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RESEARCH ARTICLE

Azolla planting reduces methane emission and nitrogen fertilizer application in double rice cropping system in southern China

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Abstract Rice paddies are a major source of methane. How to reduce the methane emission in the paddy field without decreasing the yield has become a major concern of scientists, environmental groups, and agricultural policymakers worldwide. *Azolla*, used as a dual crop in rice cultivation, has multiple agronomic benefits. However, the effects of the dual cropping of *Azolla* on methane emissions of double rice cropping paddies have not yet been reported. Here, we conducted a 3-year field experiment to evaluate the impacts of rice + *Azolla* on methane emission and rice yield in a double rice cropping system. The results indicated that the rice + *Azolla* without N fertilizer and with moderate N fertilizer (200 kg N ha^{-1} a^{-1}) significantly reduced methane emissions over the rice cycle by 12.3 and 25.3% compared with the conventional rice cropping with common N fertilizer (400 kg N ha^{-1} a^{-1}), respectively. The reason for the trend was because the dual cropping of *Azolla* has significant effect on dissolved oxygen and soil redox potential, which are key factors for methane emission in this study. The rice yield under the rice + *Azolla* with moderate N fertilizer annually averaged 12.7 Mg ha^{-1}, which was comparable with that of the conventional rice cropping with common N fertilizer. Moreover, the rice + *Azolla* with moderate N fertilizer had the lowest yield-scaled methane (25.2 kg Mg^{-1} grain yield). Here, we showed for the first time that *Azolla* planting allows sustainable rice production coupled with methane mitigation in double rice cropping systems.

Keywords *Azolla* · Double rice cropping · Grain yield · Methane

1 Introduction

Methane (CH_4) is an important trace gas that contributes to global warming (Montzka et al. 2011), with a 100-year scaled global warming potential (GWP) 28 times the forcing radiative intensity of CO_2 on a per mass basis (Myhre et al. 2013). The atmospheric concentration of CH_4 risen from 722 ppb in 1750 to 1803 ppb in 2010 (Myhre et al. 2013). Rice paddies are a significant source of atmospheric CH_4 and emit between 33 and 40 Tg CH_4 annually, accounting for 10–12% of global anthropogenic CH_4 (Ciais et al. 2013). Major concern exists regarding CH_4 emissions from rice paddies, and these emissions will continue to increase as rice production intensifies (Hussain et al. 2015).

To meet the food demand of the growing population, rice production is projected to increase from 571.9 million tons in 2001 to 771.1 million tons by 2030 (Nguyen and Ferrero 2006). In China, the production of rice accounts for 35% of the global production, and China's 30 million ha of rice-planting land accounts for approximately 20% of the world's total (FAO 2013). An annual double rice cropping system (planting two successive rice crops in 1 year) was established and is popular in southern China; the planting area there represents 56% of the total national paddy fields (Frolking et al. 2002). Generally, rice intensification requires synthetic fertilizers to achieve higher grain yields. However, the overuse of synthetic fertilizers has many adverse consequences, such as

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the eutrophication of surface water, greenhouse gas emissions, and soil acidification (Shang et al. 2011; Zhang et al. 2013). To produce more rice and simultaneously reduce agricultural environmental harm is the greatest challenge that contemporary agriculture confronts.

Decreasing synthetic fertilizer application and increasing organic matter amendments have drawn recent attentions. The free-floating water fern *Azolla* occurs in symbiotic association with a nitrogen-fixing cyanobacterium generally refers to as *Anabaena azollae*. *Azolla* has been used extensively and effectively as green manure in paddy fields (Bharati et al. 2000; Chen et al. 1997; Ying et al. 2000). As a green manure, *Azolla* could be either incorporated into paddy soil at the beginning of land preparation for rice planting or grown as a dual crop along with rice plants Fig. 1. Over the past decades, the beneficial effects of *Azolla* as a green manure in rice cultivation have been widely discussed (Kollah et al. 2016; Vlek et al. 1995). Dual cropping of *Azolla* exerts various influences on CH_4 emissions, mainly by regulating the soil physical-chemical properties. However, the CH_4 emissions influenced by the dual cropping of *Azolla* during double rice growth were poorly reported. Although several observations were made, mainly on single rice (Bharati et al. 2000; Chen et al. 1995, 1997; Ying et al. 2000) or early rice of a double rice cropping system (Ma et al. 2012), no consensus exists regarding the impact of growing *Azolla* on CH_4 emissions in paddy fields. Bharati et al. (2000) and Ma et al. (2012) reported that dual cropping of *Azolla* decreased significantly CH_4 emissions from paddy fields. Bharati et al. (2000) found that mitigation of CH_4 emission associated with the presence of *Azolla* could be related to the increase in dissolved oxygen (DO) content in the standing water, resulting in less reduced paddy soils. *Azolla* with a higher photosynthetic capacity (Wagner 1997) might release sufficient oxygen into the standing water and soil (Bharati et al. 2000), which makes paddy soil less reduced. The activity of CH_4-producing bacteria in paddy soil is inhibited by exposure to less reduced paddy soils (Conrad 2002). Conversely, increased DO oxidizes paddy soils and stimulates methanotrophic bacteria to consume CH_4 (Aulakh et al. 2001). A large proportion of the CH_4 produced may become oxidized beneath the plants due to the accumulation of CH_4 as a result of a decrease in the diffusion. Moreover, ebullition through bubbles is a common and significant mechanism of CH_4 transport, and less than 10% of CH_4 are diffused through ebullition (Aulakh et al. 2001). *Azolla* floats on the water's surface and forms a mass with a large percentage of cover on the water surface of paddy fields. This *Azolla* cover might serve as a physical barrier that prevents CH_4 transport through ebullition (Ma et al. 2012; Wang et al. 2015). The free-floating plants retard CH_4 transport through bubble ebullitive (Kosten et al. 2016). As we known, CH_4 emission is the net product of CH_4 production, oxidation, and transport in the rice soil-plant system (Conrad 2002). These effects are the benefits of mitigation of CH_4 emissions from paddy fields.

