

Greenhouse gas emissions and reactive nitrogen releases from rice production with simultaneous incorporation of wheat straw and nitrogen fertilizer

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Abstract

~~The impacts~~Impacts of simultaneous inputs of crop straw and nitrogen (N) fertilizer on greenhouse gas (GHG) emissions and ~~reactive nitrogen (Nr) releases~~N losses from rice production ~~in intensive agricultural regions~~are not well understood. A two-year field experiment was established in a rice–wheat cropping system in the Taihu Lake region (TLR) of China ~~since 2013~~ to evaluate the GHG intensity (GHGI), ~~Nr~~reactive N intensity (NrI) ~~and environmental costs~~ of ~~concurrent rice production with~~ inputs of wheat straw and N fertilizer ~~to rice paddies~~. The field experiment included five treatments of different N fertilization rates for rice production: 0 (RN0), 120 (RN120), 180 (RN180), 240 (RN240) and 300 kg N ha⁻¹ (RN300, traditional N applied rate in the TLR). Wheat straws were fully incorporated into soil before rice transplantation ~~in all treatments~~. The ~~results~~meta-analytic technique was employed to evaluate various Nr losses. Results showed that the response of rice yield to N ~~application~~rate successfully fitted a quadratic model. ~~Nitrous oxide (N₂O) emissions were increased exponentially as~~, while N fertilization rates increased, while methane (CH₄) emissions increased slightly with wheat straw rates increased. The estimated soil organic carbon sequestration rate varied from 129.58 (RN0) to 196.87 kg C ha⁻¹ yr⁻¹ (RN300). ~~Seasonal average~~promoted Nr discharges exponentially (nitrous oxide emission, N leaching and runoff) or linearly (ammonia volatilization). The GHGI of rice production ranged from 1.20 (RN240) to 1.61 ~~(RN0)~~ kg CO₂-equivalent (CO₂-eq) kg⁻¹ ~~(RN0)~~, while NrI varied from 2.14 (RN0) to 10.92 ~~(RN300)~~ g N kg⁻¹ ~~(RN300)~~. ~~Methane (CH₄ emissions) emission~~ dominated the GHGI with proportion of 70.2–88.6% ~~due to direct straw incorporation~~, while ammonia (NH₃) volatilization dominated the NrI with proportion of 53.5–57.4% ~~in all fertilization treatments~~. The ~~damage~~%. Damage costs to environment incurred by GHG and Nr

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releases from current rice production (RN300) accounted for 8.8% and 4.9% of ~~farmer's~~farmers' incomes, respectively. Cutting ~~the traditional N~~ application rate ~~of N fertilizer~~ from 300 ~~(traditional N rate)~~ to 240 kg N ha⁻¹ ~~improved~~could improve rice yield and nitrogen use efficiency by 2.14% and 10.30%, respectively, whilst simultaneously reduced GHGI by 13%, NrI by 23% and total environmental costs by 16%. Moreover, the reduction of 60 kg N ha⁻¹ improved ~~farmer's~~farmers' income by 639 ¥ ha⁻¹, which would provide them with an incentive to change ~~their traditional~~the current N application rate. Our study suggests that GHG and Nr releases, especially ~~the~~for CH₄ emission and NH₃ volatilization, from rice production in the TLR could be further ~~curbed~~reduced, considering the current incorporation pattern of wheat straw and N fertilizer.

Key words: Taihu Lake region, greenhouse gas intensity, Nr intensity, rice production, straw incorporation

1 Introduction

Rice is the staple food for the majority of the world's population. However, while industriously feeding the ~~world's~~global population, rice production is an important source of greenhouse gas (GHG) emissions and reactive nitrogen (Nr) releases (Yan et al., 2009; Chen et al., 2014). Rice production in China involves heavy methane (CH₄) emissions due to ~~current~~the water regime ~~management~~managements (e.g., continuous flooding in some regions) and straw incorporation practices (e.g., direct incorporation without any pretreatments) (Yan et al., 2009). Besides, ~~the~~ lower nitrogen use efficiency for rice cultivation in China (approximately 31%) aggravates the release of various Nr species, thus threatening ecosystem functions (Galloway et al., 2008; Zhang et al., 2012). Such a dilemma highlights the need for the simultaneous evaluation of GHG emissions and Nr losses for rice production in China. ~~And rice~~Rice cultivation in intensive agricultural regions, characterized by high inputs of N fertilizer and crop residues, should be prioritized for the implementation of such evaluation (Ju et al., 2009; Chen et al., 2014).

Taihu Lake region (TLR) is one of the most productive areas for rice production in China, largely owing to the popularity of intensive cultivation (Zhao et al., 2012a; Zhao et al., 2012b).

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Currently, rice yield of this region in some fields can reach up to 8000 kg ha⁻¹ or even higher (Ma et al., 2013; Zhao et al., 2015). However, these grain yields are achieved with a cost to environment (Ju et al., 2009). TLR generally receives 550–600 kg N ha⁻¹ yr⁻¹, with the rice-growing season accounting for nearly 300 kg N ha⁻¹ (Zhao et al., 2012b). Besides from these excessive N inputs, TLR also experiences high amounts of crop residue incorporation, which is highly encouraged by local governments (Xia et al., 2014). However, direct straw incorporation before rice transplantation triggers substantial CH₄ emissions (Ma et al., 2009; Ma et al., 2013). Besides such substantial releases of Nr and ~~GHGs~~GHG in a direct way, indirect releases during the production of various agricultural materials used for farming operations ~~in the TLR~~, are also not ignorable, due to higher input rates of these materials caused by intensive cultivation (Zhang et al., 2013; Cheng et al., 2014). This warrants the need for life-cycle assessment (LCA) of GHG emissions and Nr releases with respect to rice production in this region.

Considerable environmental costs can be caused by the direct and indirect releases of ~~GHGs~~GHG and Nr from rice production in the TLR, for instance, in the form of global warming, water eutrophication, or soil acidification (Ju et al., 2009; Xia and Yan, 2011; Xia and Yan, 2012). Previous studies have proven that environmental costs assessment could provide guidance for emerging policy priorities in mitigating certain GHG or Nr species, after quantifying both their release amounts and damage costs to ecosystems (Gu et al., 2012). However, few studies have attempted to evaluate the ~~life-cycle assessment of~~ total GHG and Nr releases, and the associated environmental costs ~~they incur from rice production in the TLR under the current conditions of,~~ with high inputs of N fertilizer and crop straw, ~~are scarce~~.

In the present study, we conducted two years of simultaneous measurements of CH₄ and

nitrous oxide (N₂O) emissions from a rice-wheat cropping system in the TLR to evaluate the impacts of simultaneous inputs of crop straw and N fertilizer on (1) net global warming potential (NGWP) and GHG intensity (GHGI), (2) total Nr losses and Nr intensity (NrI), (3) environmental costs incurred by these GHG and Nr releases ~~of~~associated with rice production, from the perspective of ~~life-cycle-assessment~~LCA.

2 Materials and methods

2.1 Experimental site

The field experiment was conducted in a paddy rice field at Changshu Agroecological Experimental Station (31°32'93"N, 120°41'88"E) in Jiangsu province, which is located in the TLR of China where the cropping system is primarily dominated by summer rice (*Oryza sativa* L.) and winter wheat (*Triticum aestivum* L.) rotation. The climate of the study area is subtropical monsoon, with a mean air temperature of 16.1°C and mean annual precipitation of 990 mm, of which 60–70% occurs during the rice-growing season. The daily mean temperature and precipitation during two rice-growing seasons from 2013 to 2014 are shown in Fig.1. The paddy soil is classified as ~~an~~ Anthrosol, which develops from lacustrine sediments. The topsoil (0–20cm) has a pH of 7.68 (H₂O). The bulk density is 1.16 g cm⁻³, the organic C content is 20.1 g C kg⁻¹, the total N is 1.98 g kg⁻¹, the available P is 11.83 mg kg⁻¹ and the available K is 126 mg kg⁻¹.

