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Article type : Primary Research Articles

Simultaneous quantification of N_2, NH_3 and N_2O emissions from a flooded paddy field under different N fertilization regimes

Running Title: Gaseous N losses from paddy fields

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Abstract: Gaseous nitrogen (N) emissions, especially emissions of dinitrogen (N_2) and ammonia (NH_3), have long been considered as the major pathways of N loss from flooded rice paddies. However, no studies have simultaneously evaluated the overall response of gaseous N losses to improved N fertilization practices due to the difficulties to directly measure N_2 emissions from paddy soils. We simultaneously quantified emissions of N_2 (using membrane inlet mass spectrometry), NH_3 and nitrous oxide (N_2O) from a flooded paddy field in southern China over an entire rice-growing season. Our field experiment included three treatments: a control treatment (no N addition) and two N fertilizer (220 kg N ha^{-1}) application methods, the traditional surface application of N fertilizer and the incorporation of N fertilizer into the soil. Our results show that over the rice-growing season, the cumulative gaseous N losses from the surface application treatment accounted for 13.5% (N_2), 19.1% (NH_3), 0.2% (N_2O) and 32.8% (total gaseous N loss) of the applied N fertilizer. Compared with the surface application treatment, the incorporation of N fertilizer into the soil decreased the emissions of NH_3, N_2 and N_2O by 14.2%, 13.3% and 42.5%, respectively. Overall, the incorporation of N fertilizer into the soil significantly reduced the total gaseous N loss by 13.8%, improved the fertilizer N use efficiency by 14.4%, increased the rice yield by 13.9% and reduced the gaseous N loss intensity (gaseous N loss/rice yield) by 24.3%. Our results indicate that the incorporation of N fertilizer into the soil is an effective agricultural management practice in ensuring food security and environmental sustainability in flooded paddy ecosystems.

Keywords: N_2 emissions; soil denitrification; gaseous N losses; incorporation of N fertilizer into the soil; flooded paddy fields

1 Introduction

Rice is a staple food and feeds nearly 50% of the global population. To meet the increasing demand for rice production by the world's growing population, large amounts of nitrogen (N) fertilizer are applied to paddy fields, particularly in China, where N consumption and rice production are the highest in the world (Ray *et al.*, 2012; Zhang *et al.*, 2015). It is estimated that the average N fertilizer application rate for rice production in China is about 240 kg N ha^{-1} season^{-1}, which is two to three times higher than the application rate in Japan (80 kg N ha^{-1} season^{-1}) and USA (140 kg N ha^{-1} season^{-1}) (Linquist *et al.*, 2014; Xia *et al.*, 2016b). Unfortunately, the nationwide approach of applying N fertilizer to the surface of flooded rice paddies in China has resulted in a significant loss of the applied N to the environment through gaseous N emissions (Zhu *et al.*, 1989; Zhao *et al.*, 2012).

The emission of dinitrogen (N_2) from soil denitrification is considered to be a major gaseous N loss pathway particularly in flooded paddy fields, where the strictly anaerobic environment promotes the complete reduction of nitrate or nitrite to N_2, through the intermediates of nitrous oxide (N_2O) and nitric oxide (NO) (Davidson & Verchot, 2000; Butterbach-Bahl *et al.*, 2013). Quantifying N_2 emissions from flooded paddy fields is therefore an essential prerequisite for the development of improved N management to increase fertilizer N use efficiency (Wang *et al.*, 2017; Zistl-Schlingmann *et al.*, 2019). In addition to N_2 emission, ammonia (NH_3) emission is another major gaseous N loss pathway in paddy fields, which, in contrast to N_2 emissions, has been well studied during the past two decades (Zhao *et al.*, 2012; Xia *et al.*, 2016a). Overall, in a three-year paddy rice field experiment in southern China, Zhao *et al.* (2012) estimated, using a ^{15}N tracer method, that the emission of N_2 and NH_3 represented 51.9% of the fertilizer N broadcast on the soil surface, whereas the loss of N via hydrological pathways (e.g., N leaching and runoff) only accounted for 7.4%. Reducing the loss of gaseous N through optimizing the fertilizer application method is therefore vital to improve both the efficiency of N fertilizer use and the rice yield (Chen *et al.*, 2014).