On the contrary, Ying et al. (2000) and Chen et al. (1995, 1997) reported that a dual cropping of *Azolla* greatly increases CH_4 emissions from paddy fields. Ying et al. (2000) found a significant decrease in DO concentration in surface water and an increase in NH_4^+-N content in paddy soil due to the presence of *Azolla*, which enhanced CH_4 production and inhibited CH_4 oxidation, thereby leading to an increase in CH_4 emissions. Otherwise, the exudation of *Azolla* root and the decomposition of dead *Azolla* could offer abundant substrates for methanogens and hence CH_4 production (Chen et al. 1997). In addition, Ying et al. (2000) reported that *Azolla* mediates CH_4 transport from the floodwater of a rice soil into the atmosphere just as rice plants did.

The benefits of the rice + *Azolla* cropping system with respect to decreasing N fertilizer application and mitigating CH_4 emissions are unknown in double rice cropping systems. The current experiment will provide unique insights regarding the rice + *Azolla* farming system in double rice cropping systems in southern China. The objectives of the study were to (1) estimate the effect of a dual cropping of *Azolla* along with double rice on CH_4 emissions from double rice cropped fields in southern China and (2) to clarify the mechanism underlying the impacts of *Azolla* on CH_4 emission.

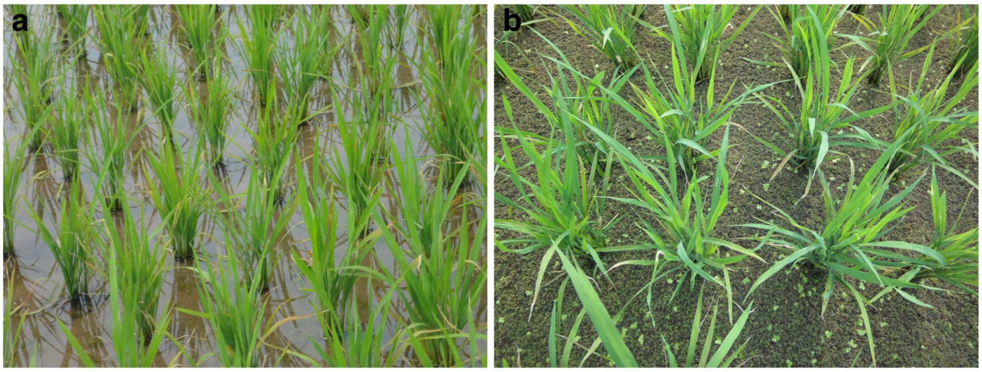

Fig. 1 Planting patterns of **a** the conventional rice cultivation system and **b** the rice + *Azolla* cropping system

2 Materials and methods

2.1 Site description

A consecutive 3-year field experiment was conducted at the experimental farm of the Soil and Fertilizer Institute of Hunan Academy of Agricultural Sciences from 2012 to 2014. This farm is located in Wanyu (29° 34′ N latitude, 112° 49′ E longitude), Huarong County, Hunan Province, southern China. The region belongs to the *Dongting Lake* Plain, which has a typical subtropical monsoon climate with an average temperature of 16–18 °C, a total rainfall of 1200–1700 mm, 262 frost-free days, and up to 1516.8 h of annual sunshine. The soil is purple calcareous clayey paddy soil developed from lake deposits. The initial properties of the paddy soil (0–20-cm depth) were sand 28.5%, silt 56.8%, clay 16.7%, bulk density 1.27 g cm^{-3}, organic carbon content 29.2 g kg^{-1}, pH (H_2O) 7.7, total nitrogen 2.95 g kg^{-1}, available phosphorus 16.4 mg kg^{-1}, and exchangeable potassium 69 mg kg^{-1}.

2.2 Experimental setup and field-management practices

The experiment consisted of four treatments: the conventional rice cultivation without N fertilizer, the conventional rice cultivation with common N fertilizer (200 kg N ha^{-1}), the rice + *Azolla* without N fertilizer, and the rice + *Azolla* with moderate N fertilizer (100 kg N ha^{-1}). The common N fertilizer application at a typical rate of 200 kg N ha^{-1} per season was in line with the local application rate. The treatments were arranged in a completely randomized block design with three replications. Twelve plots of 30 m^2 (5 × 6 m) per individual plot were used.

N fertilizer (urea) was applied in two split doses: 70% as basal fertilizer and 30% as topdressing approximately 25 days after rice seedling transplanting. A total of 85 kg P_2O_5 ha^{-1} (calcium super-phosphate) was applied in two split doses for all plots: 75 kg ha^{-1} as basal fertilizer and 10 kg ha^{-1} as topdressing 1 day after *Azolla* inoculation. A total of 100 kg K_2O ha^{-1} (potassium chloride) was used as basal fertilizer. Basic fertilizers were applied 1 day before rice transplanting. In accordance with the local water regime, flooding was initiated 3–4 days prior to rice seedling transplanting and maintained for approximately 30 days until midseason drainage to aerate the paddy soils.

2.3 Crop management

The double rice cropping system is widely practiced in southern China, especially in the rice-producing area of the middle and lower reaches of the Yangtze River. In the current study, the typical rice-rice-fallow farming system was adopted. The 30-day-old early rice seedlings (*cv. T-Liangyou 705*) were transplanted in late April and harvested in middle July, and 30-day-old late rice seedlings (*cv. Yueyou 9113*) were transplanted in middle or late July and harvested in late October.