2.2 Experimental design and field management

The field experiment included five treatments of different N fertilization rates for rice production: 0 (RN0), 120 (RN120), 180 (RN180), 240 (RN240) and 300 kg N ha⁻¹ (RN300, traditional N applied rate in the TLR). Consistent with local practices, wheat straws were harvested, chopped and fully incorporated into soil before rice transplantation in all treatments

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(Table 1). All of the treatments are laid out in a randomized block design with three replicates, and each plot covered an area of 3 m × 11 m (33 m²).

Rice is transplanted in the middle of June and harvested at the beginning of November. N fertilizer (in the form of urea) was split into three parts during the rice-growing season: 40% as basal fertilizer, 30% as ~~tillering~~tiller fertilizer, and 30% as panicle fertilizer. Phosphorus (in the form of calcium superphosphate) and potassium (in the form of potassium chloride) were applied as basal fertilizer at rates of 30 kg P₂O₅ ha⁻¹ and 60 kg K₂O ha⁻¹, respectively. All basal fertilizers were thoroughly incorporated into the soil through plowing, while topdressing fertilizers were applied evenly to the soil surface. According to local practices, the water regime of ‘flooding-midseason drainage-flooding-moist but non-waterlogged by intermittent irrigation’ was adopted. Details of the specific agricultural management practices for rice production are provided in Table 1.

2.3 Gas fluxes and topsoil organic carbon sequestration rate

The CH₄ and ~~nitrous oxide~~ (N₂O) fluxes during the rice-growing seasons of 2013 and 2014 were measured using a static chamber and gas chromatography technique. Details of the procedures used for sampling and analysis the gases were described in Xia et al. (2014).

~~Considering the fact that~~Generally, it takes long-term observations over years to decades before the soil organic carbon ~~sequestration rate (SOC SR)~~(SOC) change is detectable (Yan et al., 2011). The SOC content changes of this short-term field experiment ~~could not~~couldn't be correctly measured ~~directly, due to the high variability of SOC during the preliminary several years of the~~ experiment. Therefore, we used the following relationship between the straw input rate (kg C ha⁻¹ yr⁻¹) and SOC sequestration rate (SOC SR) (~~kg C ha⁻¹ yr⁻¹~~), obtained ~~via~~through an on-going

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long-term straw application experiment in the same region, to calculate the SOCSR in this study:

(Xia et al., 2014):

$$\text{SOC SR} = \text{Straw input rate} \times 0.0603 + 31.39 \quad (R^2 = 0.92); \quad (1)$$

This on-going long-term field experiment is also taking place at the Changshu

Agroecological Experimental Station (since 1990), which includes three straw application levels:

0, 4.5 t, and 9.0 t dry-weight $\text{ha}^{-1} \text{yr}^{-1}$ and the N application rate for rice cultivation in these

treatments is 180 kg N ha^{-1} . The estimated SOCSR (from 1990 to 2012) for these three treatments

was 10.65, 194.96 and $254.83 \text{ kg C ha}^{-1} \text{yr}^{-1}$ (Xia et al., 2014). The equation (1) was established

based on above straw input rates and the estimated SOCSR. We used the average straw input

rates the results of the two rice growing seasons to estimate the SOCSR. The on going long term

experiment and the experiment in this study received similar 22-year observation (Xia et al., 2014).

Same agricultural managements. Details of management practices were applied to the on-going

long-term experiment are described in Xia et al. (2014) and the experiment of this study.

2.4 Net global warming potential and greenhouse gas intensity

The net global warming potential (NGWP, $\text{kg CO}_2 \text{ eq ha}^{-1}$) and greenhouse gas intensity

(GHGI, $\text{kg CO}_2 \text{ eq kg}^{-1}$) of rice production in the TLR was calculated using the following

equations:

$$\text{NGWP} = \sum_{i=1}^m \text{AI}_{\text{ico}_2} + \text{CH}_4 \times 25 + \text{N}_2\text{O} \times 44/28 \times 298 - \text{SOC SR} \times 44/12; \quad (2)$$

$$\text{GHGI} = \text{NGWP}/\text{rice yield}; \quad (3)$$

Here, AI_{ico_2} denotes the GHG emissions from the production and transportation of agricultural

inputs, which are calculated by multiplying their application rates by their individual GHG

emission factors, such as synthetic fertilizers, diesel oil, electricity and pesticides (Liang, 2009;

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Zhang et al., 2013). CH₄ (kg CH₄ ha⁻¹), N₂O (kg N ha⁻¹) and SOCSR (kg C ha⁻¹ yr⁻¹) represent the CH₄ emissions and N₂O emissions from rice production, and the SOC sequestration rate, respectively.

2.5 Total Nr losses and Nr intensity

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The total Nr losses (kg N ha⁻¹) and Nr intensity (NrI, g N kg⁻¹) were calculated using the following equations:

$$\text{Total Nr losses} = \sum_{i=1}^m AI_{iNr} + (NH_3 + N_2O + N_{\text{Leaching}} + N_{\text{Runoff}})_{\text{rice}}; \quad (4)$$

$$NH_3 \text{ volatilization} = 0.17 \times \frac{N_{\text{fertilizer rate}}}{N_{\text{rate}}} + 0.64;$$

(5)

$$N \text{ runoff} = 5.39 \times \frac{N_{\text{fertilizer rate}}}{N_{\text{rate}}} \times \exp(-0.0054 \times \frac{N_{\text{fertilizer rate}}}{N_{\text{rate}}});$$

(6)

$$N \text{ leaching} = 1.44 \times \frac{N_{\text{fertilizer rate}}}{N_{\text{rate}}} \times \exp(-0.0037 \times \frac{N_{\text{fertilizer rate}}}{N_{\text{rate}}});$$

(7)

$$NrI = (1000 \times \text{Total Nr losses}) / \text{rice yield}; \quad (8)$$

Here, AI_{iNr} denotes the Nr lost (mainly through N₂O and NO_x emissions) from the production and transportation of agricultural inputs (Liang, 2009; Zhang et al., 2013), while '(NH₃+N₂O+N_{Leaching}+N_{Runoff})_{rice}' represents the NH₃ volatilization, N₂O emissions, N leaching and runoff during the rice-growing season. ~~We conducted~~ N_{rate} represents the N fertilizer application rate. Nr empirical models (Equation 5, 6, 7) derived from a meta-analysis of published literature to establish Nr empirical models to stimulate the concerning Nr losses, such as NH₃ volatilization (Equation 5), N leaching and runoff (Equation 6 and 7), from different treatments from rice production in the TLR. Specific details regarding this literature survey are provided in

Appendix A.

2.6 Total environmental costs incurred by GHG and Nr releases and farmer's income

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The total environmental costs (¥ ha⁻¹) incurred by GHG and Nr releases and farmer's income from rice production in the TLR ~~was~~were calculated based on the following equations:

$$\text{Environmental costs} = \sum_{i=1}^n (\text{Nr}_i \text{A} \times \text{DC}_i) + \text{CO}_2 \text{A} \times \text{DC}_{\text{CO}_2}; \quad (9)$$

$$\text{Farmer's income} = \text{rice yield} \times \text{rice price} - \text{input costs}; \quad (10)$$

Nr_iA (kg N) represents the release amounts of certain Nr species (i), and DC_i (¥ kg⁻¹ N) denotes the damage cost (DC) per kg of certain Nr (i). CO₂A (ton) and DC_{CO₂} (¥ ton⁻¹) represent the CO₂ emissions amount and global warming cost of CO₂, respectively. N₂O is both a GHG and ~~an~~ Nr species, but its environmental cost was calculated as a GHG here. Because the cost of N₂O emission as Nr species is to damage human health (Gu et al., 2012), but the effects of Nr losses on the damage costs of human health were not included in this study. The environmental costs mainly refer to the global warming incurred by GHG emissions, soil acidification incurred by NH₃ and NO_x emissions, and aquatic eutrophication caused by NH₃ emissions, N leaching and runoff (Xia and Yan, 2012).

