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Straw incorporation influences soil organic carbon sequestration, greenhouse gas emission, and crop yields in a Chinese rice (*Oryza sativa* L.) –wheat (*Triticum aestivum* L.) cropping system



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ABSTRACT

Crop straw management plays important roles in sustainable agriculture and environmental protection. Straw incorporation has multiple influences on soil organic carbon (SOC) sequestration, greenhouse gas (GHG) emissions, and crop yields, but these influences have rarely been studied simultaneously in a single cropping system. This study was conducted to examine the influence of long-term straw incorporation on the SOC sequestration rate, methane (CH_4) and nitrous oxide (N_2O) emissions and crop yields in a Chinese rice (*Oryza sativa* L.) -wheat (Triticum aestivum L.) cropping system in Hydragric Anthrosols under a subtropical monsoon climate. Four straw incorporation treatments were applied: wheat straw incorporation only (WS), rice straw incorporation only (RS), both wheat and rice straw incorporation (WSRS), and no straw incorporation (as a control). The SOC sequestration rate was estimated from the changes in SOC stock in the topsoil (0-20 cm) from 2007 to 2016. The emissions of CH₄ and N₂O were measured every 7 d when possible using a static chamber method from the 2013 rice season to the 2016 wheat season. Our results showed that the straw incorporation treatments significantly influenced the seasonal CH₄ and N₂O emissions and rice yield but had no influence on wheat yield. Straw incorporation significantly increased the annual topsoil SOC sequestration rate by 0.24-0.43 t C ha⁻¹ yr⁻¹ and the annual CH₄ and N₂O emissions by 44–138 kg CH₄-C ha⁻¹ yr⁻¹ and 0.68–1.49 kg N₂O-N ha⁻¹ yr⁻¹, respectively. Relative to the RS treatment, the WS and WSRS treatments significantly increased annual CH₄ emissions by 38% and 61%, respectively. Relative to the RS treatment, the WSRS treatment significantly increased the annual N₂O emissions, by 35%. The average annual yields were significantly higher in the WSRS (16.8 t ha^{-1} yr⁻¹) and RS (16.7 t ha^{-1} yr⁻¹) treatments than in the WS (15.7 t ha^{-1} yr⁻¹) and control $(15.2 \text{ t ha}^{-1} \text{ yr}^{-1})$ treatments. Across the three rotation cycles, the annual net global warming potential and greenhouse gas intensity were similar between the control and RS treatments but were significantly lower in these treatments than in the WSRS and WS treatments. These findings suggest that the RS treatment can simultaneously increase crop yields and environmental sustainability in rice-wheat cropping systems.

1. Introduction

Global warming due to increasing atmospheric greenhouse gas (GHG, mainly carbon dioxide [CO₂], methane [CH₄] and nitrous oxide

 $[N_2O]$ concentrations is one of the most prominent challenges facing agricultural production and environmental sustainability (IPCC, 2013). It is estimated that worldwide agriculture contributed 5.1–6.1 Pg CO₂-eq in 2005, which accounts for 10–12% of the total global

Abbreviations: CH₄, methane; CO₂, carbon dioxide; GHG, greenhouse gas; GHGI, greenhouse gas intensity; NGWP, net global warming potential; N₂O, nitrous oxide; RS, rice straw incorporation only; SOC, soil organic carbon; WS, wheat straw incorporation only; WSRS, both wheat and rice straw incorporation

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anthropogenic emissions of GHGs (Smith et al., 2007). Many agricultural practices have been explored to mitigate net GHGs emissions by decreasing CH₄ and N₂O emissions and/or sequestering more atmospheric CO₂ into stable soil organic carbon (SOC) pools (Chen et al., 2018; Dash et al., 2017; Linquist et al., 2012). However, the net influence of agricultural practices such as straw incorporation on the emissions of different kinds of GHGs remain unclear.

Straw incorporation is a common agricultural practice in China for improving soil physical properties and nutrients. Straw incorporation can potentially increase SOC storage and mitigate climate change (Chen et al., 2017; Ghosh et al., 2018; Lal, 2004; Zhao et al., 2018). For example, Xia et al. (2014) reported that the annual topsoil SOC sequestration rate increased by 0.18-0.24 t C ha⁻¹ yr⁻¹ in a 22-yr straw incorporation experiment. Similarly, a 15-yr study showed a positive increase in the SOC sequestration rate (0.34 t C ha⁻¹ yr⁻¹) in response to rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.) straw incorporation (Chaudhary et al., 2017).

In straw incorporated fields, easily decomposable C from straw and anaerobic soil conditions are the most important drivers of CH₄ losses. Several field experiments have shown that straw incorporation significantly increases CH₄ emissions in rice fields (Bhattacharyya et al., 2012; Ma et al., 2009; Zhang et al., 2015a; Zou et al., 2005). However, Hou et al. (2013) demonstrated that rice straw applied before wheat sowing decreased CH₄ emissions by 14–43% during the subsequent rice season in a rice–wheat cropping system. Negative correlations between CH₄ emissions during the rice season and the rate of rapeseed straw incorporation have also been reported (Li et al., 2011). In addition, straw ditch burying, ditch mulching and strip mulching decreased CH₄ emissions by 11–32% relative to the even incorporation of straw (Hu et al., 2016; Ma et al., 2009), indicating that straw incorporation method influences the magnitude of CH₄ emissions.

The reported influence of straw incorporation on N_2O emissions varies among studies (Chen et al., 2013). Straw incorporation treatments have been reported to decrease soil N_2O emissions (Bhattacharyya et al., 2012; Ma et al., 2009; Yao et al., 2009; Zou et al., 2005), have no influence on N_2O emissions (Xia et al., 2014; Zhang et al., 2017), or stimulate N_2O emissions (Li et al., 2011). The influence of straw incorporation on soil N_2O emissions can vary with season and year (Hu et al., 2016; Ma et al., 2009; Zhang et al., 2015a). For example, Hu et al. (2016) reported that wheat straw incorporation significantly increased N_2O emissions during the second wheat season in a rice–wheat cropping system. Furthermore, ditch mulching and strip mulching significantly increased N_2O emissions by a factor of 1.4–5.1 relative to even incorporation of straw (Ma et al., 2009). The above observations indicate that soil N_2O emissions are influenced not only by the rate of straw incorporation but also by the incorporation method. Long-term field observations are needed to clarify the net responses of N_2O emission to straw incorporation.