The fresh *Azolla caroliniana* was used, with 2.23–2.84% total carbon and 0.22–0.29% nitrogen, and broadcast into the floodwater surface at a rate of 1000 kg ha^{-1} 7 days after early and late rice transplanting. Initially, *Azolla* was inoculated into *Azolla*-treated plots with a percentage of 12% surface cover of flooding water. A 5- to 7-cm depth of standing water was maintained to facilitate *Azolla* growth before midseason drainage. *Azolla* grew rapidly and held a biomass up to 6802 ± 243 kg ha^{-1} with greater than 79–88% of flooding water surface less than 14 days after inoculation during the early rice seasons of 2012–2014. In the late rice season, the *Azolla* grew slowly due to high air temperature and the fresh biomass of all the *Azolla*-treated plots was increased to 6293 ± 356 kg ha^{-1} with approximately 73–84% coverage on the surface water until 20 days after inoculation. Thereafter, rice fields were waterlogged and a water regime of alternating wet-drought cycles was adopted until 7 days prior to rice harvest. Notably, most of the *Azolla* died off due to drought during the midseason drainage period, but a small amount of *Azolla* survived. At the end of the midseason drainage when paddy soils were waterlogged again, *Azolla* floated on the floodwater, grew well, and reproduced rapidly.

2.4 CH_4 flux measurements

CH_4 flux was determined using a closed chamber/gas chromatography method (Zou et al. 2005). The closed static chambers (50 × 50 × 100 cm) were made of transparent Perspex. Removable wooden boardwalks (2 m in length) were installed before gas sampling to avoid disturbing the paddy soil in all of the plots. Four rice hills were covered to measure CH_4. Sampling events were conducted in the morning (9:00–11:00) on every sampling day. A battery-operated fan was mounted on the top of the chamber to mix the interior air. Gas samples were then drawn off with a syringe with a 25-ml volume at 10-min intervals (i.e., 0, 10, 20, and 30 min after closure) and immediately transferred into 18-ml vacuumed vials. After each sampling event, the chambers were removed from their bases. The air temperature inside the chamber was recorded using a manual thermocouple thermometer (JM624, Tianjin Instrument Co. Ltd., Tianjin, China). On average, CH_4 flux measurements were recorded every 3–5 days during the early and late rice seasons. During the early stage (from shortly after transplanting to the midseason drainage) of the early and late rice seasons, the sampling event was more frequent than the late stage, and the sampling event was stopped 3–7 days prior to harvest.

We measured CH_4 concentration using a modified gas chromatograph (Agilent 7890A, CA, USA) equipped with a flame-ionization detector (FID) and a Porapak Q column (6 ft. long, 1/8 in. outer diameter, 80/100 mesh size, stainless steel column). The temperatures of the injector, column, and detector were maintained at 150, 50, and 230 °C, respectively.

2.5 Auxiliary measurements

The dissolved oxygen concentration at the soil-flood water and soil redox potential were measured at the same time as CH4 emissions were monitored. In the duration of midseason drainage, the standing water was withdrawn, and therefore, the DO measurements were paused. The dissolved oxygen concentration at the soil-flood water interface was determined using a portable dissolution oxygen meter (HI9143, HANNA Instrument, Italy). Soil redox potential (E_h) was measured by portable E_h meter (Chuan-Di Instrument & Equipment Co., Ltd., Nanjing, China), using the depolarization method. The platinum electrode was inserted in the soil at known depths at 10 cm. The rice grain yields were determined at harvest via oven drying to a constant weight at approximately 75 °C.

2.6 Data analyses

Fluxes of CH_4 (F) were computed based on the change in concentration (Δc) over a period of time (Δt). Sample sets were rejected unless they yielded an R^2 value of greater than 0.90 (Mosier et al. 2006). Cumulative CH_4 emissions over the entire rice season were estimated via the trapezoidal integration of the mean flux over time (Mosier et al. 2006)

$$F = \rho \times \left(V/A\right) \times \left(\Delta c/\Delta t\right) \times 273/(273+T) \qquad (1)$$

$$R_{CH_4} = \sum_{i=1}^{n} (Fi \times Di \times 24) \qquad (2)$$

where V is the volume of the static chamber above the enclosed soil with surface area (A), ρ is the density of CH_4, T is the mean temperature (°C), R_{CH4} is the cumulative CH_4 emission per rice season, Fi is the average of two adjacent intervals of the measurements, Di is the interval in days of the adjacent two sampling dates, and 24 are the hours in 1 day.

The collected data were analyzed using the PROC ANOVA procedure in SAS version 9.3 (SAS Institute Inc., Cary, NC, USA). Multiple comparisons among treatment means were conducted using the least significant difference (LSD) test depending on the number of treatment means compared. Tests of the significance among treatments were based on a probability level of 5%. A linear regression analysis was used to clarify the correlations between the N fertilizer application and cumulative seasonal CH_4 emissions. SigmaPlot version 12.5 (Systat Software Inc. San Jose, CA, USA) was employed for figure preparation.

3 Results and discussion

3.1 CH_4 emissions

Across 3-year measurements, the CH_4 effluxes ranged respectively from −1.96 to 47.70 mg m^{-2} h^{-1} for early rice and from −2.50 to 93.45 mg m^{-2} h^{-1} for late rice (Fig. 2a). Similar patterns of CH_4 emissions for all treatments were observed during the early and late rice growing seasons. In the current study, two major peaks of CH_4 emission were observed for all treatments in the rice seasons of 2012, 2013, and 2014 (Fig. 2a). The first major CH_4 emission peaks during the early and late rice seasons occurred in late May and early August when rice plants were at the tillering stage, when the paddy soil redox potential declined to very low value (Fig. 2c) due to consumption of the oxygen in the standing water (Fig. 2d). In addition, the CH_4 emission peaks could be associated with the increased availability of organic matter derived from the microbial decomposition of leftover plant residues (Conrad 2002; Hussain et al. 2015). In the duration of midseason drainage, CH_4 effluxes declined to approximately zero and even below (Fig. 2a). The reason for the low CH_4 effluxes could be associated with higher soil redox potential. During the midseason drainage, the standing water was withdrawn and paddy soil was aerated and thereby the soil redox status was improved, reflecting the higher soil redox potential. High soil redox potential could inhibit CH_4 production and contribute to CH_4 oxidation and hence lower CH_4 emission rate (Malyan et al. 2016). Other minor CH_4 emission peaks were observed, respectively, in late June and early or middle September during the early and late rice growing seasons when rice plants were in their flowering stages. Besides low soil redox potential (Fig. 2c) and dissolved oxygen concentration in the standing water (Fig. 2d), the minor CH_4 peaks might be ascribed to the increased substrates from the root exudates from vigorous roots of developed rice plants.