2.7 Nitrogen use efficiency and N₂O emission factor

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Nitrogen use efficiency (NUE) ~~is~~and N₂O emission factor (EF_g%) were respectively calculated by the following ~~equation~~equations (Ma et al., 2013; Yan et al., 2014):

$$\text{NUE} = (\text{U}_\text{N} - \text{U}_0) / \text{F}_\text{N}; \quad (11)$$

$$\text{EF}_\text{g}\% = (\text{E}_\text{N} - \text{E}_0) / \text{F}_\text{N}; \quad (12)$$

Here, U_N is the plant N uptake (kg ha⁻¹) measured in ~~aboveground biomass~~grain at physiological maturity in the N fertilization treatments, while U₀ is the N uptake measured in ~~aboveground~~

~~biomass~~grain in the treatment without N fertilizer addition (RN0). ~~E_N denotes the cumulative N_2O~~
~~emissions in the N fertilization treatments, while E_0 denotes the N_2O emissions in the RN0. F_N~~
~~represents the application rate of N fertilizer.~~ The N uptake in straw and grain was analysed via
concentrated sulfuric acid digestion and the Kjeldahl method (Zhao et al., 2015).

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2.8 Statistical analysis

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Differences in seasonal CH_4 , N_2O emissions and rice yield of the two rice-growing seasons
from 2013 to 2014 affected by fertilizer treatments, year and their interaction were examined by
using a two-way analysis of variance (ANOVA) (Table 2). The grain yield, seasonal CH_4 and N_2O
emissions, SOCSR and GHGI of ~~the~~ different treatments were tested by analysis of variance, and
mean values were compared by least significant difference (LSD) at the 5% level. All these
analyses were carried out using the SPSS (Version 19.0, USA).

3 Results and discussion

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3.1 Rice yield and NUE

The two-way ANOVA analyses indicated that the rice grain yields were significantly affected
by the year and fertilizer treatment (Table 2). The farmer's practice plot (RN300) had an average
rice grain yield of 8395 kg ha^{-1} , with an NUE of 31.35%, over the two growing seasons from
2013 to 2014. Compared with RN300, reducing the N fertilizer rate by 20% (RN240) slightly
improved the grain yield and NUE to 8576 kg ha^{-1} and 34.58%, respectively. Further N reduction,
without additional agricultural managements, could decrease the rice yield by 8.15% (RN180) and
15.18% (RN120) (Table 3). The response of rice yield to the synthetic N application rate in our
study successfully fitted a quadratic model (Fig.2), as has been reported in previous studies (Xia
and Yan, 2012; Cui et al., 2013a). Reducing N application ~~to a reasonable rate~~reasonably,

therefore, is considered essential to reduce environmental costs, without sacrificing grain yield (Chen et al., 2014). ~~Lowering~~Our study showed that lowering the N input adopted by local farmer (300 kg N ha⁻¹) by 20% could still enhance the grain yield and NUE, ~~without threatening food security in this study~~. However, a further reduction of N 40% (RN180) would largely undermine the rice yield (Table 3).

Further reduction in N fertilizer may be achieved with improvements of agricultural managements, Ju et al. (2009) reported that, based on knowledge-based N managements, such as optimizing ~~the~~ N fertilizer source, rate, timing and place (in accordance with crop demand), rice grain yield in the TLR was not significantly affected by a 30–60% N saving, while various Nr losses would endure a two-fold curbing. Similarly, Zhao et al. (2015) found that the NUE could be improved from 31% to 44%, even under a N reduction of 25% for rice production in the TLR, through the implementation of integrated soil-crop system managements. In the present study, the NUE was improved by 10% via a 20% N reduction, but it still falls behind the NUE values in the studies which received knowledge-based N managements. Previous studies have proven that straw incorporation exerted little ~~positive~~ impacts on grain yield. For instance, a meta-analysis conducted by Singh et al. (2005) have found that incorporation of crop straw produced no significant trend in improving crop yield in rice-based cropping systems. Moreover, based on a long-term straw incorporation experiment established since 1990 ~~at Changshu Agroecological Experimental Station in the TLR~~, Xia et al. (2014) have reported that long-term incorporation of wheat straw only increased the rice yield by 1%. Therefore, in the present study, the effects of straw incorporation on rice yield were considered as inappreciable.

3.2 CH₄, N₂O emissions and SOSCR

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Over the two rice-growing seasons from 2013 to 2014, all treatments ~~produced~~showed similar patterns of CH₄ fluxes, albeit with large inter-annual variation (Fig.3a). The seasonal average CH₄ emissions from all plots showed no significant difference, ranging from 289.53 kg CH₄ ha⁻¹ in the RN180 plot to 334.61 kg CH₄ ha⁻¹ in the RN120 plot (Table 4), much higher than observations conducted in the same region (Zou et al., 2005; Ma et al., 2013). This phenomenon can be attributed to the larger amounts of straw incorporation in this study (Table 1). Relative to the RN300 plot, CH₄ emissions from the RN240 plot decreased by 8% and 10%, during the rice-growing season of 2013 and 2014, respectively, although this effect was not statistically significant (Table 4).

Many studies have shown a clear linear relationship between CH₄ emissions and the amounts of applied organic matter (OM). ~~Such an obvious linear relationship generally occurs under the following conditions: first, the OM inputs are low (generally less than 3 Mg dry matter ha⁻¹) (Zou et al., 2005; Ma et al., 2013); second, the applied OM rates among different treatments are statistically different (Shang et al., 2011; Xia et al., 2014). (Shang et al., 2011; Xia et al., 2014).~~ It is possible that the linear response of CH₄ emissions to OM inputs can become flat or even unobvious (Fig.S1), when ~~OM is applied at higher rates (in this study, the applied rates of straw in all N fertilization treatments were higher than 4.4 Mg dry matter ha⁻¹) and these rates among treatments were not statistically different. Besides, the experimental error caused by small differences in water conditions among different treatments may also have promoted the unclear response of CH₄ emissions to straw inputs in this study (Xia et al., 2014).~~

the OM applied rates among different treatments were insignificant different (Table S1). It is unsurprising that no obvious relationship between CH₄ emissions and N fertilizer application rates

was observed in this study (Fig.S1), because the effects of N fertilization on CH₄ production, transportation and oxidation are complex. For instance, N fertilization can provide methanogens with more carbon substrates in the rhizosphere of plants by stimulating the growth of rice biomass, thus promoting CH₄ production and transportation (Zou et al., 2005; Banger et al., 2012). On the other side, N enrichment could also enhance the activities of methanotrophs, therefore enhancing CH₄ oxidation (Xie et al., 2010; Yao et al., 2012). ~~Moreover, ammonium-based fertilizer could compete with CH₄ oxidation, due to the similar size and structure between NH₄⁺ and CH₄ (Linguist et al., 2012a).~~

The N₂O fluxes were sporadic and pulse-like, and these fluxes showed large variations between different seasons, and the majority of the N₂O peaks occurred after the application of N fertilizer (Fig.3b). The two-way ANOVA analyses indicated that the seasonal N₂O emissions were significantly affected by the year, the fertilizer treatment, and their interactions during the rice-growing seasons (Table 2). The average N₂O emission, during the two rice-growing seasons, ranged from 0.05 kg N ha⁻¹ for the RN0 to 0.35 kg N ha⁻¹ for the RN300 (Table 4), which increased exponentially as the N fertilizer rate increased. this highlights that the reduction of N fertilizer rate is an effective approach to reduce the N₂O emissions (Zou et al., 2005; Zhang et al., 2012). The average N₂O emission factors varied between 0.03% and 0.1%, with an average of 0.07%, which is comparable with previous studies (0.05%–0.1%) conducted in the same region (Ma et al., 2013; Zhao et al., 2015).

