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# Effects of Dense Planting with Less Basal N Fertilization on Rice Yield, N Use Efficiency and Greenhouse Gas Emissions

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# Abstract

Rice cropping innovations for high yield with high N use efficiency (NUE) and low greenhouse gas (GHG) emissions are significant in ensuring food security and coping with climate change. The objective of this study was to investigate the comprehensive effects of dense planting with less basal N application (DR) on rice yield, NUE and GHG emissions. Field experiments were conducted at three sites in China: Shenyang, Danyang and Jinxian representing annual single rice cropping system, wheat-rice cropping system and double rice cropping system, respectively. Four planting densities with 25, 50, 75 and 100% higher each time correspondingly with about 25, 50, 75 and 100% less in basal N rate (i.e., DR1, DR2, DR3 and DR4 correspondingly) relative to traditional cropping for high yield (CK). Across three tested sites, the DR1 mode showed a large potential of NUE enhancement by 19.6% and GHG emissions mitigation by 12.2% at area- and yield-scaled with similar rice yield compared to the CK. However, further increase in planting density and decrease in basal N application caused a significant reduction in rice yield with a large increase in GHG emissions. Our results will provide important reference to rice cropping innovations for the integrated goals of food security, environmental health and climate change mitigation in China. © 2015 Friends Science Publishers

Keywords: Climate change; Food security; Resource use efficiency; Rice production; Planting density; Nitrogen fertilization

# Introduction

Rice (Oryza sativa L.) is the staple food of more than half of the world's population and this demand will increase by 8-10 million Mg per year over the next decade due to the expanding population (Seck et al., 2012). To increase rice yield will be the primary strategy for global food security due to the limited farmland area, environmental problem and climate change (Tilman et al., 2011). Increasing nitrogen (N) fertilizer input has contributed significantly to the improvement of rice yield (Cassman et al., 2003), but simultaneously resulted in serious environmental pollution due to low N use efficiency (NUE) by improper N fertilization (Chien et al., 2009). Meanwhile, rice crop represent an important source of global methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O) emissions (Zou et al., 2009; IPCC, 2013); greenhouse gas (GHG) emissions reduction from paddy field has gained increasing concerns throughout the world (Huang and Tang, 2010; Shang et al., 2011; Feng et al., 2013; Pittelkow et al., 2014). Thus, it is a great challenge for rice cropping to obtain high yield and NUE with low GHG emissions (Foley et al., 2011).

Many efforts have been made on rice cropping

innovation to increase grain yield and/or NUE (Dobermann et al., 2002; Surridge, 2004; Ladha et al., 2009; Alam et al., 2013). Evidences showed that panicle number is the key factor determining rice yield (Ottis and Talbert, 2005; Lee et al., 2010), suggesting an increase in panicle can increase rice yield (Zeng et al., 2012; Sui et al., 2013). Some experiments also demonstrated that increasing planting density to some extent could increase panicle number without adverse impact on other yield components (Latif et al., 2005; Hayashi et al., 2006; Lin et al., 2009). Thus, dense planting can be good approach to get further increases in rice yield (Ma et al., 2013; Zhao et al., 2013; Huang et al., 2013), especially for the mechanical rice transplanting, which will be dominant in rice cropping areas (CMA, 2011). Meanwhile, many improved N managements have been made to increase NUE for rice cropping systems (Dobermann et al., 2002; Peng et al., 2010; Rehman et al., 2013; Sui et al., 2013). For example, Peng et al. (2006) found that 30% reduction in N application during the early vegetative stage could double agronomic NUE without obvious reduction in rice yield compared to the farmers' practice in China. Moreover, Bhatia et al. (2012) suggested a 12.5% reduction in N application during early vegetative

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stage could increase rice yield. These studies clearly demonstrated a moderate reduction in basal N application rate rice cropping innovation for higher NUE (Peng *et al.*, 2010). Therefore, dense planting with less N application at early growing stage (e.g. basal N) may benefit rice crop for higher yield and NUE. To our knowledge, the impacts of dense planting with less basal N are not well documented on rice yield, NUE and GHG emissions (Thakur *et al.*, 2010; Zeng *et al.*, 2012; Ma *et al.*, 2013; Mahamud *et al.*, 2013; Chen *et al.*, 2014).

China is the largest country of rice consumption and production in the world, and Chinese average N rate for rice was 168 kg ha<sup>-1</sup> in 2010, about 69.0% higher than the world average (FAOSTAT, 2010; Heffer, 2013). Higher N fertilizer application result in low rice agronomic NUE in China less than 11 kg kg<sup>-1</sup> (Zhu and Jin, 2013). Meanwhile, Chinese carbon emission in total has climbed up to the highest of the world (IEA, 2009). It is very urgent and necessary to innovate in rice cropping practices for higher yield with higher NUE and lower GHG emissions in the country. Therefore, we conducted an integrated cropping experiment in three locations in major Chinese rice cropping areas: single rice cropping in Northeast China, wheat-rice cropping in middle-east China, and double rice cropping in South China. The impacts of dense planting with less basal N on rice yield, GHG emissions and N use efficiency were observed in the integrated experiment. Our objectives were to determine the rational rates of the increase in planting density and the decrease in basal N application for the integrated goals of rice cropping.