In response to the temporal change in CH_4 effluxes, the pattern of the cumulative CH_4 emissions per rice crop season was divided into three distinct phases: the rapid increase from transplanting to midseason drainage, the near-zero growth phase during the midseason drainage, and the slow increasing phase from the end of midseason drainage to rice maturity (Fig. 2b). Over the early and late rice growing seasons, the CH_4 emissions varied from 108.3 to 212.4 kg ha^{-1} and from 164.3 to 345.9 kg ha^{-1}, respectively (Fig. 2b). These values were within the magnitude range of 3–2050 kg ha^{-1} estimated by Cai et al. (2000) in major Chinese rice fields.

Across all treatments, the maximum cumulative CH_4 emission was observed under the conventional rice cropping without

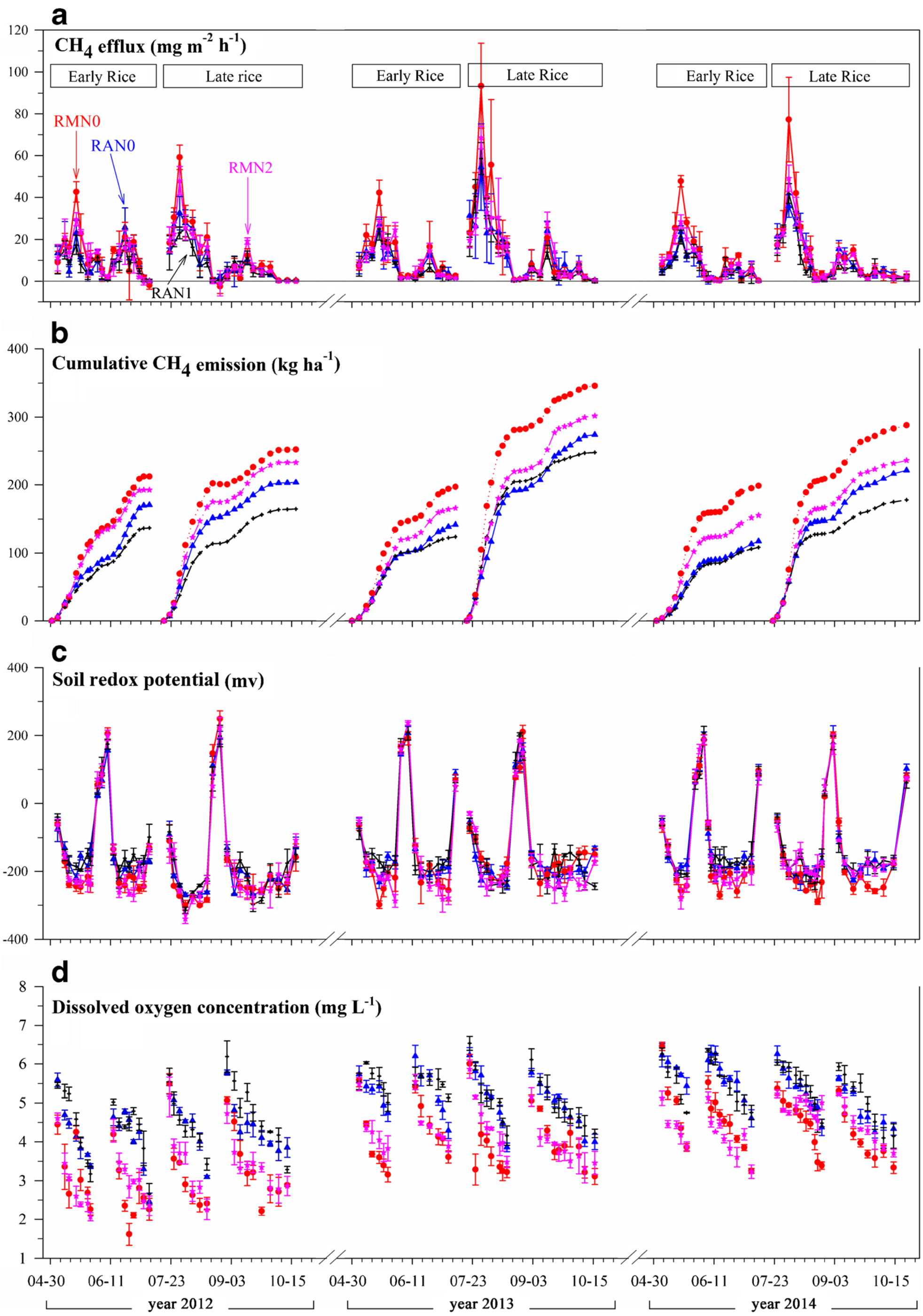

Fig. 2 Seasonal variations in CH_4 fluxes (**a**), cumulative CH_4 emissions (**b**), soil redox potential (**c**), and dissolved oxygen concentration in surface water (**d**) during the early and late rice growing seasons from 2012 to 2014. RAN0 represents the rice + *Azolla* without N fertilizer, RMN0 represents the conventional rice without N fertilizer, RAN1 represents the rice + *Azolla* with moderate N fertilizer at 100 kg ha^{-1} N, and RMN2 represents the conventional rice with common N fertilizer at 200 kg ha^{-1} N. The data shown in the panel are averages of the three replicates for individual treatment. *Vertical bars* represent the standard errors of the three replicates