The rice paddies have witnessed an increase in the SOC stock as a result of straw incorporation (Table 4). The estimated topsoil (0–20cm) SOCSR varied from 0.43013 t C ha⁻¹ yr⁻¹ for the RN0 plot to 0.197 t C ha⁻¹ yr⁻¹ for the RN300 plot ~~(Table 4). The~~. The empirical

model established through a long-term straw incorporation study in the same region was employed to evaluate the SOCSR in this study, which likely brought uncertainty into the results of this study. Under the same agricultural managements, soil and climatic conditions, cropping systems and straw types, it is reasonable to believe that the rates of straw C stabilizing into SOC (i.e. conversion efficiency of crop residue C into SOC) are similar between these two experiments (Mandal et al., 2008). It is reported that the conversion rates of crop straw to SOC in two main wheat/maize production regions in China, which have similar climatic conditions and agricultural practices, were very close, at 40.524 versus 40.607 kg SOC-C t⁻¹ dry-weight straw (Lu et al., 2009). Moreover, the current estimated SOCSR for rice production in the TLR (0.197 t C ha⁻¹), falling within the SOCSR range of 0.13–2.20 t C ha⁻¹ yr⁻¹ estimated by Pan et al. (2004) for paddy soils in China, is also comparable to the estimation of 0.17 t C ha⁻¹ yr⁻¹ from Ma et al. (2013) in a study based on a paddy field experiment with OM incorporation in the same region. Moreover, Therefore, we hold the provincial average opinion that the above SOCSR of Jiangsu province has been estimated to be 0.16–0.21 t C ha⁻¹ yr⁻¹ from the period of 1980 to 2000 (calculation method is appropriate, and the uncertainty incurred by this method unlikely affects the main conclusions of this study).

The magnitude of the SOC increase is variable depending on the straw incorporation method, the degree of tillage, the cropping systems and etc. (Yan et al., 2011; Huang & Sun, 2006; Liao et al., 2009), which is also similar to our estimation. et al., 2013). Liu et al. (2014) suggested that straw incorporation in rice-based cropping systems requires an overall consideration, due to the direct incorporation promoting substantial CH₄ emissions. When converting to CO₂ eq, the SOCSR only offsets the CH₄ emissions by 6.2–9.2% in this study (Table 4). This proportion is

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expected to increase provided that appropriate straw incorporation method (e.g., compost straw before incorporation) and conservative-tillage are adopted. Moreover, previous studies have shown that the combined adoption of conservative-tillage system with straw return had large advantages in increasing SOC stocks while reducing CH₄ emissions (Zhao et al., 2015a; Zhao et al., 2015b).

3.3 NGWP and GHGI

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The average NGWP for all treatments varied from 8656 to 11550 kg CO₂ eq ha⁻¹ (Table 4). CH₄ emissions dominated the NGWP in all treatments, with the proportion ranging from 70.23% to 88.56%, while synthetic N fertilizer production was the secondary contributor (Table 4). In addition, SOC sequestration offset the positive GWP by 5.18–6.18% in the fertilization treatments. Compared to conventional practice (RN300), the NGWP in the 20% reduction N practice (RN240) decreased by 10.64%. Therein, 6.28% came from CH₄ reduction and 4.31% from N production savings (Table 4). The GHGI of rice production ranged from 1.20 (RN240) to 1.61 (RN0) kg CO₂ eq kg⁻¹, which is higher than previous estimation of 0.24–0.74 kg CO₂ eq kg⁻¹ for rice production in other rice-upland crop rotation systems (Qin et al., 2010; Ma et al., 2013). Moreover, the GHGI of current rice production in the TLR (RW300) was estimated to be 1.45 times that of the national average value estimated by Wang et al. (2014a), at 1.38 versus 0.95 kg CO₂ eq kg⁻¹.

Such phenomenon was attributed to the following reasons. First, compared to above studies, current higher amounts of direct straw incorporation (2.9–6.2 Mg dry matter ha⁻¹), before rice transplantation in the TLR, triggered substantial CH₄ emissions (290–335 kg CH₄ ha⁻¹). Crop residue incorporation is regarded as a win-win strategy to benefit food security and mitigate

climate change, due to the fact that it possesses a large potential for carbon sequestration (Lu et al., 2009). However, the GWP of straw-induced CH₄ emissions was reported to be 3.2–3.9 times that of the straw-induced SOCSR, which indicates direct straw incorporation in paddy soils worsens rather than mitigates climate changes, in terms of GWP (Xia et al., 2014). The SOC sequestration induced by straw incorporation only offset the positive GWP by 5.2–6.2% in this study. Sensible methods of straw incorporation should therefore be developed to reduce the substantial CH₄ emissions without compromising the build-up of SOC stock in the TLR.

Second, the high N application rate (300kg N ha⁻¹) in the TLR combined with the large emission factor of N fertilizer ~~manufactureproduction~~, 8.3 kg CO₂-eq kg⁻¹ N (Zhang et al., 2013), ~~promotedmarked~~ the sector of N fertilizer production ~~to-beas~~ the secondary contributor to the GHGI (Table 4), ~~while such); this~~ sector ~~wasn't, however, was not~~ involved in above-mentioned studies. Compared to local farmer's practices (RN300), reducing the N rate by 20% (RN240) lowered the GHGI by 13%, under the condition of straw incorporation, although this effect was not statistically significant (Table 4). Compared to RN240, however, further reduction of N rate (RN180 or RN120) increased the GHGI, ~~largely~~ due to the fact that rice yield was considerably ~~underminedreduced~~ under excessive N reduction. Therefore, the joint application of reasonable N reduction and judicious method of straw incorporation would be promising in reducing the GHGI for rice production in the TLR, in consideration of the current situation of simultaneous high inputs of N fertilizer and wheat straw.

3.4 Various Nr losses and NrI

The results of the meta-analysis indicated that N₂O emissions, as well as N leaching and runoff, ~~increaseincreased~~ exponentially with an increase in N application rate (Fig.4b-d, $P < 0.01$),

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while the response of NH_3 volatilization to N rates fitted the linear model best (Fig.4a, $P < 0.01$).

~~Established models can explain the variation in the estimation of various Nr losses by 50-57%.~~

The estimated total Nr losses for all treatments varied from 39.3 to 91.7 kg N ha⁻¹ in the

fertilization treatments (Table 5), accounting for 30.1–32.8% of N application rates. NH_3

volatilization dominated the NrI, with the proportion ranging from 53.5% to 57.4%, mainly

because of the current fertilizer application method (soil surface ~~broadcasting~~broadcast) and high

temperatures in the field (Zhao et al., 2012b; Li et al., 2014). N runoff was the second most

important contributor, ~~with the proportion ranging from 25.9% to 29.7%~~ (Table 5). Using ¹⁵N

micro-plots combined with three-year field measurements, Zhao et al. (2012b) reported that the

total Nr ~~loss~~losses from rice production in the TLR, under an N rate of 300 kg N ha⁻¹, ~~was~~were 98

kg N ha⁻¹, which is comparable with our estimation of 91.69 kg N ha⁻¹ in the RN300 plot.

Similarly, Xia and Yan (2011) estimated the Nr ~~loss~~losses for life-cycle rice production in this

region to be around 90 kg N ha⁻¹. The high proportion (30.1–32.8%) of the applied N fertilizer

released as Nr from rice production in the TLR, highlights the need to adopt reasonable N

managements to increase the plant N uptake and reduce Nr losses (Ju et al., 2009).