# **Materials and Methods**

# **Experimental Site and Designs**

**Experimental site:** Field experiments were carried out in 2012 at three sites representing the major Chinese rice cropping systems. The experimental locations included were Shenyang (42°05′N, 123°21′E), Liaoning province in Northeast China with an annual single rice cropping system; Danyang (31°59′N, 119°35′E), Jiangsu province in East China with a wheat-rice cropping system; and Jinxian (28°37′N, 116°26′E), Jiangsu province in South China with a double rice cropping system. The climate information and soil properties are listed in Table 1.

**Experimental design:** All field trials were arranged as a completely randomized design with three replications. For each experimental site, there were five planting densities and N application treatments, including the traditional cropping for high yield (CK) and four new cropping modes of dense planting with less basal N and same later top-dressing N amount (DR). Taking the planting density and basal N amount in the CK mode as a baseline, the four new cropping modes were about 25, 50, 75 and 100% higher in planting density correspondingly with about 25, 50, 75 and 100% less in basal N, respectively (i.e., DR1, DR2, DR3)

and DR4 correspondingly). The 25% increase in planting density and 25% decrease in basal N rate compared to CK are about 12 seedlings per m<sup>-2</sup> and 35 kg N ha<sup>-1</sup>, respectively. The detailed information about the planting density and N application of the five treatments are listed in Table 2. Mechanized planting have become dominate rice production in China (CMA, 2011), considering the row spacing of rice transplanter widely used in experimental site cannot be changed, dense planting was implied by increasing seedlings per hill. But dense planting of cv. Y Liangyou-302 was implied by narrowing row spacing due to its normal planting with one seedling per hill. In addition, the baseline planting density is very low in Shenyang compared to the Danyang and Jinxian, thus, a larger increase in planting density was used at Shenyang to avoid yield loss resulted by less basal N application. For all treatments, N fertilizer was applied at basal, tillering and panicle initiation stages. In the DR treatments, only basal N and top-dressing N at tillering was reduced as the designed decreasing rates based on the CK baseline, while later top-dressing N at panicle initiation stage was kept the same as for CK plot.

Two rice varieties were tested for each experimental site. The rice cultivars used in Danyang site were Y Liangyou 302 (D-v1, Indica hybrid rice) and Ningjing 3 (D-v2, conventional Japonica rice). In Jinxian site, the varieties were also Y Liangyou 302 (J-v1) and Ningjing 3 (J-v2). For Shenyang site, two conventional Japonica rice varieties were tested, containing Liaoxing 1 (S-v1) and Yanjing 48 (S-v2). The seedlings were manually transplanted. The fields were irrigated 3-5 days before rice transplanting, and then one drainage episodes was implemented at late tillering stage. Afterwards, the fields were intermittently flooded until about 1-2 weeks before harvest. In addition, nursery, insecticide and herbicide management followed the local practices conducted in CK mode.

## CH<sub>4</sub> and N<sub>2</sub>O Measurements

The CH<sub>4</sub> and N<sub>2</sub>O fluxes were simultaneously measured using a static, opaque chamber method (Zou et al., 2005). In each experimental plot, a plastic frame encompassing six hills of rice plants within an area of 0.25 m<sup>2</sup> was permanently inserted into the soil, chambers with a bottom area of 0.5 m  $\times$  0.5 m and a height of 0.5 or 1.0 m (according to plant height) were temporarily installed on the frame while sampling. The chamber was covered with sponge and retro-reflective sheet to minimize temperature changes inside the chamber during gas sampling, and equipped with fans to ensure sufficient mixing of air in chamber. In addition, wooden boardwalks were placed in the rice field to prevent soil disturbance. During each gas sampling event, four gas samples were collected between 09:00 and 11:00 h at 5 min intervals by 40-mL plastic syringes via a Teflon tube that was connected to a three-port valve and the chamber headspace. The air temperature inside the chamber was monitored

Site	Annual P <sup>a</sup> (mm)	Mean T <sup>b</sup> (°C)	Soil type <sup>c</sup>	Soil organic matter (g kg <sup>-1</sup> )	Total N (g kg <sup>-1</sup> )	Alkali-N (mg kg <sup>-1</sup> )	Available K (mg kg <sup>-1</sup> )	Olsen-P (mg kg <sup>-1</sup> )
Shenyang	786	7.4	Cambisols	26.7	1.50	148.7	135.6	15.9
Danyang	1112	16.0	Fluvisols	33.6	1.78	189.7	130.0	23.8
Jinxian	2059	18.0	Acrisols	20.5	1.42	110.0	103.5	11.2