Rice–wheat cropping systems are critical to food security in South Asia and China. As the dominant types of cropping system, rice–wheat cropping systems are employed in 60% of the rice fields in southeastern China (Frolking et al., 2002). In this region, straw incorporation in fields has been strongly encouraged in recent years, as this practice has potential advantages over straw burning, including the promotion of SOC storage, the improvement of crop yields and the reduction of air pollution (Huang et al., 2013). However, straw incorporation may increase the incidence of rice disease or aggravate seed germination for wheat production, thereby having no influence or a negative influence on crop yields (Singh and Sidhu, 2014; Singh et al., 2005; Villamil et al., 2015). Therefore, to advance our understanding of future sustainable agriculture, more information concerning long-term straw incorporation on GHG emissions and crop productivity is urgently needed.

We conducted a nine-year long-term straw incorporation experiment in a rice–wheat cropping system in China. SOC was measured during the entire experiment, and soil CH_4 and NO_2 were estimated in the last three years. Indicators of comprehensive estimates of global warming, net global warming potential (NGWP) and greenhouse gas intensity (GHGI) (Mosier et al., 2006; Shang et al., 2011) were calculated. We predicted that long-term straw incorporation would increase CH_4 and N_2O emissions. The objectives of this study were to determine the influence of different straw incorporation treatments on SOC sequestration, GHGs emissions, and crop yields.

2. Materials and methods

2.1. Experimental site

The experimental site is located at the Key Field Scientific Observation & Experiment Station of Suzhou Paddy Soil Eco-environment, Ministry of Agriculture and Rural Affairs, Suzhou City, Jiangsu Province, China (31°27′45′′N, 120°25′57′′E). The site has a typical subtropical monsoon climate. The soil is developed from loessial deposits, with a clay loam texture (299 g kg⁻¹ sand, 393 g kg⁻¹ silt, and 308 g kg⁻¹ clay), and is classified as Hydragric Anthrosols according to the FAO soil taxonomy system (FAO, 2015). The initial topsoil (0–20 cm) contained 17.0 g kg⁻¹ SOC, 1.4 g kg⁻¹ total N, 129.1 mg kg⁻¹ mineral N, 88.7 mg kg⁻¹ Olsen-P, and 128.8 mg kg⁻¹ NH₄OAc-K, with a pH (H₂O) of 6.1. The initial soil bulk density was 1.1 g cm⁻³. Annually, the region receives 1094 mm of precipitation and 3039 sunshine hours. The mean annual temperature is 15.7 °C, and the effective cumulative temperature (greater than 10 °C) is 4947 °C (Zhang et al., 2015a). The daily precipitation and mean air temperature during



Fig. 1. Daily precipitation and mean air temperature during the experimental seasons from 2013 to 2016.

the experiment were recorded from 2013 to 2016 (Fig. 1).

2.2. Experimental design

The experiment was initiated in June 2007. Four straw incorporation treatments were established in a completely randomized block design with three replicates (5 \times 6.5 m for each replicate plot). The treatments included wheat straw incorporation only (WS, incorporating 4.5 t ha⁻¹ wheat straw before rice transplanting), rice straw incorporation only (RS, incorporating 6 t ha⁻¹ rice straw before wheat sowing), both wheat and rice straw incorporation (WSRS, incorporating 4.5 t ha⁻¹ wheat straw before rice transplanting plus 6 t ha⁻¹ rice straw before wheat sowing), and no straw incorporation (as a control). The blocks and plots were separated by buffer zones with widths of 2 m and 1 m, respectively. The wheat and rice straw used in the current study were produced on the study site. All straw was cut into pieces approximately 0.1 m long. Straw was uniformly incorporated into each plot using a rotary tiller (1GQN-200, Taicang Zhongxin Machinery Manufacture Ltd., Jiangsu, China) before rice transplanting or wheat sowing. In the control plots, all wheat or rice straw was removed after harvest.

2.3. Crop management

The rice cultivar Suxianging 1 and the wheat cultivar Yangmai 14 were used in this study from 2013 to 2016. Rice seeds were sown in a nursery bed each May and then manually transplanted in June. The hill spacing was 13.3×23.3 cm, and there were 3 plants per hill. The rice plants were harvested in October (Table 1). Wheat seeds were sown at 150 kg ha⁻¹ (ca. 348 seeds m⁻²) in rows 20 cm apart in November and harvested in the following May. The planting density of wheat was controlled at 270 plants m⁻² at the 4-leaf stage. Consistent with local high-yielding agricultural practices (Zhang et al., 2018), all experimental plots were managed using a flooding-drainage-intermittent irrigation water management system during the rice season (Table 1). After the rice was transplanted, all plots were submerged with water to a depth of approximately 3–5 cm for approximately 35 d. The rice fields were then drained, and no ponding water remained; subsequently, a midseason drainage was imposed for approximately 10 d. The experimental plots were then reflooded followed by intermittent irrigation until approximately 10 d before rice harvest. No plots were irrigated during the wheat season. All treatments received the same N, phosphorus and potassium fertilizers at the recommended rate of 405 kg N ha^{-1} yr⁻¹ in the form of urea, 150 kg $P_2O_5 ha^{-1}$ yr⁻¹ in the form of calcium superphosphate and 300 kg K_2O ha⁻¹ yr⁻¹ in the form of potassium chloride (Table 1). Pesticide, disease and herbicide

Table 1

Main cultivation and management stages during three annual cycles from the 2013 rice season to the 2016 wheat season.

2.4. Methane and nitrous oxide sampling and measurements

The field measurements of CH₄ and N₂O emissions were conducted from the 2013 rice season to the 2016 wheat season. Methane and N₂O were simultaneously measured in situ using the static chamber method (Cai et al., 1997). Three polyvinyl chloride frames were inserted into each replicate plot for the gas measurements. The top edge of the frame had a groove filled with water, which sealed the rim of the sampling chamber. The chamber (0.5 m long; 0.5 m wide; 1.2 m high) was equipped with two 12-V fans for air mixing and was wrapped with a layer of sponge and aluminum foil to minimize the air temperature variability inside the chamber during sampling. The gas samples were collected from 8:00 to 10:00 a.m. every 7 d in most cases; there was one 20 d interval in winter. For each measurement, four gas samples were collected at 10-min intervals during the 30-min sampling process. Using a plastic syringe, 50-mL gas samples were collected from the chambers; the samples were then intermittently transferred to 100-mL pre-evacuated gasbags (LB-301-0.1, Dalian Delin Gas Packing Co., Ltd, Liaoning, China). The samples were transported to the laboratory for analysis within 24 h.