The NrI of rice production in different plots varied between 2.14 g N kg⁻¹▲(RN0) and 10.92 g

N kg⁻¹▲(RN300), which increased significantly as the N fertilizer rate increased (Table 5). The NrI

for rice production in the TLR was estimated to be 10.92 g N kg⁻¹▲(RN300), which is 68% higher

than the national average value estimated by Chen et al. (2014), ~~largely due to the~~ as a result of

higher N fertilizer ~~inputs~~input in the TLR. Under the condition of straw incorporation, reducing

~~the~~ N application rate by 20% pulled the NrI down to 8.42 g N kg⁻¹ (RN240) (Table 5). Additional

N reduction could further lower the NrI, but the rice yield would be compromised largely (Table

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3). Previous studies have proven that direct incorporation of crop straw ~~exert unobvious~~had insignificant effects on various Nr releases (Xia et al., 2014). Because ~~crop straws usually possess high values of C/N ratio and~~ the majority of N contented in the ~~residue~~crop straw is not easily degraded by microorganisms in ~~a short-term period~~(Huang et al., 2004). Therefore the straw ~~incorporation could promote the N contained in the residues to, and can~~ be stabilized in soil in ~~a long-term period, rather than directly releasing~~being released as various Nr (Huang et al., 2004; Xia et al., 2014). For instance, a meta-analysis, integrating 112 scientific assessments of the crop residue incorporation on the N₂O emissions, has reported that the practice exerted no statistically significant effect on the N₂O releases (Shan and Yan, 2013). Therefore, the effects of wheat straw incorporation on various Nr losses were considered as negligible in this study. ~~Although no specific relationship was found between the NrI and GHGI in all treatments in this study (Table 4 and Table 5),~~

Extra attention should be paid to the interrelationship between ~~them the NrI and GHGI,~~ which could provide hints for the mitigation purpose. For instance, N fertilizer production and application is an intermediate link between the NrI and GHGI ~~and NrI~~ (Chen et al., 2014). For the NrI, N fertilization promotes various Nr releases, exponentially or linearly (Fig.4), while N production and application made a secondary contribution to the GHGI (Table 4). Such interrelationships ought to be taken into account fully for any mitigation options pursued, in order to reduce the GHG emissions and Nr discharges from rice production simultaneously (Cui et al., 2013b; Cui et al., 2014).

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3.5 Economic evaluations of GHG emissions and Nr releases and their mitigation potential

The total environmental costs associated with the GHG emissions and Nr releases varied from 1214 ¥ ha⁻¹ for the RN0 to 2399 ¥ ha⁻¹ for the RN300, which approximately accounted for 10.44–13.47% of the farmer's income and 27.05–32.47% of the input costs, respectively (Table 6). CH₄ emission and NH₃ volatilization were the dominant contributors to the total environmental costs, respectively (Table 4 and Fig.5). The total damage costs to environment accounted for 13.5% of farmer's income under the current rice production in the TLR (RN300). Cutting the N rate from 300 to 240 kg N ha⁻¹ slightly improved the farmer's income by 3.64%, while further N reduction would ~~undermine~~reduce the economic return of farmer's (Table 6).

GHG and Nr releases from rice production in the TLR are expected to possess a large potential for mitigation, due to the current situation of direct straw incorporation and higher N fertilizer inputs. Compared to traditional practice, a reduction of N application rate from 300 to 240 kg N ha⁻¹ could alleviate 12.52% for GHGI (Table 4), 22.94% for NrI (Table 5), and 15.76% for environmental costs (Table 6). Further reduction in GHG and Nr releases (especially for CH₄ emissions and NH₃ volatilization) is possible, with the implementation of knowledge-based managements (Chen et al., 2014; Nayak et al., 2015). For the mitigation of Nr releases, switching the N fertilizer application method from surface ~~broadcasting~~broadcast to deep incorporation could largely lower the NH₃ volatilization from paddy soils (Zhang et al., 2012; Li et al., 2014). Moreover, other optimum N managements, such as applying controlled-release fertilizers and ~~nitrification or~~ urease inhibitors, could also effectively increase the NUE and reducing the overall Nr losses (Chen et al., 2014). For the mitigation of GHG emissions, rather than being directly incorporated before rice transplantation, crop residues should be preferentially decomposed under aerobic conditions or used to produce biochar through pyrolysis, which could effectively reduce

CH₄ emissions (Linguist et al., ~~2012b~~2012; Xie et al., 2013). Moreover, these pre-treatments are also beneficial for carbon sequestration and ~~food security~~yield production (Woolf et al., 2010; Linguist et al., ~~2012b~~2012).

Most previous studies have merely focused on the quantification of GHG and Nr releases from food production from the perspective of environment assessments (Zhao et al., 2012b; Ma et al., 2013; Zhao et al., 2015). The perspective of economic evaluation is seldom implemented, which goes against encouraging farmer to participate in the abatement of GHG and Nr releases on their own initiative (Xia et al., 2014). The current pattern of rice production in the TLR incurs great costs to the environment, which accounted for 13.47% of the net economic return that farmer ultimately acquire (Table 6). Such an evaluation facilitates the translation of highly specialized scientific conclusions into monetary-based information that is more familiar and accessible for ~~farmer~~farmers, and therefore likely encouraging them to adopt eco-friendly agricultural managements (Wang et al., 2014b). Profitability is generally considered the main driver for farmer to change their management approach. Compared to traditional N application rate, a reduction of 20% would make environmental costs savings of 14%, whilst simultaneously improving the economic return of farmer's by 648 ¥ ha⁻¹ (Table 6). This represents an incentive for ~~farmer~~farmers to optimize their N fertilizer application rates, provided that such information is available to them.

Considering the fact that no specific carbon- and Nr-mitigation incentive programs, like the 'Carbon Farming Initiative' in Australia (Lam et al., 2013), ~~has~~have been launched in China, an ecological compensation incentive mechanism (~~national subsidy program~~) should be established by governments. ~~This~~This should be a national subsidy program with a special compensation and

award fund to cover the extra mitigation costs induced by the adoption of knowledge-based
mitigation managements for farmers (Xia et al., 2016). Such a program would provide
~~farmer~~farmers with a tangible incentive, thus guiding them towards gradually adopting
~~knowledge-based~~the mitigation managements, ~~that~~which could effectively curb GHG emissions
and Nr losses, but likely exert little positive effects on improving ~~farmer's~~their net economic
return (Xia et al., 2014). Examples include the composing of crop straws aerobically, or their use
to produce biochar before incorporation (Xie et al., 2013), and encouraging the application of deep
placement of N fertilizer (Wang et al., 2014b), as well as the application of enhanced-efficiency N
fertilizers during the rice-growing season (Akiyama et al., 2010).

4 Conclusions

Our results demonstrated that producing ~~per-unit-of~~ rice yield in the TLR released
~~higher~~substantial GHG and Nr ~~in the TLR, than that in other rice upland cropping systems~~, which
largely attributed to the current ~~situation-of~~ direct straw incorporation and excessive ~~nitrogen~~N
fertilizer inputs. CH₄ emissions and NH₃ volatilization dominated the GHG and Nr releases,
respectively. Reducing ~~the~~ N application rate by 20% from the tradition level (300 kg N ha⁻¹)
could effectively decrease the GHG emissions, Nr releases and the damage costs to the
environment, while increased the rice yield and improved farmer's income ~~as well simultaneously~~.
Agricultural managements, such as making straw decompose aerobically before its incorporation
and optimizing the application method of N fertilizer, ~~could~~showed large potentials to further
reduce the GHG (e.g., CH₄ emission) and Nr releases (~~especially CH₄ emissions and~~ e.g., NH₃
volatilization) from rice production in ~~the TLR~~this region. Further studies are needed to evaluate
the comprehensive effects of these managements on GHG emissions, Nr releases and farmer's

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economic returns.