 Table 1: Climate pattern and soil characteristics of three experimental sites

<sup>a</sup>mean values of annual precipitation during the experimental year

<sup>b</sup>mean values of daily mean temperature during the experimental year

classification of World Reference Base for Soil Resources 2006

Table 2: Rice varieties, planting density and nitrogen fertilization amount and timing for each treatment of each experimental site

Site	Variety	Treatment	Ν	fertilizatio	n rate and tir	ning (kg N ha <sup>-1</sup> )	Planting density			
	-		Total	Basal	Tillering	Panicle initiation	Seedling spacing (cm)	Seedling number (m <sup>-2</sup> )		
Shenyang	Liaoxing-1	СК	270	135	67.5	67.5	30×16.6	60.2		
	-	DR1	229.5	108	54	67.5	30×16.6	80.3		
		DR2	189	81	40.5	67.5	30×16.6	100.4		
		DR3	148.5	54	27	67.5	30×16.6	120.5		
		DR4	108	27	13.5	67.5	30×16.6	140.6		
	Yanjing-48	CK	270	135	67.5	67.5	30×16.6	60.2		
		DR1	229.5	108	54	67.5	30×16.6	80.3		
		DR2	189	81	40.5	67.5	30×16.6	100.4		
		DR3	148.5	54	27	67.5	30×16.6	120.5		
		DR4	108	27	13.5	67.5	30×16.6	140.6		
Danyang	Y Liangyou-302	CK	150	60	30	60	25×15	26.7		
		DR1	127.5	45	22.5	60	25×13.1	30.5		
		DR2	105	30	15	60	25×11.2	35.7		
		DR3	82.5	15	7.5	60	25×9.4	42.6		
		DR4	60	0	0	60	25×7.5	53.3		
	Ningjing-3	CK	210	84	42	84	25×15	53.3		
		DR1	178.5	63	31.5	84	25×15	66.7		
		DR2	147	42	21	84	25×15	80.0		
		DR3	115.5	21	10.5	84	25×15	93.3		
		DR4	84	0	0	84	25×15	106.7		
Jinxian	Y Liangyou-302	CK	180	90	54	36	25×15	26.7		
		DR1	144	67.5	40.5	36	25×13.1	30.5		
		DR2	108	45	27	36	25×11.2	35.7		
		DR3	72	22.5	13.5	36	25×9.4	42.6		
		DR4	36	0	0	36	25×7.5	53.3		
	Ningjing-3	CK	225	112.5	67.5	45	25×15	53.3		
		DR1	180	84.4	50.6	45	25×15	66.7		
		DR2	135	56.3	33.8	45	25×15	80.0		
		DR3	90	28.1	16.9	45	25×15	93.3		
		DR4	45	0	0	45	25×15	106.7		

CK: traditional cropping for high yield; DR1, DR2, DR3 and DR4: four new cropping modes of dense planting with less basal N application

during gas collection and calibrated for flux calculation. The frequency of flux measurements was generally every 7 days over the two growing seasons, except for drainage period closed to harvest.

Concentrations of CH<sub>4</sub> and N<sub>2</sub>O in gas samples were analyzed using a gas chromatography (Agilent 7890A, USA) equipped with a flame ionization detector (FID) and an electron capture detector (ECD). Nitrogen and a gas mixture of argon and methane (Ar-CH<sub>4</sub>) were used as the carrier gas for CH<sub>4</sub> and N<sub>2</sub>O, respectively. CH<sub>4</sub> and N<sub>2</sub>O fluxes were determined by linear regression of gas concentration versus chamber closure time and adjusted for area and volume of the chamber. Sample sets were rejected unless they yielded a linear regression value of  $r^2 > 0.90$ . Seasonal cumulative CH<sub>4</sub> and N<sub>2</sub>O emissions were sequentially accumulated from the emissions between every two adjacent intervals of the measurements (Zou *et al.*, 2005).

The global warming potential (GWP) was calculated as CO<sub>2</sub> equivalents for a time horizon of 100 years using the IPCC factors (GWP = CH<sub>4</sub> × 34 + N<sub>2</sub>O × 298, IPCC, 2013). Yield-scaled GWP was determined by dividing GWP by rice grain yield (Pittelkow *et al.*, 2013).

# **Plant Sampling and Analysis**

At harvest,  $4 \text{ m}^2$  areas in the middle of each plot were harvested to determine grain yield. All grain yields were adjusted to a moisture content of 14% fresh weight. At maturity, 10 hills were sampled for each plot to determine aboveground biomass, panicle number, spikelets per panicle, 1000-grain weight and filled grain rate. Nitrogen use efficiency was defined as partial factor productivity of applied N (PFP<sub>N</sub>, kg grain per kg N applied, kg kg<sup>-1</sup>).