N fertilizer, whose CH_4 emissions were 196.8–212.4 and 252.3–345.9 kg ha^{-1} over the early and late rice seasons, respectively (Fig. 2b). In comparison with the conventional rice cropping without N fertilizer, the rice + *Azolla* without N fertilizer decreased drastically CH_4 emissions by 19.8–40.9 and 19.3–23.0% during the early and late rice seasons, respectively (Fig. 2b). The results were supported by a previous study (Ma et al. 2012). Ma et al. (2012) found that a 20.4% decrease in CH_4 emission associated with the presence of *Azolla* compared with the plots without *Azolla* in subtropical paddy fields in Fuzhou Plain, southern China. The low CH_4 flux from the plots with *Azolla* could be a combination of higher dissolved oxygen content in the standing water and soil redox potential. In the current experiment, compared with the conventional rice without N fertilizer, the rice + *Azolla* without N fertilizer markedly enhanced the dissolved oxygen at the soil-water interface by 30.0–42.8 and 24.1–44.8% in the early and late rice seasons, respectively (Fig. 2d). The leaching of oxygenated water by percolation in flooded paddy soil would inhibit methane-oxidizing bacteria through improvement of soil redox status. In this study, the rice + *Azolla* without N fertilizer enhanced the soil redox potential by 14.5–19.8 and 12.7–19.4% during the early and late rice growing seasons, respectively (Fig. 2c). Earlier study demonstrated that *Azolla* with the high photosynthesis capacity could release abundant oxygen into the standing water (Wagner 1997), and hence increased dissolved oxygen concentration. Similarly, previous studies reported that growing *Azolla* increased dissolved oxygen concentration in the standing water and improved the soil redox status (Bharati et al. 2000; Ma et al. 2012). High dissolved oxygen in the standing water might retard CH_4 emission from rice field by promoting CH_4 oxidation at the soil-water interface (Kosten et al. 2016; Conrad 2002). We found that CH_4 efflux was negatively correlated with dissolved oxygen concentration in standing water ($r = -0.653$, $P < 0.001$; Fig. 3a). Additionally, a strong inverse relationship between CH_4 efflux and soil redox potential was observed in this study ($r = -0.712$, $P < 0.0001$; Fig. 3b). Further reduction in CH_4 emission was observed under the rice + *Azolla* with moderate N fertilizer at 100 kg N ha^{-1}. The rice + *Azolla* with moderate N fertilizer application inhibited CH_4 emissions by 25.2–30.2 and 17.8–29.5% during the early and late rice, respectively, compared with the conventional rice with common N fertilizer (Fig. 2b). The rice + *Azolla* with N fertilizer at 100 kg N ha^{-1} slightly increased the dissolved oxygen concentration in the standing water (Fig. 2d) and soil redox potential (Fig. 2c). In addition, the *Azolla* in conjunction with urea N might have stronger capacity to oxidize methane compared with *Azolla* only (Prasanna et al. 2002). In the current study, N fertilizer application decreased CH_4 emissions from double rice cropping paddies. The conventional rice with common N fertilizer decreased CH_4 emissions by 9.4–21.8 and 7.6–17.9% during early and late rice seasons relative to the conventional rice without N fertilizer, which aligns with the findings of previous studies (Xie et al. 2009; Yao et al. 2012; Zou et al. 2005). In contrast, a pot experiment conducted by Ying et al. (2000) found that the pots with *Azolla* decreased remarkably the dissolved oxygen concentration in standing water relative to the pots without *Azolla*. Such discrepancy might be the limit of pot experiment.

3.2 Rice yields and yield-scaled CH_4 emissions

Across the 3-year measurements, grain yields of early rice ranged from 3.4 to 6.0 Mg ha^{-1}, whereas those of late rice ranged from 5.3 to 8.4 Mg ha^{-1} (Fig. 4). In the present study, the dual cropping of *Azolla* positively affected the rice yields of both seasons. The rice + *Azolla* without N fertilizer increased rice yields by 2.3–12.7 and 3.0–4.6% for early and late rice, respectively, compared with the conventional rice without N fertilizer across the 3-year experiment (Fig. 4). Except for the early rice yield in 2012, no significant difference was observed between these conditions (Fig. 4). This might be N supply by *Azolla* through the biological N fixation. The daily rate of N fixation was estimated as 0.6–0.7 kg ha^{-1} (Cissé and Vlek 2003b), and thereby, the biological N fixation amounted to 45 kg N ha^{-1} each season in the current experiment. Earlier studies have demonstrated that dual cropping of *Azolla* increases rice yields (Bharati et al. 2000; Fosu-Mensah et al. 2015). As reviewed by Kollah et al. (2016), N supply due to *Azolla* through biological nitrogen fixation might be associated with the increase in grain yields. The rice + *Azolla* with moderate N fertilizer had a positive and significant influence on grain yields. Over the 3-year measurement, the rice + *Azolla* with moderate N fertilizer increased significantly grain yields by 21.1–35.3% compared with the conventional rice without N fertilizer (Fig. 4). As expected, N fertilizer application stimulated rice growth and enhanced rice yields. Based on the annual rice cycle, over the 3-year average, the grain yield of the rice + *Azolla* with moderate N fertilizer totalled 12.7 ± 0.9 Mg ha^{-1}, which was only 8.6% lower than that of the conventional rice with common N fertilizer (Table 1). The conventional rice cultivation with common N fertilizer led to