Acknowledgements

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Supplementary material

Supplementary material (Appendix A) associated with this article can be found, in the online version.

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Table 1. Field experimental treatments and agricultural management practices during the rice-growing seasons of 2013 and 2014 in the ~~TLR~~Taihu Lake region.

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| Treatment ^a | RN0 | RN120 | RN180 | RN240 | RN300 |
|------------------------|-----|-------|-------|-------|-------|
|------------------------|-----|-------|-------|-------|-------|

| | | | | | |
|---|------------------------|-----------|-----------|-----------|-----------|
| Chemical fertilizer | | | | | |
| application rate | 0:30:60 | 120:30:60 | 180:30:60 | 240:30:60 | 300:30:60 |
| (N:P ₂ O ₅ :K ₂ O, kg ha ⁻¹) | | | | | |
| Split N application ratio | --- | 4:3:3 | 4:3:3 | 4:3:3 | 4:3:3 |
| Straw application rate | 3.94/2.88 ^b | 4.49/4.65 | 4.93/5.18 | 5.33/5.87 | 5.81/6.17 |
| (Mg dry matter ha ⁻¹) | | | | | |
| Water regime ^c | F-D-F-M | F-D-F-M | F-D-F-M | F-D-F-M | F-D-F-M |
| Density (10 ⁴ plants ha ⁻¹) | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |

^aRN0, RN120, RN180, RN240 and RN300 represent ~~nitrogen~~N application rates of 0, 120, 180, 240, 300 kg N ha⁻¹, respectively.

^b3.94/2.88 denote that straw application rates during the rice-growing seasons of 2013 and 2014 are 3.94 and 2.88 Mg dry matter ha⁻¹, respectively.

^cF, flooding; D, midseason drainage; M, moist but non-waterlogged by intermittent irrigation.

Table 2. Two-way ANOVA for the effects of fertilizer (F) application and year (Y) on CH₄ and N₂O emissions, and rice grain yields in rice paddies.

| Factor | df | CH ₄ (kg ha ⁻¹) | N ₂ O (kg N ha ⁻¹) | Yield (kg ha ⁻¹) |
|--------|----|--|---|------------------------------|
|--------|----|--|---|------------------------------|

| | | SS | F | P | SS | F | P | SS | F | P |
|-------|----|-------|------|------|------|-------|--------|----------|-------|--------|
| F | 4 | 8739 | 0.79 | 0.55 | 0.33 | 12.46 | < 0.01 | 39297547 | 32.96 | < 0.01 |
| Y | 1 | 4492 | 1.62 | 0.22 | 0.11 | 16.41 | < 0.01 | 2810414 | 9.43 | < 0.01 |
| F×Y | 4 | 2532 | 0.23 | 0.92 | 0.18 | 7.1 | < 0.01 | 750639 | 0.63 | 0.65 |
| Model | 9 | 15763 | 0.63 | 0.77 | 0.62 | 10.52 | < 0.01 | 42858600 | 15.97 | < 0.01 |
| Error | 16 | 20 | | | 0.13 | | | 5962260 | | |

Table 3. Rice yield and nitrogen use efficiency (NUE) for the two rice-growing seasons from 2013 to 2014 in the TLR Taihu Lake region

| Year | Treatment ^a | Yield (kg ha ⁻¹) | NUE (%) |
|------|------------------------|------------------------------|---------|
| 2013 | RN0 | 4829 ± 207 | --- |

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| | | | |
|------------------|-------|--------------------------|-------|
| | RN120 | 7079 ± 645 | 23.40 |
| | RN180 | 7655 ± 601 | 28.12 |
| | RN240 | 8273 ± 569 | 33.61 |
| | RN300 | 8029 ± 101 | 30.63 |
| 2014 | RN0 | 5919 ± 131 | --- |
| | RN120 | 7598 ± 1077 | 23.86 |
| | RN180 | 7768 ± 570 | 21.19 |
| | RN240 | 8880 ± 435 | 35.54 |
| | RN300 | 8761 ± 369 | 32.07 |
| Two-year average | RN0 | 5374 ± 617d ^b | --- |
| | RN120 | 7339 ± 843c | 23.63 |
| | RN180 | 7711 ± 527bc | 24.66 |
| | RN240 | 8576 ± 562a | 34.58 |
| | RN300 | 8395 ± 468ab | 31.35 |

687 ^aDefinitions of the treatment codes are given in the footnotes of Table 1.

688 ^bMean±SD; different letters within the same column indicate a significant difference at $p<0.05$.

Table 4. The net global warming potential (NGWP) and greenhouse gas intensity (GHGI) for the two rice-growing seasons from 2013 to 2014 in the TLR Taihu Lake region

| Year | Treatment ^a | CH ₄ emission | N ₂ O emission | SOCSR | Irrigation | N fertilizer production | Others | NGWP | GHGI |
|------|------------------------|-------------------------------------|---------------------------|--|--|----------------------------|--------|-------|--|
| | | kg CH ₄ ha ⁻¹ | kg N ha ⁻¹ | kg C ha ⁻¹ yr ⁻¹ | kg CO ₂ eq ha ⁻¹ | | | | kg CO ₂ eq kg ⁻¹ |
| 2013 | RN0 | 306.07 ± 41 ^b | 0.08 ± 0.01 | 129.58 | 1170 | 0 | 217 | 8601 | 1.78 |
| | RN120 | 317.26 ± 92 | 0.10 ± 0.01 | 154.07 | 1170 | 996 | 265 | 9845 | 1.39 |
| | RN180 | 287.8 ± 12 | 0.13 ± 0.01 | 171.54 | 1170 | 1494 | 277 | 9568 | 1.25 |
| | RN240 | 273.27 ± 36 | 0.14 ± 0.06 | 185.50 | 1170 | 1992 | 291 | 9670 | 1.17 |
| | RN300 | 305.13 ± 90 | 0.16 ± 0.03 | 196.87 | 1170 | 2490 | 285 | 10927 | 1.36 |
| 2014 | RN0 | 307.22 ± 47 | 0.02 ± 0.05 | 129.58 | 1256 | 0 | 240 | 8711 | 1.47 |
| | RN120 | 351.96 ± 28 | 0.09 ± 0.02 | 154.07 | 1256 | 996 | 276 | 10805 | 1.42 |

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| | RN180 | 291.25 ± 18 | 0.24 ± 0.04 | 171.54 | 1256 | 1494 | 280 | 9795 | 1.26 |
| | RN240 | 317.65 ± 28 | 0.34 ± 0.12 | 185.50 | 1256 | 1992 | 303 | 10972 | 1.24 |
| | RN300 | 343.8 ± 61 | 0.53 ± 0.21 | 196.87 | 1256 | 2490 | 301 | 12169 | 1.39 |
| Two-year | RN0 | 306.65 ± 39a | 0.05 ± 0.05b | 129.58c | 1213 | 0 | 229 | 8656 | 1.61 ± 0.25a |
| average | RN120 | 334.61 ± 64a | 0.09 ± 0.02b | 154.07bc | 1213 | 996 | 271 | 10322 | 1.40 ± 0.16b |
| | RN180 | 289.53 ± 14a | 0.18 ± 0.07ab | 171.54ab | 1213 | 1494 | 279 | 9679 | 1.25 ± 0.09bc |
| | RN240 | 295.46 ± 38a | 0.24 ± 0.14ab | 185.50ab | 1213 | 1992 | 297 | 10321 | 1.20 ± 0.08cd |
| | RN300 | 324.47 ± 72a | 0.35 ± 0.25a | 196.87a | 1213 | 2490 | 293 | 11550 | 1.38 ± 0.21bc |

^aDefinitions of treatment codes are given in the footnotes of Table 1.