# **Statistical Analysis**

Data were analyzed using SPSS 16.0 for the one-way and two-way analysis of variance (ANOVA) for grain yield, yield components, seasonal CH<sub>4</sub> and N<sub>2</sub>O emissions, GWP and yield-scaled GWP. The differences among treatments were examined based by least significant difference (LSD) test. Statistical significance was determined at the 5% level.

## Results

#### Grain Yield, Yield Components and N Use Efficiency

Although the difference in grain yield between the treatments varied with the tested varieties and locations, similar trend on yield of dense planting with less basal N was found (Fig. 1a). No significant difference in grain yield was found between the CK and the DR1, which correspondingly averaged 8.7 and 8.6 Mg ha<sup>-1</sup>, respectively. However, a decreasing trend for rice yields was found with the further increase in planting density and decrease in basal N dose. On an average of all varieties of the three sites, grain yields in DR2, DR3 and DR4 plots were 5.6, 5.8 and 11.0% lower than of CK plot, respectively. In contrast, the effect of cropping pattern and cropping mode × variety on grain yield was only significant at Jinxian (P<0.01) (Table 4).

Dense planting with less basal N application significantly enhanced PFP<sub>N</sub> for all varieties at each location (P<0.01) (Fig. 1b; Table 4). Across the varieties at three sites, PFP<sub>N</sub> in the DR1, DR2, DR3 and DR4 plots were 19.6, 40.0, 85.1 and 168.8% higher than of CK plot, respectively. Relative to the CK, DR treatments produced lower panicle number except for Y Liangyou-302 in Danyang where all DR plots had greater panicle number (Table 3). Overall, mean panicle number for DR1, DR2, DR3 and DR4 were 1.6, 2.2, 7.8 and 8.8% lower than of CK across all locations. The effects of cropping pattern on panicle number were significant at Shenyang and Jinxian (P<0.01) (Table 4), while the two-way interaction effects of cropping mode  $\times$ variety were significant at all locations (P<0.05) (Table 4). No significant difference was found in spikelet per panicle and 1000-grain weight between the treatments (Table 3 and 4). Dense planting with less basal N application significantly increased filled grains rate and total tiller number (P<0.05) but reduced aboveground biomass at all three locations (Table 3 and 4).

## Seasonal Patterns of CH<sub>4</sub> and N<sub>2</sub>O Fluxes

The temporal trends of  $CH_4$  fluxes were similar among the varieties but varied greatly among the locations (Fig. 2). At Shenyang and Jinxian sites,  $CH_4$  fluxes increased rapidly after transplanting with the maximum at about 25–50 days



**Fig. 1:** Grain yield (a) and NUE of partial factor productivity (PFP<sub>N</sub>) (b) of different varieties under different treatments at three locations. S-v1 and S-v2 are Liaoxing-1 and Yanjing-48 at Shenyang, D-v1 and D-v2 represent Y Liangyou-302 and Ningjing-3 at Danyang, and J-v1 and J-v2 denote Y Liangyou-302 and Ningjing-3 at Jinxian. Curves are the mean values of all varieties in three sites

after transplanting (DAT), and then dropped dramatically due to mid-season drainage (Fig. 2a, b, e and f). At Danyang site,  $CH_4$  fluxes were temporary increased after transplanting and then stable rate until the peak fluxes occurred at approximately 50 DAT (Fig. 2c and d).

Low  $N_2O$  fluxes occurred when field was flooded before mid-season drainage in all experimental plots (Fig. 3). However, distinct peaks in  $N_2O$  fluxes were observed after mid-season drainage. Afterwards, fluctuating patterns in  $N_2O$  fluxes were found until harvest due to intermittent flooding in rice field.

## CH<sub>4</sub> and N<sub>2</sub>O emissions

The differences in cumulative CH<sub>4</sub> emissions between CK and DR treatments varied with varieties and locations (Fig. 4a). In most of the experimental plots, DR1 or DR2 achieved the lowest CH4 emissions, which correspondingly averaged 6922.1 and 7536.0 kg  $CO_2$  eq ha<sup>-1</sup> compared with 7854.7 kg  $CO_2$  eq ha<sup>-1</sup> of CK across the three sites. Generally, there was no significant difference in CH<sub>4</sub> emission between CK and DR treatments for different varieties and sites (Table 4). Large spatial variance was found in the CH<sub>4</sub> emissions, the lowest CH<sub>4</sub> emission occurred at Shenyang, and the highest emissions at Danyang. Total N<sub>2</sub>O emissions significantly decreased with increase in planting density and decrease in basal N application (P<0.05) (Fig. 4b, Table 4). Mean N<sub>2</sub>O emissions for CK, DR1, DR2, DR3 and DR4 were averaged 337.3, 273.5, 227.1, 190.2 and 174.5 kg CO<sub>2</sub> eq ha<sup>-1</sup>, respectively.