The gas samples were simultaneously analyzed for CH₄ using a gas chromatograph (7890A, Agilent Technologies, Santa Clara, CA, USA) equipped with a flame ionization detector and for N₂O using a ⁶³Ni electron capture detector. The oven and two detectors were operated at 60 °C and 300 °C, respectively. Nitrogen and argon – CH₄ (95:5) mixtures were used as carrier gases for CH₄ and N₂O, respectively. The CH₄ and N₂O fluxes were calculated from the linear increases in the CH₄ and N₂O concentrations over time ($r^2 > 0.90$, n = 4) as described previously (Zhang et al., 2015b).

2.5. Soil sampling and analysis

Soil bulk density (0–20 cm) was calculated as the average soil bulk density of the 0–5-, 5–10-, 10–15-, and 15–20-cm layers, which was measured from five randomly distributed, undisturbed soil samples from each plot. The soil samples were collected using a 100 cm^3 metallic cylinder at wheat maturity from 2013 to 2016. Composite topsoil samples (0–20 cm) were collected with five replications per plot using a 5 cm-diameter core sampler. Root detritus and gravel were removed, and the soil was then air-dried and ground to pass through a 2 mm sieve. A subsample was further ground to pass through 0.15 mm sieve to determine total N and SOC concentrations using the micro-Kjeldahl method and the wet oxidation-redox titration method, respectively (Bao, 2000).

Cropping season	Stage	Date (dd-mm-yy)				
		2013–2014	2014-2015	2015–2016		
Rice	Basal fertilization (90 kg N ha ⁻¹ , 90 kg P_2O_5 ha ⁻¹ and 90 kg K_2O ha ⁻¹)	10-Jun-13	12-Jun-14	11-Jun-15		
	Field flooding and puddling	14-Jun-13	15-Jun-14	14-Jun-15		
	Seedling transplanting and field flooding	17-Jun-13	18-Jun-14	17-Jun-15		
	Tillering fertilization (67.5 kg N ha^{-1})	24-Jun-13	26-Jun-14	23-Jun-15		
	Midseason drainage	22-Jul-13	22-Jul-14	23-Jul-15		
	Field reflooding and intermittent irrigation	31-Jul-13	1-Aug-14	2-Aug-15		
	Panicle initiation fertilization (67.5 kg N ha ^{-1} and 90 kg K ₂ O ha ^{-1})	3-Aug-13	4-Aug-14	4-Aug-15		
	No irrigation	15-Oct-13	16-Oct-14	13-Oct-15		
	Harvesting	25-Oct-13	28-Oct-14	26-Oct-15		
Wheat	Basal fertilization (90 kg N ha ⁻¹ , 60 kg P_2O_5 ha ⁻¹ and 60 kg K_2O ha ⁻¹)	2-Nov-13	5-Nov-14	6-Nov-15		
	Sowing	5-Nov-13	7-Nov-14	11-Nov-15		
	Elongation and booting fertilization(54 kg N ha ^{-1} and 60 kg K ₂ O ha ^{-1})	27-Feb-14	1-Mar-15	5-Mar-16		
	Panicle initiation fertilization (36 kg N ha $^{-1}$)	27-Mar-14	31-Mar-15	28-Mar-16		
	Harvesting	30-May-14	31-May-15	25-May-16		

2.6. Crop yield measurements

To avoid any edge influence, yield samples of rice or wheat at the maturity stage were manually measured by harvesting 4 m^2 areas in the middle of each plot. The crop yields of rice and wheat were adjusted to water contents of 140 g kg^{-1} and 125 g kg^{-1} fresh weight, respectively.

2.7. Calculations and statistics

The SOC stock (t C ha⁻¹) of the topsoil (0–20 cm) was calculated as follows (Ellert and Bettany, 1995):

$$SOC \ stock = SOC \ concentration \times \rho_b \times H \tag{1}$$

Where, ρ_b is the soil bulk density (g cm⁻³) and *H* is the depth of the sampling soil (20 cm).

The SOC sequestration rate (t C ha⁻¹ yr⁻¹) during the experiment was calculated using the following equation:

$$SOC sequestration rate = \frac{SOC \ stock_{2016} - \ SOC \ stock_{2007}}{T}$$
(2)

Where, *SOC stock*₂₀₁₆ and *SOC stock*₂₀₀₇ refer to the SOC stock in 2016 and the initial year (t C ha⁻¹), respectively, and *T* (9) refers to the experiment from 2007 to 2016.

The NGWP was developed to provide a comprehensive estimate of the global warming influence of different gases (Mosier et al., 2006). The annual NGWP (kg CO₂-eq ha⁻¹ yr⁻¹) equals the total CO₂ emission equivalents minus the change in SOC in the crop system (IPCC, 2013).

$$NGWP = CH_4 \times 28 + N_2O \times 265 - SOC$$
 sequestration rate $\times 44/12$

(3)

The GHGI reflects the relative global warming influence due to crop production. The annual GHGI (kg CO_2 -eq t⁻¹ crop yield yr⁻¹) was calculated as the ratio between NGWP and crop yield in accordance with the method of Shang et al. (2011) as follows:

$$GHGI = \frac{NGWP}{yield}$$
(4)

The average and standard deviation for each data set were calculated based on the data from the triplicate plots. Two-way analysis of variance (ANOVA) in conjunction with the least significant difference test was used to determine the significance of the effects of treatment, sampling year and their interaction on GHG emissions, crop yields, soil properties, NGWP and GHGI. All the data were analyzed using the SPSS 13.0 software package (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Soil organic carbon sequestration

After nine years of continuous straw incorporation, the topsoil (0-20 cm) SOC concentrations in the straw incorporation treatments were significantly higher than those in the control (no straw incorporation) treatment. Relative to SOC under the control treatment, that under the WS, RS and WSRS treatments was increased by 10%, 12% and 19%, respectively (Fig. 2). Relative to the control treatment, WSRS significantly decreased soil bulk density by 8%. The SOC stock in all treatments ranged from 44.0 to 48.2 t C ha⁻¹ in 2016. Relative to the control treatment, straw incorporation significantly increased the topsoil SOC stock by 5–9%.

On average, the annual topsoil SOC sequestration rate in the control, WS, RS, and WSRS treatments was 0.79, 0.98, 1.07, and 1.17 t C ha⁻¹ yr⁻¹, respectively (Table 2). Relative to the control treatment, straw incorporation significantly increased the annual SOC sequestration rate by 0.24–0.43 t C ha⁻¹ yr⁻¹ (32–58%). No significant difference in annual SOC sequestration rate was observed among the straw incorporation treatments.