Compared with surface application, the incorporation of N fertilizer into the soil is considered to be effective in reducing the loss of gaseous N while maintaining or even increasing the crop yield (Gaihre *et al.*, 2015; Xia *et al.*, 2016a; Zhang *et al.*, 2017). A meta-analysis conducted by Xia *et al.* (2016a) demonstrated that the incorporation of N fertilizer into the soil significantly decreased the emission of NH_3 from paddy fields by 26.4% and increased the yield of rice by 7.0%. Yao *et al.* (2017) reported that using a slow-release urea fertilizer incorporated into the soil decreased N_2O emissions from paddy fields by 40.9–56.8%.

Few studies have simultaneously quantified the response of N_2 emissions to different N fertilization practices. This is because the direct measurement of N_2 fluxes from soils had not been feasible given the high background concentrations of N_2 in the atmosphere (Groffman *et al.*, 2006; Li *et al.*, 2014). However, this shortcoming can be overcome by using ^{15}N-labeled fertilizers or, for flooded rice paddies, membrane inlet mass spectrometry (MIMS), which measures the concentration of dissolved N_2 in floodwaters (Li *et al.*, 2014). Previous studies have reported a trade-off between NH_3 and N_2 emissions with respect to their responses to the incorporation of fertilizer into the soil during the early stages of rice growth (Freney *et al.*, 1990; Li *et al.*, 2014). Li *et al.* (2014) reported that the incorporation of N fertilizer into the soil decreased the emission of NH_3 by 34.7%, but increased the emission of N_2 by 47.2% compared with the surface application of urea during the first 21 days after rice transplantation. However, no study has explored whether this trade-off (between NH_3 or N_2O and N_2 emissions) exists over the entire rice-growing season. This has impeded the assessment of the long-term effectiveness of incorporating N fertilizer into the soil in reducing N loss from paddy fields.

N_2 emissions from denitrification process in terrestrial and aquatic systems are currently measured using acetylene (C_2H_2) inhibition methods, ^{15}N tracers and mass balance approaches (Groffman *et al.*, 2006). However, all of these methods have their own limitations. For example, the C_2H_2 inhibition method may underestimate denitrification because of the simultaneous inhibition of the reduction of N_2O to N_2. By contrast, the ^{15}N tracer method usually overestimates N_2 emissions because the addition of $^{15}NO_3^-$ increases substrate availability for denitrifying microorganisms. The mass balance approach may accumulate systematic errors in the calculation of N_2 emissions (Gu *et al.*, 2015). In contrast to these methods, the MIMS and N_2/Ar technique allows a precise, direct measurement of N_2 fluxes from flooded environments (Kana *et al.*, 1994). This method provides rapid measurements (<2 min) of the concentration of N_2 in small volumes of water (<7 ml), and, thus, the calculation of N_2 fluxes, without the addition of ^{15}N tracers. The dissolved N_2 in water samples can be measured by MIMS at a precision of <0.03% for N_2/Ar when Ar is used as a conservative tracer to minimize the variations in the measurement of N_2.

The MIMS and N_2/Ar approach has been successfully used to quantify N_2 fluxes in aquatic and marine systems (Kana *et al.*, 1994; Groffman *et al.*, 2006; Zhao *et al.*, 2015). Recently Li *et al.* (2014) applied MIMS and the N_2/Ar approach to the direct quantification of N_2 emissions during the early stages of rice growth in a flooded paddy field in southern China. Using the same method, Wang *et al.* (2017) reported that the cumulative N_2 emissions over the rice-growing season represented 11.9% of the N fertilizer applied and showed that N_2 fluxes was significantly correlated with the concentration of NO_3^- in floodwaters, but not with the concentration of NO_3^- in soils. However, no study has yet reported N_2 emissions measured by MIMS and the N_2/Ar method simultaneously with the measurement of NH_3 and N_2O emissions over a full rice-growing season.

We simultaneously quantified N_2 (using MIMS and the N_2/Ar method), NH_3 and N_2O emissions from a flooded paddy field under two different N fertilizer application methods (surface application versus the incorporation of N fertilizer into the soil) in southern China over an entire rice-growing season. Our hypotheses were: (1) the cumulative losses of N_2 from the applied N fertilizer are higher than the losses of NH_3; (2) the losses of gaseous N (N_2+NH_3+N_2O) account for at least 50% of the N fertilizer applied; and (3) compared with surface application, the incorporation of N fertilizer into the soil decreases the emission of both NH_3 and N_2O, but increases the emission of N_2 over the whole rice-growing season.