## Area- and Yield-scaled GWP

Similar trends were found in the area- and yield-scaled GWP among the treatments (Fig. 4c and d). The lowest mean values of area- and yield-scaled GWP were found at the DR1, which were both 12.2% lower than those in the CK mode. However, the area- and yield-scaled GWP increased along with further increase in planting density and the

Site	Variety	Treatment	Panicle	Spikelet number	1000-grain	Filled grains	Aboveground	Total tiller	
	•		number (m <sup>-2</sup> )	(per panicle )	weight (g)	rate (%)	biomass (g m <sup>-2</sup> )	number (m <sup>-2</sup> )	
Shenyang	Liaoxing-1	CK	312.58 a	150.44 a	21.17 a	71.56 b	1848.34 a	382.72 a	
	·	DR1	292.58 ab	157.04 a	21.00 a	75.20 ab	1707.27 b	356.81 ab	
		DR2	312.30 a	147.69 a	21.82 a	75.48 ab	1652.06 b	371.54 a	
		DR3	267.66 b	156.74 a	21.37 a	77.77 ab	1587.40 b	319.85 bc	
		DR4	270.62 b	151.33 a	21.83 a	81.49 a	1621.57 b	315.12 c	
	Yanjing-48	CK	510.96 a	80.71 a	22.37 a	74.25 c	1741.75 a	673.09 a	
		DR1	470.13 ab	75.42 a	22.66 a	77.08 bc	1615.97 ab	599.87 b	
		DR2	436.74 b	81.27 a	23.37 a	79.70 abc	1546.12 b	524.78 c	
		DR3	434.49 b	82.93 a	22.97 a	84.35 ab	1611.19 b	492.70 c	
		DR4	426.01 b	70.29 b	23.41 a	86.60 a	1581.00 b	464.74 c	
Danyang	Y Liangyou-302	CK	220.00 b	215.60 a	23.73 a	83.39 b	1834.28 c	406.78 a	
		DR1	241.67 ab	219.90 a	23.63 a	88.05 ab	2147.39 abc	412.43 a	
		DR2	265.00 ab	221.05 a	24.32 a	91.28 a	2309.29 a	386.86 a	
		DR3	250.00 ab	224.95 a	24.29 a	89.49 ab	1883.21 bc	362.53 a	
		DR4	276.67 a	216.83 a	23.89 a	85.25 ab	2278.28 ab	373.18 a	
	Ningjing-3	CK	350.71 a	114.65 a	25.69 a	78.63 b	1828.80 a	485.33 a	
		DR1	358.79 a	119.02 a	25.24 a	77.49 b	1735.63 ab	483.78 a	
		DR2	336.16 a	110.02 a	25.14 a	90.85 a	1653.21 ab	415.33 ab	
		DR3	341.82 a	107.26 a	25.54 a	84.22 ab	1733.84 ab	419.22 ab	
		DR4	306.26 b	118.90 a	25.70 a	89.09 ab	1517.60 b	357.00 b	
Jinxian	Y Liangyou-302	CK	200.00 a	183.97 a	21.75 a	50.52 b	997.88 ab	364.41 a	
		DR1	195.83 ab	179.08 a	22.84 a	55.89 a	1000.93 a	338.02 a	
		DR2	186.95 ab	193.28 a	21.91 a	56.41 a	943.28 ab	332.21 ab	
		DR3	172.48 b	188.34 a	22.21 a	58.51 a	879.63 b	291.24 bc	
		DR4	182.22 ab	186.57 a	22.57 a	59.43 a	924.44 ab	275.56 c	
	Ningjing-3	CK	271.41 a	92.64 ab	25.57 a	76.46 b	1048.67 a	360.83 a	
		DR1	260.61 ab	97.12 a	24.43 a	79.42 ab	921.68 ab	333.22 ab	
		DR2	247.39 b	88.82 ab	25.87 a	80.82 ab	922.10 ab	309.94 ab	
		DR3	231.11 b	79.43 b	25.52 a	84.25 ab	806.28 bc	295.38 bc	
		DR4	197.17 c	89.02 ab	24.60 a	86.65 a	633.26 c	248.86 c	

 Table 3: Grain yield components, aboveground biomass and total tiller number of each rice variety under different treatments in three experimental sites

ANOVA analysis was conducted among different treatments in the same variety and location and values followed by different letters are significantly different at P<0.05