Fig. 2. Soil organic carbon (SOC) concentration, soil bulk density, and SOC stock within the 0–20-cm soil depth in different straw incorporation treatments in 2016. The error bars show the standard deviations of three replicates. The different letters indicate a significant difference at P < 0.05 according to the least significant difference (LSD) multiple-range test.

3.2. Methane emissions

All treatments exhibited similar seasonal patterns of CH₄ fluxes during the rice season, which ranged from -137 to 17190 g CH_4 -C ha⁻¹ d^{-1} (Fig. 3). During the rice season under flooding, the CH₄ fluxes increased after rice transplanting, peaked approximately four to five weeks after transplanting, and then decreased sharply, subsequently stabilizing at a lower flux rate in the later rice season until harvest. The greatest CH₄ fluxes were observed in the WSRS treatment and were 11142, 17172 and 7686 g CH₄-C ha⁻¹ d⁻¹ in 2013, 2014 and 2015, respectively. The seasonal total CH₄ emissions during the rice season depended greatly on straw incorporation treatment, year and their interaction (Table 3). The average CH₄ emissions were significantly higher during the 2014 rice season than during the other two rice seasons. Across the three rice seasons, CH₄ emissions under the control, RS, WS, and WSRS treatments were 111, 152, 213, and 247 kg CH₄-C ha^{-1} season⁻¹, respectively (Table 4). Relative to the control treatment, straw incorporation markedly increased seasonal CH₄ emissions by 37-122%. Relative to the RS treatment, the WS and WSRS treatments significantly increased the seasonal CH₄ emissions by 42% and 62%, respectively.

There were no clear trends in CH₄ fluxes during the wheat season (Fig. 3). The CH₄ fluxes during the three wheat seasons in the control, WS, RS, and WSRS treatments were -6.84–14.0 g CH₄-C ha⁻¹ d⁻¹, -4.39–12.8 g CH₄-C ha⁻¹ d⁻¹, -6.46–17.0 g CH₄-C ha⁻¹ d⁻¹, and -4.82–22.0 g CH₄-C ha⁻¹ d⁻¹, respectively. The seasonal total CH₄ emissions varied significantly with treatment and year but there was no interaction effect of treatment and year (Table 3). The average CH₄ emissions were significantly lower in the control and WS treatments than in the RS and WSRS treatments during the wheat season.

Table 2

Mean annual soil organic carbon (SOC) sequestration rate, methane (CH_4) and nitrous oxide (N_2O) emissions, crop yields and their estimated global warming potential (GWP) and greenhouse gas intensity (GHGI) during three annual cycles from the 2013 rice season to the 2016 wheat season.

Treatment	SOC sequestration rate (t C $ha^{-1} yr^{-1}$)	CH_4 (kg CH_4 -C ha^{-1} yr ⁻¹)	$ m N_2O$ (kg $ m N_2O$ -N ha ⁻¹ yr ⁻¹)	Yield (t ha ^{-1} yr ^{-1})	NGWP (kg CO_2 -eq ha ⁻¹ yr ⁻¹)	GHGI (kg CO_2 -eq t ⁻¹ crop yield yr ⁻¹)
Control WS RS WSRS	$0.74 \pm 0.09 b$ $0.98 \pm 0.08 a$ $1.07 \pm 0.09 a$ $1.17 \pm 0.14 a$	112 ± 38.0 c 216 ± 70.5 a 156 ± 46.9 b 250 ± 78.4 a	$\begin{array}{l} 1.63 \ \pm \ 0.12 \ {\rm c} \\ 2.34 \ \pm \ 0.20 \ {\rm b} \\ 2.31 \ \pm \ 0.28 \ {\rm b} \\ 3.12 \ \pm \ 0.44 \ {\rm a} \end{array}$	$\begin{array}{l} 15.2 \ \pm \ 0.73 \ \mathrm{b} \\ 15.7 \ \pm \ 1.06 \ \mathrm{b} \\ 16.7 \ \pm \ 0.70 \ \mathrm{a} \\ 16.8 \ \pm \ 0.75 \ \mathrm{a} \end{array}$	9500 ± 3889 b 19533 ± 7211 a 13052 ± 4792 b 22716 ± 8112 a	627 ± 258 b 1228 ± 381 a 779 ± 269 b 1353 ± 475 a

* Mean \pm SD (n = 3); different letters within the same column indicate significant differences in variable means among treatments during the 2013–2016 seasons based on the least significant difference (LSD) multiple-range test (P < 0.05).



Fig. 3. Seasonal variation in methane (CH₄) fluxes in different straw incorporation treatments from the 2013 rice season to the 2016 wheat season. The error bars show the standard deviations of three replicates.

When averaged across the three annual rotation cycles, the annual CH₄ emissions in the control, RS, WS, and WSRS treatments were 112, 156, 216, and 250 kg CH₄-C ha⁻¹ yr⁻¹, respectively (Table 2). Relative to the control treatment, straw incorporation significantly increased the annual CH₄ emissions by 44–138 kg CH₄-C ha⁻¹ yr⁻¹ (39–123%). Relative to the RS treatment, the WS and WSRS treatments significantly increased the annual CH₄ emissions by 38% and 61%, respectively.

3.3. Nitrous oxide emissions

During the rice season, all treatments exhibited comparable seasonality in N_2O fluxes (Fig. 4). A strong peak in N_2O flux was observed during the midseason drainage period in each of the 2013 and 2015 rice seasons, whereas the corresponding N_2O fluxes in 2014 exhibited no such peak. Two-way ANOVAs showed that the total seasonal N_2O

emissions were significantly influenced by treatment and year (Table 3). When averaged across three rice seasons, the seasonal total N₂O emissions ranged from 0.55 kg N₂O-N ha⁻¹ season⁻¹ in the control treatment to 1.40 kg N₂O-N ha⁻¹ season⁻¹ in the WSRS treatment (Table 4). Relative to the control treatment, straw incorporation significantly increased the average seasonal N₂O emissions by 58–155%. The seasonal N₂O emissions in the RS treatment were similar to those in the WSRS and WS treatments.

In general, the WS treatment yielded higher N_2O fluxes than the control treatment during the early wheat season, whereas the RS and WSRS treatments yielded higher N_2O fluxes than the control treatment during the later wheat season (Fig. 4). The total N_2O emissions during the wheat season varied significantly with treatment and year (Table 3). No significant difference was observed between the control and RS treatments. Relative to the RS treatment, the WS and WSRS treatments

Table 3

Results of a two-way ANOVA of the influences of straw incorporation (S) treatment and year (Y) on methane (CH_4) and nitrous oxide (N_2O) emissions and yield in rice-wheat cropping systems.