^bMean±SD; different letters within same column indicate a significant difference at $p<0.05$.

Table 5. The seasonal average ~~various reactive N (Nr)~~ losses and ~~reactive N intensity (NrI)~~ for the two rice-growing seasons from 2013 to 2014 in the ~~TLR~~Taihu Lake region

| Treatment ^a | NH ₃ | N | N | N ₂ O | NO _x | Total Nr | NrI |
|------------------------|-----------------------|--------|----------|------------------|-----------------|----------|----------------------|
| | volatilization | runoff | leaching | emission | emission | losses | |
| | kg N ha ⁻¹ | | | | | | g N kg ⁻¹ |
| RN0 | 0.64 | 5.39 | 1.44 | 0.07 | 3.96 | 11.50 | 2.14 |
| RN120 | 21.04 | 10.30 | 2.24 | 0.12 | 5.62 | 39.32 | 5.36 |
| RN180 | 31.24 | 14.25 | 2.80 | 0.21 | 6.44 | 54.93 | 7.12 |
| RN240 | 41.44 | 19.70 | 3.50 | 0.27 | 7.26 | 72.17 | 8.42 |
| RN300 | 51.64 | 27.24 | 4.37 | 0.38 | 8.07 | 91.69 | 10.92 |

^aDefinitions of treatment codes are given in the footnotes of Table 1.

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Table 6. The ~~seasonal average~~ economic ~~evaluation indicators (two-season average)~~ for rice production of the ~~two~~ growing seasons from 2013 to 2014 in the ~~TLR~~Taihu Lake region (unit: ¥ ha⁻¹)

| Treatment ^a | Yield income ^b | Input costs ^c | Farmer's income ^d | Environmental costs ^e | |
|------------------------|---------------------------|--------------------------|------------------------------|----------------------------------|-------------|
| | | | | GHG emissions | Nr releases |
| RN0 | 16125 | 4493 | 11632 | 1143 | 71 |
| RN120 | 22020 | 6104 | 15916 | 1363 | 376 |
| RN180 | 23130 | 6542 | 16588 | 1278 | 535 |
| RN240 | 25725 | 7277 | 18448 | 1362 | 700 |
| RN300 | 25185 | 7385 | 17800 | 1525 | 874 |

^aDefinitions of treatment codes are given in the footnotes of Table 1.

^bYield income = rice yield × rice price.

^cInput costs denote the economic input of purchasing various agricultural materials and hiring labours.

^dFarmer's income = Yield income – ~~input~~Input costs.

^eEnvironmental costs denoted the sum of the acidification costs, eutrophication costs and global warming costs incurred by GHG emissions and Nr releases. The cost prices of GHG and Nr releases are as followed: GHG emission, 132 ¥ t⁻¹ CO₂eq (Xia et al., 2014); NH₃ volatilization, 13.12 ¥ kg⁻¹ N; N leaching, 6.12 ¥ kg⁻¹ N; N runoff, 3.64 ¥ kg⁻¹ N; NO_x emission, 8.7 ¥ kg⁻¹ N (Xia and Yan, 2011).

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Figure captions

Fig. 1. Seasonal variations in the daily precipitation and the temperature during the two rice-growing seasons of (a) 2013 and (b) 2014.

Fig.2. Relationship between N fertilizer application rate and seasonal average rice grain yield over the two rice-growing seasons of 2013 and 2014 in the TLR, Taihu Lake region.

The vertical bars represent standard errors ($n=6$).

Fig.3. Seasonal variations in (a) CH₄ and (b) N₂O fluxes during the two rice-growing seasons from 2013 to 2014 in the TLR, Taihu Lake region. The arrow indicates N fertilizer application.

The vertical bars represent standard errors ($n=3$).

Fig.4. Relationship between N fertilizer application rate and (a) NH₃ emissions volatilization, (b) N runoff, (c) N leaching and (d) N₂O emissions for rice production in the TLR Taihu Lake region. These relationships were obtained through a meta-analysis.

Fig.5. Seasonal average total environmental costs incurred by greenhouse gas (GHG) emissions and reactive N (Nr) losses for rice production in TLR Taihu Lake region.

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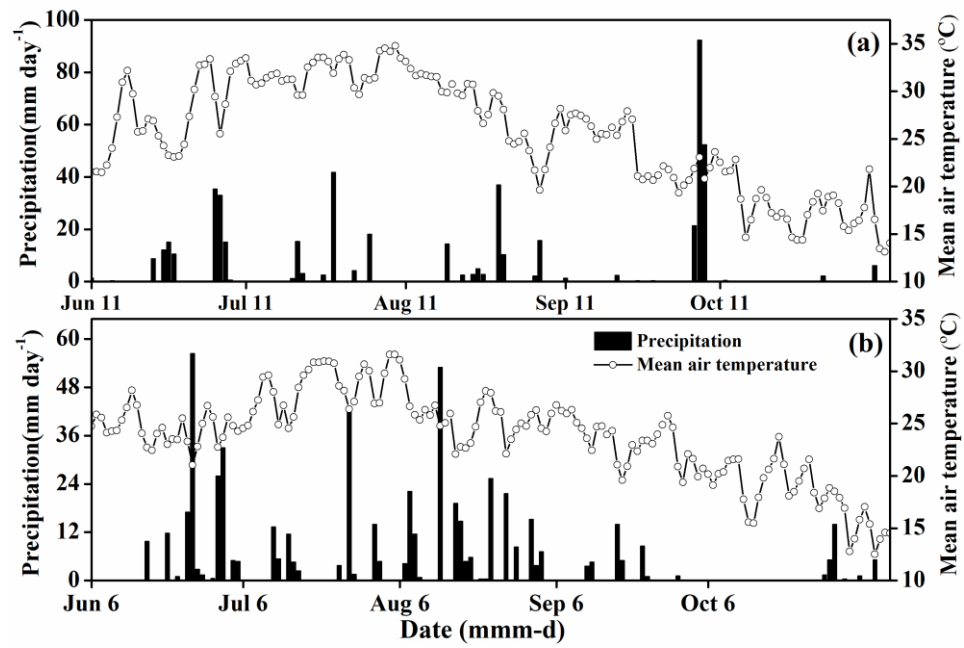


Fig.1

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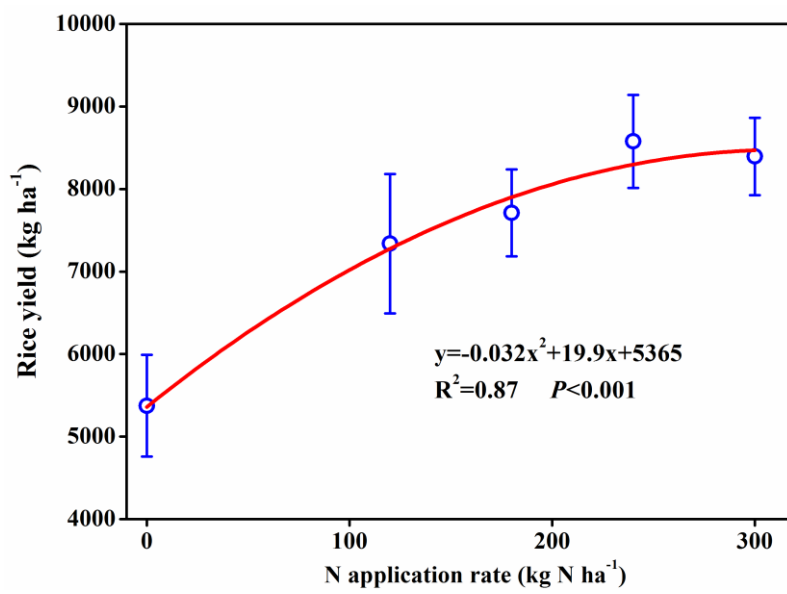