2 Materials and methods

2.1 Study site

The field experiment was conducted at Changshu Agroecological Experimental Station (31.53° N, 120.68° E), Chinese Academy of Sciences, Jiangsu Province, China. This station is located in the Yangtze River Delta, which has a subtropical monsoon climate with a mean annual temperature of 15°C and a mean annual precipitation of 1100 mm. The dominant cropping system in this region is a rotation of summer rice and winter wheat. The soil at the experimental station is classified as an Anthrosol developed from lacustrine sediments. The topsoil (0–15 cm) has an organic carbon content of 20.3 g kg^{-1}, a total N content of 2.1 g kg^{-1}, a pH (H_2O) of 7.4 and a bulk density of 1.1 g cm^{-3}.

2.2 Field treatments and management practices

The field experiment was conducted during the rice-growing season in 2012. It included a control treatment (no N addition, CK) and two N fertilizer application treatments: the traditional surface application of N fertilizer (SA) and the incorporation of N fertilizer into the soil (IA). The three treatments were arranged in a randomized block design, each with three replicates. An earth ridge (30 cm width) covered by plastic film was used to separate the 4.5 m × 5 m plots. The plastic film was placed into the soil to a depth of 20 cm to prevent the exchange of water between the plots. Following local agricultural practice, the application rate of N fertilizer in the form of urea was 220 kg N ha^{-1}, which was applied in three stages during the rice-growing season (Table 1). The split is 50% as a basal fertilizer (BF), 30% as a tillering fertilizer (TF) and 20% as a panicle initiation fertilizer (PIF).

For the SA treatment, urea was broadcast evenly over the soil surface during the fertilization process. For the IA treatment, the basal N fertilizer (urea), applied before transplanting the rice seedlings, was broadcast over the soil surface and then immediately incorporated into the soil (about 3–5 cm) by plowing. By contrast, the N fertilizer (urea) was broadcast over the soil surface at the tillering and panicle initiation stage and was not incorporated for either treatment. Phosphorus and potassium fertilizers were incorporated into the soil as basal fertilizers before N fertilizer application in all treatments at rates of 90 kg P_2O_5 ha^{-1} and 90 kg K_2O ha^{-1}, respectively.

The rice seedlings were transplanted on June 17 and harvested on October 26 (130 days) (Table 1). Following local agricultural practice, the fields were flooded to a depth of 5 cm immediately after the application of the basal fertilizer and remained flooded for 40 days. The fields were drained for one week to promote plant root growth, and then re-flooded and kept flooded until October 14. The paddy fields were then kept moist, but not waterlogged, by intermittent irrigation until rice was harvested. The fields were flooded 113 days in total. During the wheat-growing season, prior to the rice-growing season investigated here, fertilizer N was surface applied at 200 kg N ha^{-1} (local practice) to all the plots at the split ratio of 5:3:2. This was to avoid any residual effects of different N fertilization in the wheat-growing season on the response of gaseous N losses to different N fertilization regimes in the rice-growing season.

2.3 N_2 fluxes

Soil N_2 fluxes were measured based on the semi-*in situ* intact soil core incubation method (Li *et al.*, 2014). We randomly collected three intact soil cores (10 cm depth) from each plot of the three treatment areas (27 soil cores in total) during each sampling event using a PVC core sampler (25 cm depth and 7 cm inner diameter). The soil cores were then sealed at the base with rubber stoppers (5 cm height) and immediately submerged in water collected from the corresponding plots. About 20 L of floodwater were collected from each plot for the incubation of the soil cores.

The collected floodwater was used to fully fill the PVC cores (12 cm depth) in the laboratory to drive all the bubbles out the cores. The cores were then capped with airtight tops equipped with inlet and outlet Teflon tubes for sampling. The soil cores were placed around a carousel housing four magnets, which gently rotated the stirring bars suspended 6 cm from the top of each soil core at 14 rpm. This mixed the overlying water to simulate the flow of floodwater under field conditions without disturbing the soil surface (Li *et al.*, 2013; Li *et al.*, 2014; Zhao *et al.*, 2015). The incubation temperature for the soil cores was set according to the floodwater temperature during field sampling.