Cropping season	Factors	df	CH4 (kg CH	₄ -C ha ⁻¹ sease	¹ season ⁻¹) N ₂ O (kg N ₂ O-N ha ⁻¹ season		ason ⁻¹)	n^{-1}) Yield (t ha^{-1} season ⁻¹)			
			SS	F	Р	SS	F	Р	SS	F	Р
Rice	S	3	99858	68.4	< 0.001	3.61	39.6	< 0.001	6.08	12.2	< 0.001
	Y	2	94154	96.8	< 0.001	3.74	61.6	< 0.001	13.1	39.4	< 0.001
	S imes Y	6	7705	2.56	0.05	0.11	0.62	0.71	0.79	0.80	0.58
	Model	11	201027	37.6	< 0.001	7.47	22.3	< 0.001	20.0	10.9	< 0.001
	Error	24	11674			0.73			3.99		
Wheat	S	3	1.78	20.7	< 0.001	2.31	21.9	< 0.001	3.38	2.96	0.06
	Y	2	1.41	24.5	< 0.001	2.82	40.1	< 0.001	1.80	2.36	0.12
	S imes Y	6	0.20	0.12	0.99	0.28	1.33	0.28	2.29	1.00	0.45
	Model	11	3.20	10.2	< 0.001	5.41	14.0	< 0.001	7.47	1.78	0.12
	Error	24	0.69			0.84			9.14		

Table 4

Seasonal methane (CH₄) and nitrous oxide (N₂O) emissions and grain yields in different straw incorporation treatments during three annual cycles from the 2013 rice season to the 2016 wheat season.

Year	Treatment	Rice season CH ₄ (kg CH ₄ -C ha ^{-1} season ^{-1})	$ m N_2O~(kg~N_2O-N~ha^{-1})$ season ⁻¹)	Yield (t ha ^{-1} season ^{-1})	Wheat season CH ₄ (kg CH ₄ -C ha ⁻¹ season ⁻¹)	N ₂ O (kg N ₂ O-N ha ⁻¹ season ⁻¹)	Yield (t ha ^{-1} season ^{-1})
2013–2014	Control WS RS WSRS	79.1 ± 5.52 c 156 ± 23.8 ab 118 ± 8.41 b 190 ± 30.0 a	$\begin{array}{l} 0.43 \ \pm \ 0.06 \ {\rm c} \\ 0.74 \ \pm \ 0.08 \ {\rm b} \\ 1.02 \ \pm \ 0.20 \ {\rm ab} \\ 1.20 \ \pm \ 0.23 \ {\rm a} \end{array}$	$\begin{array}{l} 10.0 \ \pm \ 0.47 \ \mathrm{ab} \\ 9.58 \ \pm \ 0.02 \ \mathrm{b} \\ 10.4 \ \pm \ 0.29 \ \mathrm{a} \\ 10.5 \ \pm \ 0.32 \ \mathrm{a} \end{array}$	$\begin{array}{l} 0.21 \ \pm \ 0.07 \ \mathrm{b} \\ 0.32 \ \pm \ 0.09 \ \mathrm{b} \\ 0.65 \ \pm \ 0.15 \ \mathrm{a} \\ 0.74 \ \pm \ 0.26 \ \mathrm{a} \end{array}$	$\begin{array}{l} 1.19 \ \pm \ 0.12 \ \mathrm{b} \\ 1.58 \ \pm \ 0.18 \ \mathrm{a} \\ 1.16 \ \pm \ 0.15 \ \mathrm{b} \\ 1.75 \ \pm \ 0.25 \ \mathrm{a} \end{array}$	$\begin{array}{l} 4.78 \ \pm \ 0.34 \ a \\ 5.20 \ \pm \ 1.01 \ a \\ 6.03 \ \pm \ 0.56 \ a \\ 5.38 \ \pm \ 0.54 \ a \end{array}$
2014–2015	Control WS RS WSRS	$\begin{array}{l} 158 \ \pm \ 14.2 \ c \\ 300 \ \pm \ 23.9 \ a \\ 211 \ \pm \ 21.3 \ b \\ 341 \ \pm \ 42.9 \ a \end{array}$	$\begin{array}{l} 0.32 \ \pm \ 0.02 \ c \\ 0.55 \ \pm \ 0.07 \ b \\ 0.82 \ \pm \ 0.11 \ a \\ 1.04 \ \pm \ 0.19 \ a \end{array}$	$\begin{array}{l} 10.9 \ \pm \ 0.47 \ \mathrm{b} \\ 11.3 \ \pm \ 0.41 \ \mathrm{ab} \\ 12.1 \ \pm \ 0.46 \ \mathrm{a} \\ 12.1 \ \pm \ 0.30 \ \mathrm{a} \end{array}$	$\begin{array}{l} 0.36 \ \pm \ 0.10 \ \mathrm{b} \\ 0.50 \ \pm \ 0.08 \ \mathrm{b} \\ 0.85 \ \pm \ 0.19 \ \mathrm{a} \\ 0.97 \ \pm \ 0.21 \ \mathrm{a} \end{array}$	$\begin{array}{l} 1.30 \ \pm \ 0.09 \ \mathrm{c} \\ 1.72 \ \pm \ 0.19 \ \mathrm{b} \\ 1.44 \ \pm \ 0.18 \ \mathrm{bc} \\ 2.21 \ \pm \ 0.34 \ \mathrm{a} \end{array}$	$\begin{array}{rrrr} 4.40 \ \pm \ 0.59 \ a \\ 5.56 \ \pm \ 0.50 \ a \\ 5.08 \ \pm \ 0.37 \ a \\ 5.02 \ \pm \ 0.59 \ a \end{array}$
2015–2016	Control WS RS WSRS	95.5 ± 6.40 c 183 ± 11.8 a 128 ± 21.8 b 210 ± 23.4 a	$\begin{array}{l} 0.89 \ \pm \ 0.08 \ \mathrm{c} \\ 1.31 \ \pm \ 0.15 \ \mathrm{b} \\ 1.58 \ \pm \ 0.20 \ \mathrm{ab} \\ 1.95 \ \pm \ 0.37 \ \mathrm{a} \end{array}$	$\begin{array}{l} 10.2 \ \pm \ 0.70 \ \mathrm{b} \\ 10.1 \ \pm \ 0.50 \ \mathrm{b} \\ 10.8 \ \pm \ 0.33 \ \mathrm{ab} \\ 11.2 \ \pm \ 0.17 \ \mathrm{a} \end{array}$	$\begin{array}{l} 0.69 \ \pm \ 0.13 \ \mathrm{b} \\ 0.85 \ \pm \ 0.17 \ \mathrm{ab} \\ 1.12 \ \pm \ 0.19 \ \mathrm{a} \\ 1.19 \ \pm \ 0.26 \ \mathrm{a} \end{array}$	$\begin{array}{l} 0.74 \ \pm \ 0.05 \ \mathrm{b} \\ 1.11 \ \pm \ 0.10 \ \mathrm{a} \\ 0.92 \ \pm \ 0.11 \ \mathrm{ab} \\ 1.20 \ \pm \ 0.28 \ \mathrm{a} \end{array}$	$\begin{array}{l} 5.22 \ \pm \ 0.99 \ a \\ 5.31 \ \pm \ 0.74 \ a \\ 5.70 \ \pm \ 0.41 \ a \\ 6.00 \ \pm \ 0.27 \ a \end{array}$
2013–2016 [*]	Control WS RS WSRS	111 ± 37.0 c 213 ± 68.6 a 152 ± 46.7 b 247 ± 76.5 a	$\begin{array}{l} 0.55 \ \pm \ 0.27 \ {\rm c} \\ 0.87 \ \pm \ 0.36 \ {\rm b} \\ 1.14 \ \pm \ 0.37 \ {\rm ab} \\ 1.40 \ \pm \ 0.49 \ {\rm a} \end{array}$	$\begin{array}{l} 10.4 \ \pm \ 0.62 \ \mathrm{b} \\ 10.3 \ \pm \ 0.80 \ \mathrm{b} \\ 11.1 \ \pm \ 0.83 \ \mathrm{a} \\ 11.3 \ \pm \ 0.70 \ \mathrm{a} \end{array}$	$\begin{array}{l} 0.42 \ \pm \ 0.23 \ \mathrm{b} \\ 0.56 \ \pm \ 0.26 \ \mathrm{b} \\ 0.87 \ \pm \ 0.25 \ \mathrm{a} \\ 0.96 \ \pm \ 0.29 \ \mathrm{a} \end{array}$	$\begin{array}{l} 1.08 \ \pm \ 0.27 \ {\rm c} \\ 1.47 \ \pm \ 0.31 \ {\rm b} \\ 1.17 \ \pm \ 0.26 \ {\rm c} \\ 1.72 \ \pm \ 0.51 \ {\rm a} \end{array}$	$\begin{array}{rrrr} 4.80 \ \pm \ 0.70 \ a \\ 5.35 \ \pm \ 0.69 \ a \\ 5.60 \ \pm \ 0.57 \ a \\ 5.47 \ \pm \ 0.60 \ a \end{array}$