**Table 4:** ANOVA F values for the effects of cropping mode (M) and variety (V) and the interaction of cropping mode with variety on the yield components, aboveground biomass and total tiller number, grain yield, N use efficiency, seasonal greenhouse gas emissions and yield-scaled global warming potential (GWP) at three sites

	df	PN	SN	GW	FGR	AB	TTN	GY	PFP <sub>N</sub>	CH <sub>4</sub>	N <sub>2</sub> O	GWP	Yield-scaled GWP
Shenyang													
Μ	4	12.59**	1.61	$1.82^{**}$	8.15*	8.99**	20.35**	0.94	273.64**	0.29	4.32*	0.18	0.29
V	1	647.07**	1052.17**	32.15**	7.23**	6.44*	333.44**	13.68**	12.93**	9.09**	37.53**	12.91**	6.41*
M×V	4	3.53*	1.72	0.34	0.34	0.99	6.33**	0.50	0.69	0.34	2.38	0.44	0.31
Danyang													
М	4	0.34	0.09	0.26	3.04*	0.86	3.11*	2.16	121.43**	0.29	$6.68^{**}$	0.26	0.49
V	1	80.65**	391.63**	23.52**	2.86	32.12**	$5.78^{*}$	10.53**	181.55**	0.63	1.24	0.68	0.08
M×V	4	3.32*	0.49	0.45	1.43	4.23*	0.88	0.72	4.64**	0.55	2.68	0.50	0.36
Jinxian													
М	4	$8.78^{**}$	0.47	0.06	5.85**	5.18**	12.53**	22.21**	625.39**	0.96	7.62**	1.03	2.61
V	1	90.24**	839.62**	33.60**	347.79**	$4.74^{*}$	1.15	1.43	196.32**	2.41	0.30	2.32	2.78
M×V	4	3.10*	2.04	0.89	0.22	2.24	0.37	9.73**	63.31**	0.69	1.46	0.69	2.64

PN: panicle number; SN: spikelet number per panicle; GW: 1000-grain weight; FGR: filled grains rate; AB: aboveground biomass; TTN: total tiller number; GY: grain yield;  $PFP_N$ : partial factor productivity of applied N. The symbols \* and \*\* indicate significant effects at P<0.05 and P<0.01, respectively

decrease in basal N application, especially for the yieldscaled GWP. Substantial spatial variances were detected in the yield-scaled GWP. The lowest mean yield-scaled GWP occurred at Shenyang with 220.7 kg  $CO_2$  eq  $Mg^{-1}$ , followed by Danyang (1137.7 kg  $CO_2$  eq  $Mg^{-1}$ ) and Jinxian (1824.9 kg  $CO_2$  eq  $Mg^{-1}$ ) on an average for all varieties and treatments of each site (Fig. 4d).

## Discussion

The effects of planting density or N application rate on rice production and GHG emissions have been studied by numerous researchers. Most of these studies described the frequently yield losses caused by sparse planting (Tsujimoto *et al.*, 2009; Ye *et al.*, 2012; Huang *et al.*, 2013),



**Fig. 2:** Seasonal variation of CH<sub>4</sub> fluxes of each variety at three locations. (a) and (b) are Liaoxing-1 and Yanjing-48 at Shenyang, (c) and (d) represent Y Liangyou-302 and Ningjing-3 at Danyang, and (e) and (f) denote Y Liangyou-302 and Ningjing-3 at Jinxian. Error bars represent the standard error of three replicates



**Fig. 3:** Seasonal variation of  $N_2O$  fluxes of each variety at three locations. (a) and (b) are Liaoxing-1 and Yanjing-48 at Shenyang, (c) and (d) represent Y Liangyou-302 and Ningjing-3 at Danyang, and (e) and (f) denote Y Liangyou-302 and Ningjing-3 at Jinxian. Error bars represent the standard error of three replicates

environmental risk and low NUE induced by excessive basal N input (Peng *et al.*, 2010) in rice production of China. Present study explicitly emphasized the effect of planting density with less basal N application to develop cropping mode with at least high yield and NUE, or reduce area- and yield-scaled GHG emissions in rice field.

Although a moderate increase in planting density and basal N application didn't cause significant reduction in rice yield, further increase in planting density and decrease in basal N application induced a large decrease in rice yield (Fig. 1a). The main reason to the yield change was decline in yield component of panicle number (Table 3), than grain number and grain weight among the treatments (Table 3). Compared to the traditional cropping mode, our results demonstrated that the reduction in panicle number with 25% less basal N application might be compensated by 25% higher planting density. The later top-dressing N application amounts were the same with the CK mode, which might be enough for the rice plant demand during the later growing stages (Kamiji *et al.*, 2011; Sui *et al.*, 2013). Further decrease in basal N application might have significantly inhibited rice tillering (Zhong *et al.*, 2003), which couldn't be compensated by the increase in planting density.