Water samples were taken four times during the 6 h incubation period (at 0, 2, 4 and 6 h). Three replicate water samples (15 ml) were used for N_2/Ar analysis. For this, we filled 5 ml Exetainer vials (Labco, Lampeter, UK) directly from the outlet tube of each core, allowing overflow to avoid contamination of the samples with atmospheric N_2. To avoid further biological activity, the water samples were immediately treated with 20 μL 5% HgCl solution and then capped without headspace. The Exetainer vials were stored under water at a temperature of 1–2°C below the incubation temperature to prevent outgassing before analysis. A gravity feeding system automatically compensated for the volume of sampled water by adding the respective amount of previously sampled field floodwater. The physicochemical properties of the incubated soil (e.g., the inorganic N content and pH) were determined immediately after incubation.

Dissolved N_2 and Ar in the water samples were measured using MIMS (Bay Instruments, Easton, MD, USA), according to the procedure described in Kana *et al.* (1994) and Li *et al.* (2014). The changes in N_2 concentration were determined based on the changes in the measured N_2/Ar ratio multiplied by the concentration of Ar in water at air saturation, which was determined from solubility equations taking into account the water temperature (Weiss, 1970). The detection limit of MIMS for measuring changes in N_2/Ar is 0.03%.

The N_2 flux (μmol N_2 $L^{-1}h^{-1}$) of each soil core was calculated using four-point linear regression. The rates were then linearly prorated for the surface area of each core. The N_2 fluxes in the soil cores were corrected according to the fluxes measured in three control cores containing only distilled water (Li *et al.*, 2014; Zhao *et al.*, 2015). The N_2/Ar technique does not distinguish N_2 production from the reduction of N_2 by biological N fixation. Therefore, the changes in the N_2 concentration with time represent the net N_2 flux. The production of N_2 is derived from both denitrification and anammox processes, which are not distinguished by the N_2/Ar technique. Shan *et* *al.* (2016) reported that denitrification was the dominant pathway (76.8–92.5%) for the production of N_2 in paddy soils collected from the same study area and therefore we refer to the net N_2 production derived from "denitrification". The N_2 fluxes were measured once a week for a total of 13 measurements during the rice-growing season and more frequently after N fertilization events for all treatment plots. The N_2 fluxes were calculated by linear interpolation between sampling times and the cumulative N_2 emissions were calculated by summing all the daily fluxes over the rice-growing season.

2.4 NH_3 and N_2O fluxes

The sponge tracking and KCl extraction methods were used *in situ* to measure the emission of NH_3 from the paddy field (He *et al.*, 2002). Two sponges were spiked with 20 mL of a glycerol–phosphoric acid (H_3PO_4) mixture containing 40 mL of glycerol, 50 mL of concentrated H_3PO_4 and 910 mL of deionized water. The sponges were then inserted into a container (20 cm long and 20 cm inner diameter) in each plot (three containers per plot and 3 × 9 containers for all plots). One sponge was inserted into the base of the container (5 cm above the floodwater surface) and was used to trap the NH_3 volatilized from the paddy field. The other sponge was fitted into the top of the container to protect the sponge at the base from contamination by atmospheric NH_3. The container with two sponges was placed in the paddy field for 24 hours during the measurement of the NH_3 flux, which took place on the same day as the measurement of the N_2 flux. The NH_3 trapped in the lower sponge was extracted with 1 M KCl and then measured using a flow injection analyzer (Skalar Analytical, Breda, the Netherlands).

N_2O fluxes were measured using static chamber and gas chromatography techniques. In each plot, a chamber (50 cm × 50 cm × 50 cm, or 50 cm × 50 cm × 100 cm, depending on the height of the crop) was placed on a PVC frame inserted into the soil before the first gas measurement and remained in the soil throughout the experimental period. For each sampling activity, four gas samples were obtained from the chamber headspace using syringes (20 ml) at the time intervals of 10 min. Sampling was completed between 9:00 to 11:00 am. All the gas samples in the syringes were directly analyzed using gas chromatography within 12 hours of sampling. The N_2O and NH_3 fluxes for the days between the sampling times were calculated by linear interpolation and the cumulative emissions were calculated by summing all the daily fluxes over the rice-growing season.

