR-S – inbred rice without straw incorporation; HR-S – hybrid rice without straw incorporation; IR + S – inbred rice with straw incorporation; HR + S – hybrid rice with straw incorporation. Error bars represent the standard deviation of the mean (n = 3). Different lowercase letters represent statistical significance among treatments at $P < 0.05$

without straw incorporation due to a large amount of C inputs that increased substrate availability for methanogenesis (Jiang et al. 2018, 2019, Wang et al. 2019). Our results showed that straw addition just promoted CH_4 emissions, but did not alter the mitigation effect of HR cultivars on CH_4 emissions compared with IR cultivars (Figure 3). Previous studies have shown that straw incorporation could increase CH_4 production as C availability limits methanogenesis (Conrad 2007, Jiang et al. 2019, Wang et al. 2019). In contrast, CH_4 oxidation is limited by both CH_4 and O_2 availability (Conrad 2007). Furthermore, limitation by O_2 is most likely to occur for methanotrophs in continuously flooded systems (Megonigal et al. 2004). Thus, we speculate that HR cultivars with a large root system could increase O_2 availability and promote CH_4 oxidation regardless of the use of straw incorporation (Bhattacharyya et al. 2019).

Several limitations should be noted for the present study. First, HR cultivars with higher yield produced a larger amount of straw residues compared with IR cultivars due to greater biomass production. Therefore, more straw incorporation with HR cultivars may promote CH_4 emissions in the following rice-growing season. However, it is unclear whether the mitigating effects of HR on CH_4 emissions over IR could persist over time (Jiang et al. 2017, 2018). Second, more rice cultivars should be included to more extensively testing the effects of HR on CH_4 emissions in comparison with IR (Gutierrez et al. 2013, Zheng et al. 2018). Third, it should be noted that different experimental conditions between pots and fields may affect the response of CH_4 emissions to rice cultivars. Thus, multi-year and multi-site field experiments should be conducted in the future to evaluate the mitigation potential of HR for CH_4 emissions and its spatial-temporal variations (Zhang et al. 2015, Jiang et al. 2017).

In conclusion, HR produced a higher grain yield but reduced cumulative CH_4 emissions when compared with IR, regardless of straw management techniques. Although HR promoted the abundance of methanogens relative to IR, the abundance of methanotrophs was enhanced more by HR. Furthermore, the increased root biomass may contribute to stronger CH_4 oxidation for HR than IR. Because of this, hybrid rice cultivation may benefit both rice production and CH_4 mitigation in China.

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