* Mean \pm SD (n = 3); different letters within the same column indicate a significant difference at P < 0.05 according to the least significant difference (LSD) multiple-range test.

significantly increased the seasonal $\mathrm{N_2O}$ emissions by 26% and 47%, respectively.

Across the three annual cycles, the annual N₂O emissions were 1.63, 2.31, 2.34, and 3.12 kg N₂O-N ha⁻¹ yr⁻¹ in the control, RS, WS, and WSRS treatments, respectively (Table 2). Relative to the control treatment, the straw incorporation treatments increased the annual N₂O emissions by 0.68–1.49 kg N₂O-N ha⁻¹ yr⁻¹ (42–91%). Relative to the RS treatment, the WSRS treatment significantly increased the annual N₂O emissions by 35%.

3.4. Rice and wheat yields

The rice and wheat yields ranged from 10.3-11.3 t ha⁻¹ season⁻¹ to 4.8–5.6 t ha⁻¹ season⁻¹ during the three annual cycles (Table 4). The straw incorporation treatments significantly influenced rice yield but not wheat yield (Table 3). The average rice yields were significantly higher in the RS and WSRS treatments (7% and 9%, respectively) than in the control treatment. The average annual yields of rice and wheat were 15.2, 15.7, 16.7, and 16.8 t ha⁻¹ yr⁻¹ in the control, WS, RS, and WSRS treatments, respectively (Table 2). Relative to the RS treatment,

the WS treatment significantly decreased annual yields by 6%, whereas no significant difference was observed between the RS and WSRS treatments.

3.5. Annual net global warming potential and greenhouse gas intensity

Net global warming potential was calculated to estimate the potential climatic influence of different GHG emissions under different straw incorporation treatments (Table 2). According to the NGWP results, all field treatments acted as GHG sources. Methane was the most important GHG and should be mitigated first in rice–wheat cropping systems. Net global warming potential was similar between the control and RS treatments and was significantly lower in these treatments than in the WSRS and WS treatments. Relative to the RS treatment, the WS and WSRS treatments significantly increased the annual NGWP by 50% and 74%, respectively.

No significant difference in GHGI was observed between the control and RS treatments, whereas relative to the control treatment, the WS and WSRS treatments significantly increased GHGI by 96% and 116%, respectively. In addition, relative to the RS treatment, the WS and



Fig. 4. Seasonal variation in nitrous oxide (N₂O) fluxes in different straw incorporation treatments from the 2013 rice season to the 2016 wheat season. The error bars show the standard deviations of three replicates.

WSRS treatments significantly increased GHGI by 58% and 74%, respectively.

4. Discussion

4.1. Influence of straw incorporation on nitrous oxide emissions

In the present study, long-term straw incorporation significantly increased seasonal N2O emissions under all treatments and seasons except the RS treatment during the wheat season (Table 4). Factors such as straw type and rate, straw incorporation time and method. N fertilization application, and soil and climatic conditions have been shown to influence the responses of N₂O emissions to straw incorporation (Venterea et al., 2012). Some short-term experiments found that straw incorporation had negative or no influence on N2O emissions in rice--wheat cropping systems due to increases in the soil C:N ratio (Bhattacharyya et al., 2012; Yao et al., 2009; Zou et al., 2005). Crop straw with a C:N ratio greater than 40 may lead to temporary microbial immobilization of soil N, which can decrease substrate availability for N₂O production (Millar et al., 2004). However, sufficient N fertilizer application, especially during the early cropping season, can partially compensate for this immobilization of soil N (Bird et al., 2001). In the study region, a high N fertilizer application rate is often used during the early season of crop growth; this application accounts for 70% (i.e., 157.5 kg N ha⁻¹) and 50% (*i.e.*, 90 kg N ha⁻¹) of the seasonal N input in the rice and wheat seasons, respectively (Table 1). In addition, the subsequent N released from straw decomposition during the middle and late cropping seasons may increase N₂O production (Wu et al., 2011). In the present study, increased N₂O production was evidenced from the significant increase in soil total N concentrations after six years of continuous straw incorporation (Table A1). Consistent with this finding, Bird et al. (2001) reported that a labile pool of available N formed after four to six years of rice straw incorporation, which resulted in a diminished dependency on N fertilizer. These observations clarify the high seasonal N2O emissions under long-term straw incorporation in rice-wheat cropping systems.