2.5 Modeling N fluxes

Overall, 13 sampling activities were conducted to measure the fluxes of gaseous N in all treatment plots during the rice-growing season. In order to assess whether the sampling frequency was adequate, we constructed linear mixed effects models (LMMs) based on the relationship between the measured gaseous N fluxes and corresponding properties of the floodwater (the NH_4^+ and NO_3^- concentrations and pH) and environmental variable (air temperature) during the 13 sampling activities ($13 \times 3_{\text{treatment}} \times 3_{\text{replicate}} = 117$) (Table S1 and Fig. S4). The LMMs were used to simulate the gaseous N fluxes during the rice-growing season based on the data derived from high-frequency floodwater sampling activities (3–4 times per week and totally 84 times over the season) (Figs S4 and S5). During each sampling activity, 5 floodwater samples (100 mL each) were randomly collected from each plot and then homogeneously mixed as one sample, which was analyzed for the NH_4^+ and NO_3^- concentrations and pH. The cumulative gaseous N emissions calculated based on the LMM model simulated N fluxes (Fig. S5) were compared with those derived from the fluxes measured in the field (Fig. 1) to examine the possible uncertainties due to the relatively low sampling frequency. More detail about the construction of the LMMs is given in the Supporting Information.

2.6 Collection and analysis of auxiliary data

The daily mean air temperature and precipitation during the rice-growing season were measured by an automatic weather station at the experimental station (Fig. S1). The concentration of NH_4^+ and NO_3^- in floodwater and soil samples was measured using a flow injection analyzer (Skalar Analytical), while the pH was measured with a portable instrument (Hach Company, Loveland, CO, USA). The fertilizer use efficiency (NUE, %) was calculated using the equation:

$$NUE = 100(U_N - U_0)/F_N \quad (1)$$

where U_N is the above-ground (straw and grain) N content (kg N ha^{-1}) measured in the fertilized plots and U_0 is the above-ground N content in the control treatment plots. F_N represents the N fertilizer application rate (220 kg N ha^{-1}). The gaseous N loss intensity (GNLI, g N kg^{-1} grain) was calculated by dividing the total gaseous N loss by the grain yield:

$$GNLI = 1000(\text{gaseous N loss}/\text{grain yield}) \quad (2)$$

where the gaseous N loss denotes the total of the three gaseous N losses (kg N ha^{-1}).

At the mature stage (October 26), the aboveground biomass of the rice plants in each plot was all harvested and separated into straw and grain, and then air-dried and weighed. A subsample of the straw and grain was then prepared and oven dried for 24h at 80 °C before being ground and analyzed for the N content using the concentrated sulfuric acid digestion and Kjeldahl method (Zhao *et al.*, 2012).

2.7 Statistical analyses

The differences in the seasonal N_2, NH_3 and N_2O emissions, grain yield, aboveground N uptake, NUE and GNLI among the treatments were tested by one-way ANOVA followed by the least significant difference test at the 5% level since the data was normally distributed. The figures were prepared using Origin (version 10.0).

3 Results

3.1 NH_4^+ and NO_3^- concentrations in floodwater

Among all the treatments, the NH_4^+ and NO_3^- concentrations in floodwater of the paddy field ranged from 0.13 to 29.8 mg N L^{-1} and 0.007 to 0.67 mg N L^{-1}, respectively (Fig. 1d,e). Each N fertilization event clearly increased the NH_4^+ and NO_3^- concentrations in the floodwater. For different N fertilization periods, compared to the incorporated application (IA) treatment, the surface application of N fertilizer (SA) treatment increased the average NH_4^+ concentration in the floodwater by 6.5–22.4% during all N fertilization periods ($P > 0.05$) (Table S2). In contrast, the average NO_3^- concentration in IA treatment was significantly higher than that in SA treatment during the basal fertilizer (BF) period ($P < 0.05$), although no significant difference was found between these two treatments during the tillering fertilization (TF) and panicle initiation fertilization (PIF) periods.