Fig.2

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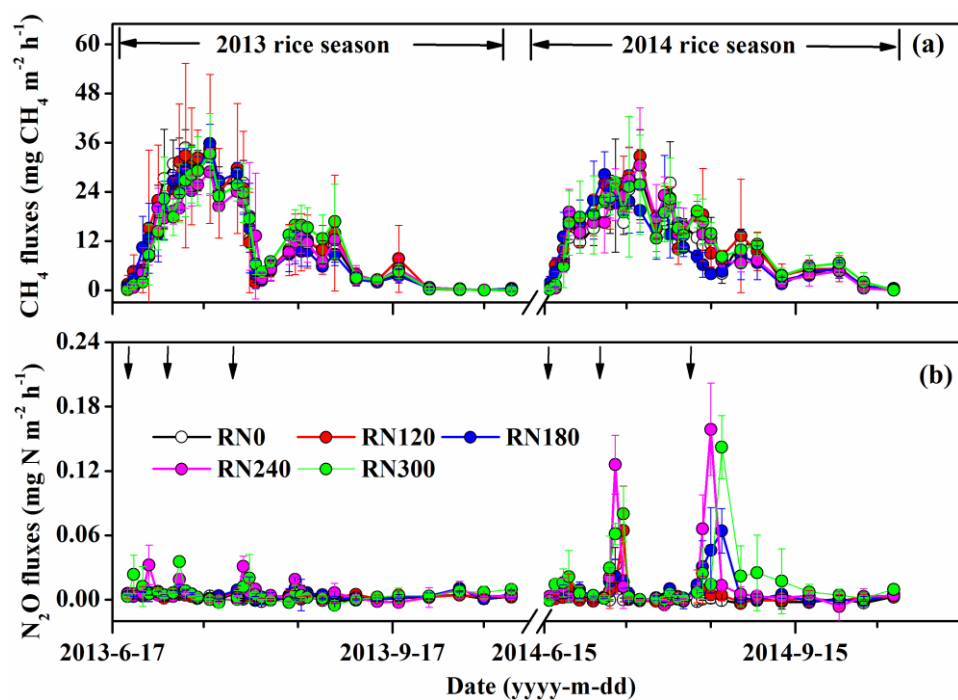


Fig.3

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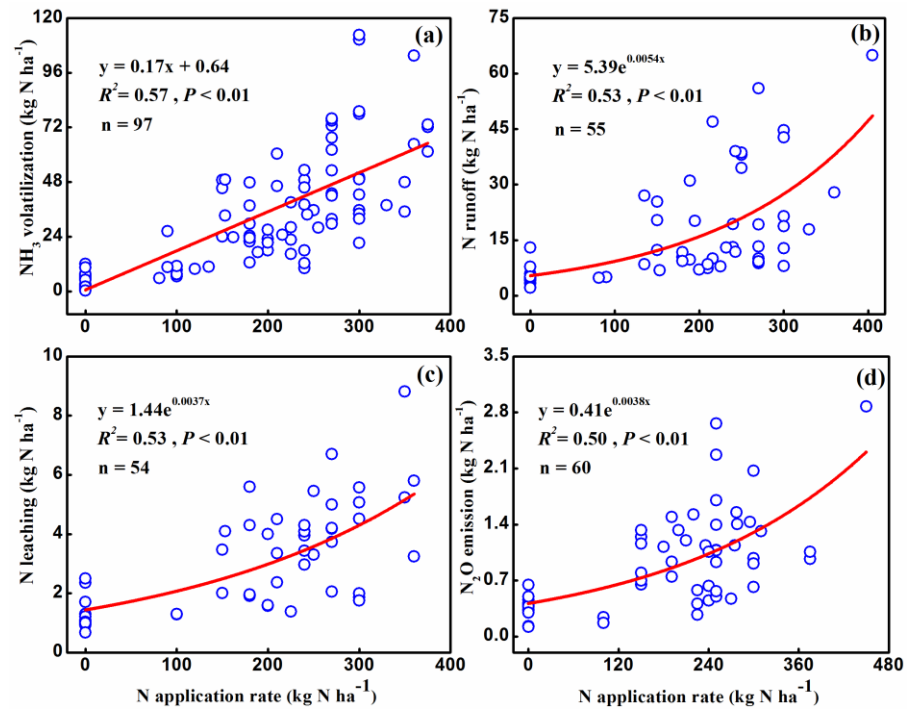


Fig.4

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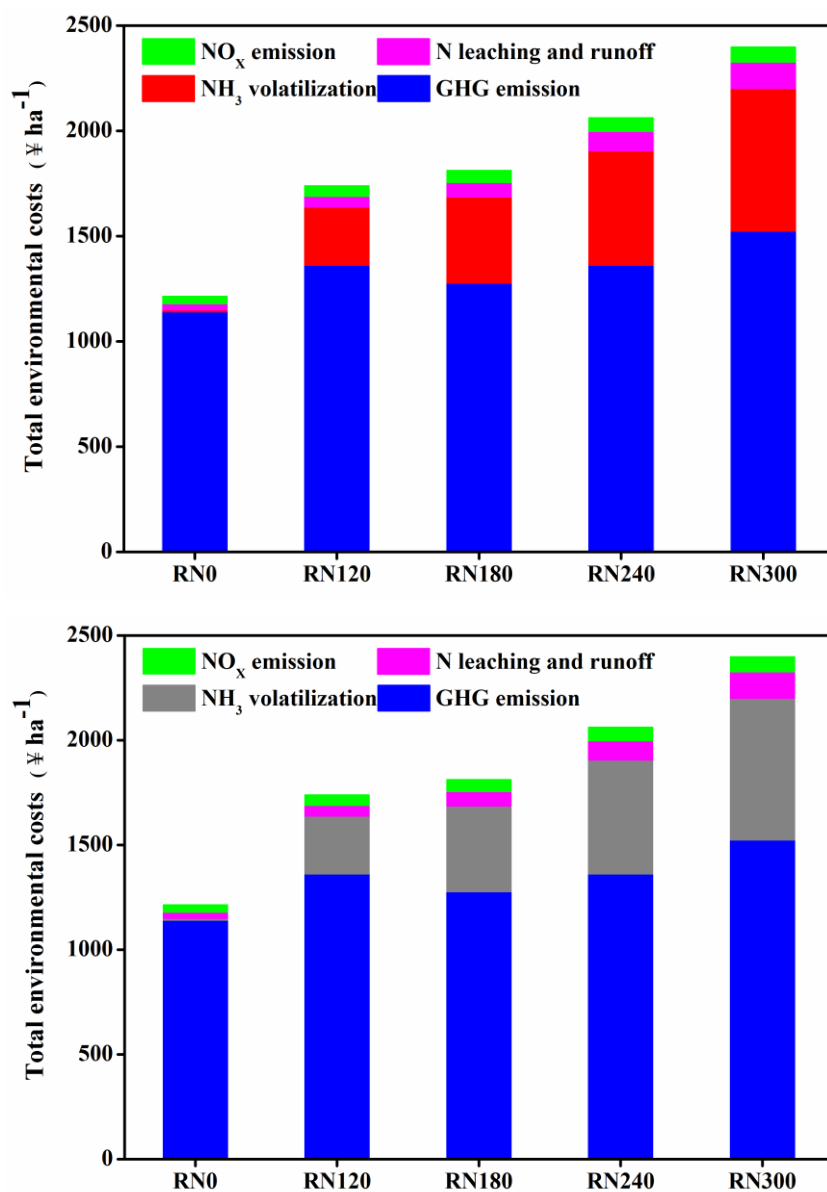


Fig.5

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