The annual N₂O emissions reported in this study agree well with previous extensive measurements in the same area (Xia et al., 2014; Yao et al., 2009; Zhang et al., 2015b). As expected, relative to the control treatment, straw incorporation significantly increased the annual N₂O emissions by 42–91% across the three rotation cycles (Table 2). Our results are consistent with previous studies showing that N fertilizer application is one of the most important factors contributing to N₂O emissions from agricultural fields (Hoben et al., 2011; Venterea et al., 2012). A previous study found that after three years of rice straw incorporation, the N rate in the rice season can be decreased by as much as 25% (30 kg N ha⁻¹ season ⁻¹) without decreasing rice yield (Singh and Sidhu, 2014). Therefore, we speculate that N₂O emissions under long-term straw incorporation can be decreased by decreasing N fertilizer rate, especially during the late crop growth season. More data from field experiments are needed.

4.2. Influence of straw incorporation on methane emissions

Straw incorporation significantly stimulated CH_4 emissions during the rice season. This result is consistent with some previous studies (Bhattacharyya et al., 2012; Ma et al., 2009; Zou et al., 2005) but inconsistent with others (Hou et al., 2013; Li et al., 2011). Generally, high SOC concentration and straw incorporation stimulate CH_4 emissions (Kimura et al., 2004; Wang et al., 1999). After analyzing 28 soils from different fields, Wang et al. (1999) reported a significant positive correlation between soil CH_4 production and SOC concentration. Increases in CH_4 production by wheat straw incorporation before rice transplanting have been mainly attributed to the availability of wheat straw as a labile organic substrate that is easily degraded into CH_4 (Ma et al., 2009; Zou et al., 2005). Tang et al. (2016) showed that undecomposed rice straw in the off-rice season can be expected to result in marked CH₄ production during the subsequent rice season. To our knowledge, there are two potential reasons for the higher CH₄ emissions during the rice season under straw incorporation than under the control treatment in the present study. First, the higher CH₄ emissions might result from the greater SOC concentration under long-term straw incorporation than under the control treatment. After six years of continuous straw incorporation, the SOC concentration significantly increased before rice transplanting (Table A1), which may have led to the higher CH₄ emissions. Second, wheat straw incorporation before rice transplanting or rice straw incorporation before wheat sowing may have provided sufficient C substrate for CH₄ production during the rice season, although most of the rice straw can be expected to have already decomposed during the off-rice season before the subsequent rice season (Tang et al., 2016). In the present study, the annual CH₄ emissions in the RS treatment were significantly lower than those in the WS and WSRS treatments, suggesting that caution is required when incorporating wheat straw with the goal of mitigating annual CH₄ emissions.

In agreement with previous studies (Ma et al., 2009; Zou et al., 2005), our results highlight the importance of mitigating CH_4 emissions during the early rice season. To achieve high crop yields, conventional transplanted rice in combination with a typical flooding-drainage-intermittent water regimen were applied in this study. The drainage occurred during the middle rice season. However, researchers have recently suggested that shifting from transplanted rice to direct-seeded rice, applying early season drainage or alternating between wet and dry irrigation could effectively decrease CH_4 emissions during the rice season (Islam et al., 2018; LaHue et al., 2016). Additional estimates of the possible tradeoffs between decreased CH_4 emissions and decreased yields and/or increased N_2O emissions are needed.

4.3. Influence of straw incorporation on soil organic carbon sequestration

The SOC stock in this study varied from 44.0 to $48.2 \text{ t C} \text{ ha}^{-1}$ in 2016; this range falls within the range $(39.4-50.5 \text{ t C ha}^{-1})$ reported in this region based on a 22-yr straw incorporation experiment (Xia et al., 2014). Greater SOC stock in the straw incorporation treatments than in the control treatment can be attributed mainly to the greater SOC concentration in the straw incorporation treatments than in the control treatment rather than to changes in soil bulk density (Fig. 2). From 2007 to 2016, the annual topsoil SOC sequestration rate in this rice--wheat cropping system ranged from 0 .79 to 1.17 t C ha⁻¹ yr⁻¹. These values are within the SOC sequestration rate $(0.13-2.20 \text{ t C ha}^{-1} \text{ yr}^{-1})$ reported by Pan et al. (2004) but are greater than those (0.01-0.34 t C $ha^{-1} yr^{-1}$) reported by Xia et al. (2014) and Chaudhary et al. (2017). The higher SOC sequestration rates in the present study probably resulted from higher fertilization rate in our study than in the previous studies, which was accompanied by higher quantities of crop straw and root exudation (Xia et al., 2014; Chaudhary et al., 2017). High crop straw and root exudation inputs have been identified as among the main drivers of SOC accumulation (Jacinthe et al., 2002; Huang and Sun, 2006; Chen et al., 2018). However, no significant differences in annual SOC sequestration rate were observed among the long-term straw incorporation treatments (Table 2; Jacinthe et al., 2002; Xia et al., 2014). These findings implied that in addition to straw incorporation, other agricultural practices should be applied to further increase SOC sequestration (Schapel et al., 2018).

4.4. Influence of straw incorporation on crop yields

Straw incorporation is one of the traditional practices employed in rice–wheat cropping systems; however, the reported influence of straw incorporation on rice and wheat yields is inconsistent among studies. Singh et al. (2005) concluded that straw incorporation did not increase rice or wheat yield in the tropics. In contrast, after conducting a meta-