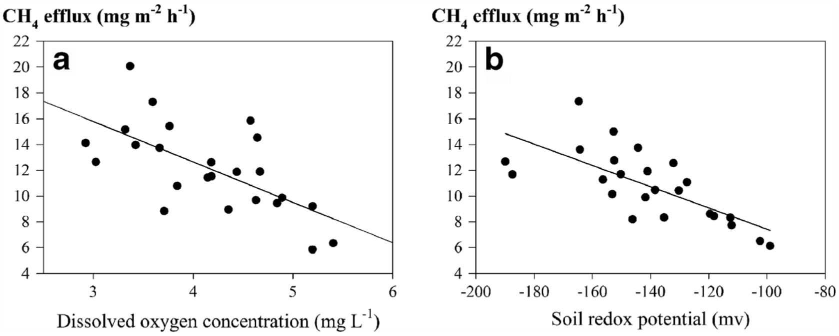

Fig. 3 Correlation between the average CH_4 efflux and corresponding dissolved oxygen concentration (**a**) and soil redox potential (**b**). RAN0 represents the rice + *Azolla* without N fertilizer, RMN0 represents the conventional rice without N fertilizer, RAN1 represents the rice + *Azolla* with moderate N fertilizer at 100 kg ha^{-1} N, and RMN2 represents the conventional rice with common N fertilizer at 200 kg ha^{-1} N. The correlation shows a tendency for the seasonal CH_4 emissions to decrease with the either increase in dissolved oxygen concentration or soil redox potential. For dissolved oxygen concentration, $y = -3.13x + 25.19$ ($n = 24$, $r = -0.653$, $P < 0.001$); for soil redox potential, $y = -0.083x - 0.806$ ($n = 24$, $r = -0.712$, $P < 0.001$)

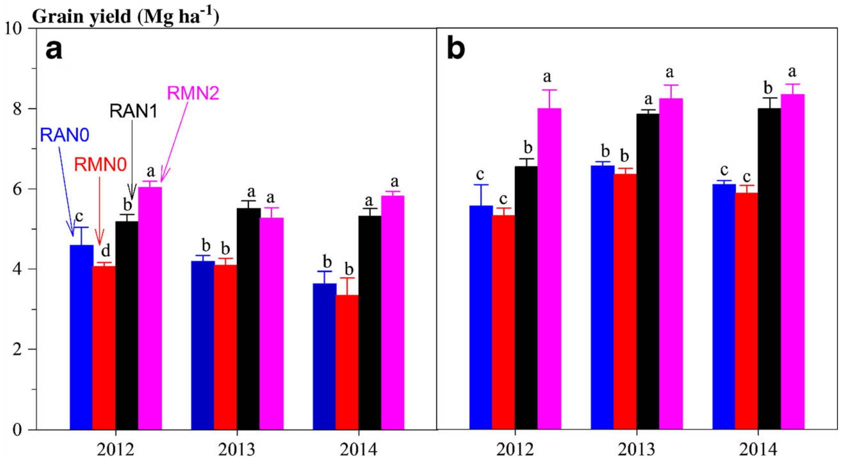

Fig. 4 Rice grain yields for early (**a**) and late (**b**) rice from 2012 to 2014. RAN0 represents the rice + *Azolla* without N fertilizer, RMN0 represents the conventional rice without N fertilizer, RAN1 represents the rice + *Azolla* with moderate N fertilizer at 100 kg N ha^{-1}, and RMN2 represents the conventional rice with common N fertilizer at 200 kg N ha^{-1}. The data shown in the panel are averages of the three replicates for individual treatment. *Vertical bars* represent the standard errors. *Different lowercase letters* represent the significant differences at $P < 0.05$

the highest rice grain yields, with 3-year averages of 5.7 ± 0.3 Mg ha^{-1} for early rice and 8.21 ± 0.4 Mg ha^{-1} for late rice (Fig. 4 and Table 1). The grain yields of the rice + *Azolla* with moderate N fertilizer were comparable to those of the conventional rice with common N fertilizer, decreased grain yields of early and late rice by 7.0 and 9.8%, respectively (Table 1). This might be *Azolla* N supply and the conservation of urea N via immobilization-remobilization in the presence of *Azolla* enhanced N availability for rice plants (Cissé and Vlek 2003a), and thereby increased rice growth. The results were interesting regarding the 50% reduction in synthetic N fertilizer. The application of N fertilizer enhanced rice grain yields in our study, as other works have reported (Zou et al. 2005). A significant increase was observed in grain yields when synthetic N was applied, regardless of the dual cropping of *Azolla*.

To better evaluate the rice + *Azolla* cropping system, it is important to consider options that have the potential to improve rice production and reduce CH_4 emission. Yield-scaled CH_4 emission was previously introduced to evaluate CH_4 emission and rice yields (Mosier et al. 2006). Based on the annual rice cycle, the lowest yield-scaled CH_4 emission was determined with regard to the rice + *Azolla* with moderate N fertilizer (25.2 ± 2.9 kg Mg^{-1} grain yield) and followed in ascending order by the conventional rice with common N fertilizer (30.8 ± 3.4 kg Mg^{-1} grain yield), the rice + *Azolla* without N fertilizer (37.2 ± 2.7 kg Mg^{-1} grain yield), and the conventional rice without N fertilizer (51.3 ± 3.6 kg Mg^{-1} grain yield) (Table 1). Similarly, Bharati et al. (2000) found that the lowest yield-scaled CH_4 emission (20.6 kg CH_4 Mg^{-1} grain yield) was associated with the plot with the *Azolla* dual crop, supporting our findings. However, the rice + *Azolla*

Table 1 The 3-year (from 2012 to 2014) average grain yields, CH_4 emissions, and yield-scaled CH_4 emissions influenced by *Azolla* as a dual crop combination with urea N