Reduction in N application usually promotes NUE especially for  $PFP_N$  (Peng *et al.*, 2010). The results of present study also exhibited that  $PFP_N$  significantly increased as N rate reduced (Fig. 1b). However, excessive reducing basal N application could cause significant



**Fig. 4:** Total CH<sub>4</sub> (a) and N<sub>2</sub>O (b) emissions, area-scaled global warming potential (GWP) (c) and yield-scaled GWP (d) of each variety at three locations. S-v1 and S-v2 are Liaoxing-1 and Yanjing-48 at Shenyang, D-v1 and D-v2 represent Y Liangyou-302 and Ningjing-3 at Danyang, and J-v1 and J-v2 denote Y Liangyou-302 and Ningjing-3 at Jinxian. Curves are the mean values of all varieties in three sites

decrease in yield, though dense planting could compensate the negative impacts partially (Fig. 1a). Thus, there was a trade-off between the effects of N application and planting density on rice yield and NUE. Generally, sparse planting density with high N application exist in Chinese major rice cropping areas (Peng et al., 2002; Huang et al., 2013), resulting in relative low NUE. In order to increase NUE, less N application was recommended to the rice farmer, which might induce yield loss. Recently, mechanical rice transplanting are widely applied in China (CMA, 2011), however, the planting density was not enough for high yield. Thus, mechanical transplanting with a high planting density was recommended to the rice farmer, but this occurred with great lodging risk due to high N application rate. Our results indicated that dense planting with less basal N application can be a good rice cropping technique for mechanical transplanting for the trade-off between rice yield and NUE.

Many efforts have been made on the effects of rice planting density on CH<sub>4</sub> emission (Chareonsilp *et al.*, 2000; Minamikawa *et al.*, 2005; Chen *et al.*, 2013), and the impacts of N application (Schimel, 2000; Cai *et al.*, 2007; Pittelkow *et al.*, 2013). However, very few studies reported about the integrated effects of planting density and N application, though dense planting is mostly recommended to be applied with a reduction in N application. About a 25% increase in planting density and a similar decrease in basal N application could decrease CH<sub>4</sub> emissions, however, further corresponding increase in density and decrease in N tended to increase CH<sub>4</sub> emissions of present study (Fig. 4a). Generally, dense planting with less basal N application might reduce CH<sub>4</sub> emission due to the lower aboveground biomass and total tiller number (Table 3) for CH<sub>4</sub> production and transport in comparison with the CK (Huang et al., 1997; Aulakh et al., 2000). On the other hand, less N application might suppress N availability for CH<sub>4</sub> oxidation (Bodelier et al., 2000; Krüger and Frenzel, 2003; Wang et al., 2003; Cai et al., 2007), which might stimulate CH4 emission. A moderate decrease less than 25% in N application might not cause limitation to CH4 oxidation (Cai et al., 2007). However, a large reduction in basal N application might cause significant limitation in soil N availability for CH4 oxidation during rice early growing and later stages (Bodelier et al., 2000). Thus, a moderate increase in planting density with less basal N application could make a trade-off between CH<sub>4</sub> production and oxidation, resulting in the lowest CH<sub>4</sub> emissions level among the treatments at the three tested sites. Recent findings from literature exhibits that N rates with optimal grain yield have no net effect on CH<sub>4</sub> emission due to the counter-balanced by the positive effect of N addition on CH<sub>4</sub> production and oxidation (Banger et al., 2012; Linquist et al., 2012; Feng et al., 2013; Pittelkow et al., 2014). That may be true in farmer's fields, but specifically as in present study for CK and DR1 with optimal yield, only basal and tillering N was decreased and the panicle N application was the same for both CK and DR1, which may results in inhibitory effect of reduced N application on CH<sub>4</sub> oxidation only occurred at tillering stage; in addition, the negative effect of reduced N application on CH4 oxidation may be modified by deficiency of oxygen for Methanotrophs due to continuous flooding at tillering stage (Banger et al., 2012). Therefore, the effect of less basal N input on CH<sub>4</sub> oxidation may be less significant than less N applied at all the stages. Previous studies mainly focus on the effect of total N rates on CH<sub>4</sub> emission in rice field, but the ratio of applied N at different crop stages is critical to rice grain yield (Peng *et al.*, 2010), future research need to detect the effect of N management with high yield on CH<sub>4</sub> emission. As planting density increased with reduced basal N application; dense planting could enhance plant N uptake (Wang *et al.*, 2003), which may cause the increase of N shortage of Methanotrophs, how planting density impact CH<sub>4</sub> oxidation might represent an area for future research.