analysis, Huang et al. (2013) claimed that straw incorporation significantly increased rice yields by 5.2% in China. In the present study, rice yield varied greatly on straw incorporation treatment and year, whereas wheat yield was not significantly influenced by straw incorporation. Across the three rice seasons, average rice yield was significantly higher in the WSRS and RS treatments than in the WS and control treatments (Table 3), showing that rice straw incorporation before wheat sowing increased subsequent rice yields, whereas wheat straw incorporation did not influence subsequent rice yields. This phenomenon might be caused primarily by two factors. First, the observed higher yields under rice straw incorporation than under the other treatments could result from the increased availability of both soil and fertilizer N. Our results showed that rice straw incorporation before wheat sowing provided large quantities of soil N before subsequent rice transplanting (Table A1). In contrast, wheat straw incorporation before rice transplanting may result in the short-term immobilization of soil and fertilizer N, which could ultimately inhibit rice growth (Huang et al., 2013). Second, high air and soil temperature during the rice season could increase straw decomposition (Sierra, 2012). The phytotoxins (e.g., organic acids and reducing substances) released during fresh wheat straw decomposition directly decrease rice growth during the early season (Tanaka and Nishida, 1998; Xu et al., 2009; Wu et al., 2011). However, as these two negative effects of wheat straw incorporation on rice yield can be compensated by long-term straw incorporation or high N fertilizer application rate (Bird et al., 2001; Huang et al., 2013), no significant difference in rice yield was observed between the WS (10.3 t ha^{-1} season⁻¹) and control (10.4 t ha^{-1} season⁻¹) treatments in this study. Consistent with previous results in rice-wheat cropping systems (Xia et al., 2014), the long-term use of straw incorporation resulted in higher annual crop yields than did the control treatment, which showed that straw incorporation can play a critical role in promoting the long-term sustainability of crop production systems. Our results confirm that rice straw incorporation before wheat sowing can contribute to higher annual crop productivity than will no straw incorporation.

4.5. Influence of straw incorporation on net global warming potential and greenhouse gas intensity

In this study, all the straw incorporation treatments were possible GHG sources, which is consistent with previous findings (Ma et al., 2009; Bhattacharyya et al., 2012; Hu et al., 2016). These results showed that straw incorporation can be expected to intensify rather than mitigate climate change, without consideration of the carbon emissions to the atmosphere caused by straw burning. Compared to straw burning, however, *in situ* straw incorporation to the soil *via* tillage is much simpler and easier due to the low labor required and the very short turnaround time between grain harvest and rice transplanting/wheat sowing (Singh and Sidhu, 2014). In the present study, no significant

AppendixA

differences in NGWP and GHGI were found between the RS and control treatments; however, relative to the RS treatment, the WS and WSRS treatments yielded significantly higher NGWP and GHGI. These findings suggest that relative to the WS or WSRS treatment, the RS treatment in rice–wheat cropping systems increases crop productivity while decreasing GHG emissions. Future studies should address the increases in NGWP with experimental duration due to decreased SOC sequestration (Powlson et al., 2008) and/or increased CH₄ emissions.

5. Conclusions

This study provided a comprehensive investigation of the potential influences of different straw incorporation treatments over long-term use on both GHG emissions and system productivity in a Chinese rice-wheat cropping system in Hydragric Anthrosols under a subtropical monsoon climate. Relative to the control treatment, long-term straw incorporation significantly increased annual CH₄ and N₂O emissions and the topsoil SOC sequestration rate. The RS treatment yielded lower annual CH₄ emissions than the WS and WSRS treatments. Annual N₂O emissions were lower in the RS and WS treatments than in the WSRS treatment, and average annual yield was greater in the RS and WSRS treatments than in the WS and control treatments. Moreover, annual NGWP and GHGI were similar between the control and RS treatments and were significantly lower in these treatments than in the WS and WSRS treatments. Altogether, these results suggest that relative to the WS or WSRS treatment, the RS treatment has the potential to simultaneously increase crop yields while decreasing GHG emissions. The valuable long-term observations from a rice-wheat system in this study provided insights into environmental-friendly straw incorporation management, but comparisons with other ecosystems and crop straw incorporation methods will be useful to further develop sustainable agriculture.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Table A1

Soil organic carbon (SOC), total nitrogen (N) and the C:N ratio in different straw incorporation treatments at wheat maturity in 2013, 2014 and 2015.

Treatment	2013 SOC (g kg ⁻¹)	Total N (g kg $^{-1}$)	C:N ratio	2014 SOC (g kg ⁻¹)	Total N (g kg $^{-1}$)	C:N ratio	2015 SOC (g kg ⁻¹)	Total N (g kg $^{-1}$)	C:N ratio
Control WS RS WSRS	$\begin{array}{l} 17.2 \ \pm \ 0.63 \ c \\ 18.1 \ \pm \ 0.35 \ b \\ 18.4 \ \pm \ 0.84 \ ab \\ 19.3 \ \pm \ 0.22 \ a \end{array}$	$\begin{array}{l} 1.45 \ \pm \ 0.05 \ \mathrm{c} \\ 1.57 \ \pm \ 0.04 \ \mathrm{b} \\ 1.58 \ \pm \ 0.03 \ \mathrm{b} \\ 1.70 \ \pm \ 0.06 \ \mathrm{a} \end{array}$	$\begin{array}{l} 11.8 \ \pm \ 0.22 \ a \\ 11.5 \ \pm \ 0.55 \ a \\ 11.7 \ \pm \ 0.73 \ a \\ 11.3 \ \pm \ 0.43 \ a \end{array}$	$\begin{array}{rrrr} 17.6 \ \pm \ 0.88 \ c \\ 18.8 \ \pm \ 0.85 \ b \\ 19.1 \ \pm \ 0.20 \ ab \\ 20.0 \ \pm \ 0.80 \ a \end{array}$	$\begin{array}{l} 1.50 \ \pm \ 0.01 \ \mathrm{c} \\ 1.65 \ \pm \ 0.07 \ \mathrm{b} \\ 1.64 \ \pm \ 0.02 \ \mathrm{b} \\ 1.74 \ \pm \ 0.03 \ \mathrm{a} \end{array}$	$\begin{array}{rrrr} 11.7 \ \pm \ 0.53 \ a \\ 11.4 \ \pm \ 0.55 \ a \\ 11.7 \ \pm \ 0.25 \ a \\ 11.5 \ \pm \ 0.56 \ a \end{array}$	$\begin{array}{rrrr} 17.8 \ \pm \ 0.27 \ c \\ 19.3 \ \pm \ 1.45 \ b \\ 19.6 \ \pm \ 0.45 \ ab \\ 20.8 \ \pm \ 0.67 \ a \end{array}$	$\begin{array}{l} 1.54 \ \pm \ 0.07 \ \mathrm{c} \\ 1.70 \ \pm \ 0.02 \ \mathrm{b} \\ 1.68 \ \pm \ 0.06 \ \mathrm{b} \\ 1.80 \ \pm \ 0.04 \ \mathrm{a} \end{array}$	$\begin{array}{l} 11.6 \ \pm \ 0.41 \ a \\ 11.4 \ \pm \ 0.75 \ a \\ 11.7 \ \pm \ 0.46 \ a \\ 11.5 \ \pm \ 0.11 \ a \end{array}$

*Mean \pm SD (n = 3); different letters within the same column indicate a significant difference at P < 0.05 according to the least significant difference (LSD) multiple-range test.

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