Treatment	Early rice		Late rice		Annual rice cycle[a]		
	Grain yield (Mg ha^{-1})	CH_4 emission (kg ha^{-1})	Grain yield (Mg ha^{-1})	CH_4 emission (kg ha^{-1})	Grain yield (Mg ha^{-1})	CH_4 emission (kg ha^{-1})	Yield-scaled CH_4 (kg Mg^{-1} grain yield)
RAN0	4.1 ± 0.5 b	143.1 ± 17.2 c	6.0 ± 0.5 c	233.1 ± 44.3 bc	10.2 ± 0.5 c	376.2 ± 40.1 c	37.2 ± 2.7 b
RAN1	5.3 ± 0.2 a	123.0 ± 11.1 d	7.5 ± 0.7 b	196.7 ± 47.5 c	12.7 ± 0.9 b	319.7 ± 42.0 d	25.2 ± 2.9 d
RMN0	3.8 ± 0.4 b	202.5 ± 26.4 a	5.9 ± 0.5 c	295.3 ± 54.0 a	9.7 ± 0.7 d	497.9 ± 44.2 a	51.3 ± 3.6 a
RMN2	5.7 ± 0.3 a	171.1 ± 19.7 b	8.2 ± 0.4 a	257.2 ± 45.8 ab	13.9 ± 0.4 a	428.3 ± 37.2 b	30.8 ± 3.4 c
$LSD_{0.05}$	0.381	18.622	0.508	46.137	0.628	39.324	3.065

Mean ± SD; different lowercase letters indicate the significant differences ($P < 0.05$) based on LSD multiple range tests. RAN0 represents the rice + *Azolla* without N fertilizer, RMN0 represents the conventional rice without N fertilizer, RAN1 represents the rice + *Azolla* with moderate N fertilizer, and RMN2 represents the conventional rice with common N fertilizer

[a] The CH_4 emissions during the winter fallow were near zero. Therefore, the annual emission is equivalent to the sum of the CH_4 emission from the early and late rice paddies

without N fertilizer increased the yield-scaled CH_4 emissions by 20.8% relative to the conventional rice with common N fertilizer (Table 1). In light of sustainable rice production, a dual cropping of *Azolla* alone is not expected to be adopted in double rice cropping systems in southern China. Annual grain yields of the rice + *Azolla* with moderate N fertilizer were up to 12.7 ± 0.9 Mg ha^{-1}, which was 8.6% lower than those of the conventional rice with common N fertilizer; however, the 25.4% decrease in CH_4 emissions is exciting. Above all, a combination dual cropping of *Azolla* with moderate N fertilizer had a slight decrease in rice yields versus the conventional rice with common N fertilizer; however, the effect was compensated by a substantial decrease in CH_4 emissions from paddy soils.

4 Conclusions

Azolla is applied as a dual crop combined with early and late rice in double rice cropping systems in southern China. The present experiment indicated that dual cropping of *Azolla* drastically decreases CH_4 emissions from double rice cropping paddies relative to the conventional rice cultivation system. Compared with the conventional rice with common N fertilizer, the rice + *Azolla* without N fertilizer and with moderate N fertilizer decreased CH_4 emissions by 11.5–24.5 and 25.2–30.2% during the early rice season and by 6.2–12.7 and 14.8–29.5% during the late rice season, respectively. The rice + *Azolla* with moderate N fertilizer decreased CH_4 emission per unit of grain yield. The interaction between the dual cropping of *Azolla* and N fertilizer application significantly influenced the maintenance of rice production while mitigating CH_4 emissions. Significant reductions in CH_4 emissions, considerable decreases in N fertilization, and comparable rice grain yields indicate that a dual cropping of *Azolla* combined with N fertilizer are possible ways to help both rice production and climate change.

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References

Aulakh MS, Wassmann R, Rennenberg H (2001) Methane emissions from rice fields—quantification, mechanisms, role of management, and mitigation options. Adv Agron 70:193–260. doi:10.1016/S0065-2113(01)70006-5

Bharati K, Mohanty SR, Singh DP, Rao VR, Adhya TK (2000) Influence of incorporation or dual cropping of *Azolla* on methane emission from a flooded alluvial soil planted to rice in eastern India. Agric Ecosyst Environ 79:73–83. doi:10.1016/S0167-8809(99)00148-6

Cai ZC, Tsuruta H, Minami K (2000) Methane emission from rice fields in China: measurements and influencing factors. J Geophys Res-Atmos 105:17231–17242. doi:10.1029/2000jd900014

Chen GX, Huang GH, Huang B, Wu J, Yu KW, Xu H, Xue XH (1995) CH_4 and N_2O emission from a rice field and effect of *Azolla* and fertilization on them. Chinese Journal of Applied Ecology 6:378–382. doi:10.13287/j.1001-9332.1995.0077 (in Chinese)

Chen GX, Huang GH, Huang B, Yu KW, Wu J, Xu H (1997) Nitrous oxide and methane emissions from soil–plant systems. Nutr Cycl Agroecosyst 49:41–45. doi:10.1023/A:1009758900629

Ciais P, Sabine C, Bala G, Bopp L, Brovkin V, Canadell J, Chhabra A, DeFries R, Galloway J, Heimann M, Jones C, Quéré CL, Myneni RB, Piao S, Thornton P (2013) Carbon and Other Biogeochemical Cycles. In: Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM (eds) Climate Change 2013: The Physical Science Basis Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge

Cissé M, Vlek PLG (2003a) Conservation of urea-N by immobilization-remobilization in a rice-*Azolla* intercrop. Plant Soil 250:95–104. doi:10.1023/A:1022878306316

Cissé M, Vlek PLG (2003b) Influence of urea on biological N_2 fixation and N transfer from *Azolla* intercropped with rice. Plant Soil 250:105–112. doi:10.1023/A:1022830423154