The response of microbial processes of CH<sub>4</sub> emission in the rice soils and rice plant growth to N application was interacted by crop management and climate conditions (Ramakrishnan et al., 2001; Zou et al., 2005; Xie et al., 2010). This study covered three major Chinese rice cropping areas with different climate features, soil properties, rice varieties and previous crop managements. Moreover, the CH<sub>4</sub> flux pattern differed among locations; thus, the impacts of DR treatment on CH<sub>4</sub> emission in these zones could be different. The lowest CH<sub>4</sub> emissions occurred at DR1 or DR2 at Shenyang and Jinxian (Fig. 4a), CH<sub>4</sub> flux peak in these two sites all appeared at tillering stage (Fig. 2a, b, e and f), thus, the negative of reduced basal N application on CH<sub>4</sub> oxidation at tillering stage may play important role in CH<sub>4</sub> emission throughout the crop growth stage. According to Ding and Cai (2003), if the soil organic carbon is less than 20 g kg<sup>-1</sup>, N application can stimulate soil CH<sub>4</sub> production, the soil organic carbon were all less than 20 g kg<sup>-1</sup> in Shenyang and Jinxian (Table 1), thus, CK treatment could enhance CH<sub>4</sub> production and thereby CH<sub>4</sub> emissions compared to the DR treatments. In addition, there are many early rice residues incorporated into late rice fields at Jinxian, the higher basal N application may stimulate the decomposition of rice residues that enhance CH<sub>4</sub> production (Shang et al., 2011). At Danyang for Y Liangyou-302, DR treatments tend to reduce CH<sub>4</sub> emissions (Fig. 4a) due to DR treatments not only may suppress CH<sub>4</sub> oxidation by reduced N application but also produce higher aboveground biomass and total tiller number that stimulate CH4 production and transport. But for Ningjing-3, the effect of DR treatments on CH<sub>4</sub> emission (Fig. 4a) was similar to results found at other two locations.

As expected, N<sub>2</sub>O emissions increased along with increasing N rates (Fig. 4b). Previous studies have showed a non-linear response of N<sub>2</sub>O emissions to N rates due to N<sub>2</sub>O emissions increased abruptly at N rates with increase in plant N uptake capacity (Hoben *et al.*, 2011; Kim *et al.*, 2013; Pittelkow *et al.*, 2013). Contrastingly, our results demonstrated a linear response of N<sub>2</sub>O emissions to N rates, though dense planting could enhance plant N absorption (Wang *et al.*, 2003) that may further decrease soil N availability for N<sub>2</sub>O production under less basal N treatment. This is probably associated with the maximum N rates as for CK treatment in our experiments did not overtaken rice plant maximum N uptake capacity, and also reduced N only occurred at early stage with low N<sub>2</sub>O emissions due to continuous flooding irrigation, while the same N fertilizer was applied at panicle initiation stage following peak of N<sub>2</sub>O emission (Fig. 4b). The effect of N rates on N<sub>2</sub>O emission have been most studied, yet little reports about the interaction of rice varieties with the response of N2O emission to N rates, though the response of plant growth to N rates is much differed among varieties with different N absorption capability (Huang et al., 2008; Taylaran et al., 2009). In the present study, the response of  $N_2O$  emissions to N rates in Y Liangyou-302 was significantly higher than in Ningjing-3 at both Danyang and Jinxian (Fig. 4b), it may be attributed to the higher N use efficiency of Y Liangyou-302 resulting in much available soil N for microbial N<sub>2</sub>O production. At Shenyang, the difference in N<sub>2</sub>O emissions of Liaoxing-1 among different N rates was more obvious than Yanjing-48 (Fig. 4b), the latter is a variety with strong tillering capacity (Table 3) that could absorb much N even at relative high N rates (e.g. CK), resulting in low available soil N for N<sub>2</sub>O production.

Yield-scaled GWP is increasingly used to evaluate the integrated effects of cropping practices on GWP and food production (Liang et al., 2013; Pittelkow et al., 2014). For rice, yield-scaled GWP is mainly decided by CH<sub>4</sub> emission and rice yield, since CH<sub>4</sub> emission is the major contributor to GWP (Linquist et al., 2012; Feng et al., 2013; Pittelkow et al., 2013). It was reported that yield-scaled GWP was minimized at optimal N rates with maximum yield (Pittelkow et al., 2014). In the present study, DR1 with a moderate increase in planting density and less basal N application could reduce CH<sub>4</sub> emission with an unchanged rice yield as compared to the CK and other DR treatments, resulting in lowest yield-scaled GWP (Fig. 4d). Further increase in planting density and decrease in basal N application could neither reduce CH<sub>4</sub> emission nor increase rice yield, thus, their yield-scaled GWP were higher than DR1 and CK (Fig. 4d).

# Conclusion

Rice crop is facing multiple challenges of farmland shortage, resource scarcity and global warming. Consequently, a lot of efforts have been made to the innovation of rice cropping technique for high yield with high resource use efficiency and low greenhouse gas emission. Recently, mechanical rice cropping with dense planting begins to dominate in rice cropping systems, and less N application at rice early growing stage is recommended to increase N use efficiency for rice production. This study conducted in the major Chinese rice cropping areas, indicated that a moderate increase in planting density with less basal N application can significantly increase N use efficiency and decrease CH<sub>4</sub> emission with an unchanged or even increased rice yield. Further increase in planting density and decrease in basal N might decrease rice yield with a higher CH4 emission. Thus it may be concluded that rational dense planting with less N application at early stage can be a candidate of climate smart rice cropping to mitigate climate

change with increase in resource use efficiency and acceptable high crop yield.

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