Conrad R (2002) Control of microbial methane production in wetland rice fields. Nutr Cycl Agroecosyst 64:59–69. doi:10.1023/A:1021178713988

FAO (2013) Food and Agriculture Organization statistical database. Agricultural data available @ http://faostat3.fao.org

Fosu-Mensah BY, Vlek PLG, Manske G, Mensah M (2015) The influence of *Azolla pinnata* on floodwater chemistry, grain yield and nitrogen uptake of Rice in Dano. Southwestern Burkina Faso J Agr Sci 7. doi:10.5539/jas.v7n8p118

Frolking S, Qiu J, Boles S, Xiao X, Liu J, Zhuang Y, Li C, Qin X (2002) Combining remote sensing and ground census data to develop new maps of the distribution of rice agriculture in China. Global Biogeochemical Cycles 16:38-31–38-10. doi:10.1029/2001gb001425

Hussain S, Peng S, Fahad S, Khaliq A, Huang J, Cui K, Nie L (2015) Rice management interventions to mitigate greenhouse gas emissions: a review. Environ Sci Pollut Res Int 22:3342–3360. doi:10.1007/s11356-014-3760-4

Kollah B, Patra AK, Mohanty SR (2016) Aquatic microphylla *Azolla*: a perspective paradigm for sustainable agriculture, environment and global climate change. Environ Sci Pollut R 23:4358–4369. doi:10.1007/s11356-015-5857-9

Kosten S, Pineiro M, de Goede E, de Klein J, Lamers LP, Ettwig K (2016) Fate of methane in aquatic systems dominated by free-floating plants. Water Res 104:200–207. doi:10.1016/j.watres.2016.07.054

Ma Y-Y, Tong C, Wang W-Q, Zeng C-S (2012) Effect of *Azolla* on CH_4 and N_2O emissions in Fuzhou Plain paddy fields. Chin J Eco-Agric 20:723–727. doi:10.3724/sp.j.1011.2012.00723 (in Chinese)

Malyan SK, Bhatia A, Kumar A, Gupta DK, Singh R, Kumar SS, Tomer R, Kumar O, Jain N (2016) Methane production, oxidation and mitigation: a mechanistic understanding and comprehensive evaluation of influencing factors. Sci Total Environ. doi:10.1016/j.scitotenv.2016.07.182

Montzka SA, Dlugokencky EJ, Butler JH (2011) Non-CO_2 greenhouse gases and climate change. Nature 476:43–50. doi:10.1038/nature10322

Mosier AR, Halvorson AD, Reule CA, Liu XJ (2006) Net global warming potential and greenhouse gas intensity in irrigated cropping systems in northeastern Colorado. J Environ Qual 35:1584–1598. doi:10.2134/jeq2005.0232

Myhre G, Shindell D, Bréon F-M, Collins W, Fuglestvedt J, Huang J, Koch D, Lamarque J-F, Lee D, Mendoza B, Nakajima T, Robock A, Stephens G, Takemura T, Zhang H (2013) Anthropogenic and natural radiative forcing. In: Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM (eds) Climate Change 2013: The Physical Science Basis Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge

Nguyen NV, Ferrero A (2006) Meeting the challenges of global rice production. Paddy & Water Environment 4:1–9. doi:10.1007/s10333-005-0031-5

Prasanna R, Kumar V, Kumar S, Yadav AK (2002) Methane production in rice soil is inhibited by cyanobacteria. Microbiol Res 157:1–6. doi:10.1078/0944-5013-00124

Shang QY, Yang XX, Gao CM, Wu PP, Liu JJ, Xu YC, Shen QR, Zou JW, Guo SW (2011) Net annual global warming potential and greenhouse gas intensity in Chinese double rice-cropping systems: a 3-year field measurement in long-term fertilizer experiments. Glob Chang Biol 17:2196–2210. doi:10.1111/j.1365-2486.2010.02374.x

Vlek PLG, Diakite MY, Mueller H (1995) The role of *Azolla* in curbing ammonia volatilization from flooded rice systems. Nutr Cycl Agroecosyst 42:165–174. doi:10.1007/BF00750511

Wagner GM (1997) *Azolla*: a review of its biology and utilization. Bot Rev 63:1–26. doi:10.1007/Bf02857915

Wang C, Li S, Lai DYF, Wang W, Ma Y (2015) The effect of floating vegetation on CH_4 and N_2O emissions from subtropical paddy fields in China. Paddy Water Environ 13:425–431. doi:10.1007/s10333-014-0459-6

Xie B, Zheng X, Zhou Z, Gu J, Zhu B, Chen X, Shi Y, Wang Y, Zhao Z, Liu C, Yao Z, Zhu J (2009) Effects of nitrogen fertilizer on CH_4 emission from rice fields: multi-site field observations. Plant Soil 326:393–401. doi:10.1007/s11104-009-0020-3

Yao ZS, Zheng XH, Dong HB, Wang R, Mei BL, Zhu JG (2012) A 3-year record of N_2O and CH_4 emissions from a sandy loam paddy during rice seasons as affected by different nitrogen application rates. Agric Ecosyst Environ 152:1–9. doi:10.1016/j.agee.2012.02.004

Ying Z, Boeckx P, Chen GX, Cleemput OV (2000) Influence of *Azolla* on CH_4 emission from rice fields. Nutr Cycl Agroecosyst 58:321–326. doi:10.1023/A:1009871308968

Zhang FS, Chen XP, Vitousek P (2013) Chinese agriculture: an experiment for the world. Nature 497:33–35. doi:10.1038/497033a

Zou JW, Huang Y, Jiang JY, Zheng XH, Sass RL (2005) A 3-year field measurement of methane and nitrous oxide emissions from rice paddies in China: effects of water regime, crop residue, and fertilizer application. Glob Biogeochem Cycles 19:9. doi:10.1029/2004gb002401