3.2 N_2 fluxes

We observed similar N_2 flux patterns among the fertilization treatments during the rice-growing season (Fig. 1a). Among all the treatments, the N_2 fluxes ranged from −0.07 to 0.71 kg N ha^{-1} day^{-1}. The highest N_2 flux was observed for the SA treatment, which occurred shortly after the PIF application. The N_2 fluxes in the CK treatment were consistently lower than those in the other two treatments. N fertilization significantly increased the N_2 fluxes in the both SA and IA treatments, with N_2 emission peaks occurring after each fertilization event. With respect to different N fertilization periods, IA treatment increased the average N_2 flux by 15.1% compared with SA treatment during the BF period ($P > 0.05$), but decreased the flux by 5.2% ($P > 0.05$) and 20.7% ($P < 0.05$) during the TF and PIF periods, respectively (Fig. 2a). The average N_2 fluxes in SA (0.45 kg N ha^{-1} day^{-1}) and IA (0.43 kg N ha^{-1} day^{-1}) treatments over the rice-growing season did not differ statistically, but were significantly higher than the CK treatment. The cumulative N_2 emissions over the rice-growing season ranged from 24.1 (CK) to 53.7 (SA) kg N ha^{-1} among all treatments (Fig. 3a). Compared with the control treatment, N fertilization significantly increased N_2 emissions by 94–123%. The seasonal N_2 emission for IA treatment was 13.3% lower than that from SA treatment ($P > 0.05$), although the average N_2 flux for SA treatment was 14.0% lower than for IA treatment during the BF period ($P > 0.05$) (Fig. 2a).

3.3 NH_3 fluxes

The NH_3 fluxes ranged from 0.08 to 3.24 kg N ha^{-1} day^{-1} across all treatments (Fig. 1b). The highest NH_3 flux was observed for SA treatment, which appeared shortly after the BF application. The average NH_3 flux from IA treatment was lower than the SA treatment by 20.1, 7.9 and 2.3% during BF, TF and PIF periods, respectively (Fig. 2b), albeit not significantly. The average NH_3 flux over the rice-growing season in SA and IA treatments was not statistically different at 0.79 and

0.68 kg N ha^{-1} day^{-1}, respectively, which was 4.2 and 3.6 times the average NH_3 flux of the control treatment. The cumulative NH_3 emissions over the rice-growing season varied from 20.1 (CK) to 62.1 (SA) kg N ha^{-1} among different treatments. The NH_3 emissions from the N fertilization treatments were significantly higher (165–209%) than for the CK treatment (Fig. 3b). The incorporation of N fertilizer into the soil significantly decreased NH_3 emissions by 14.2% compared with the surface application method.

3.4 N_2O fluxes

The N_2O fluxes across all treatments varied from −0.0007 to 0.0456 kg N ha^{-1} day^{-1} (Fig. 1c). The highest N_2O flux was observed for the SA treatment, with peak emissions occurring upon the PIF application. The N_2O fluxes from the control treatment were negligible and consistently lower than those observed for the SA and IA treatments. The average N_2O fluxes for the IA treatment were 57.2 and 63.9% lower than the SA treatment across the TF and PIF periods ($P > 0.05$), respectively (Fig. 2c). As a result, the average N_2O fluxes over the rice-growing season for the SA treatment (0.0072 kg N ha^{-1} day^{-1}) were nearly twice as high as for the IA treatment (0.0038 kg N ha^{-1} day^{-1}). The CK treatment had negligible cumulative N_2O emissions (0.05 kg N ha^{-1}), but the emissions were much higher in the N fertilization treatments (0.25–0.43 kg N ha^{-1}) (Fig. 3c). The incorporation of N fertilizer into the soil decreased N_2O emissions by 42.5% compared with the surface application treatment ($P > 0.05$) (Fig. 3c).

3.5 Cumulative gaseous N losses and gaseous N loss intensity based on field measurements

The cumulative gaseous N emissions over the rice-growing season ranged from 44.2 (CK) to 116.3 (SA) kg N ha^{-1} among different treatments (Fig. 3d). Compared with the CK treatment, N fertilization significantly increased the total gaseous N emissions by 180–195%. Compared with SA treatment, IA treatment significantly decreased the total gaseous N emissions by 13.8%. Accompanying the lower gaseous N losses, the plant N uptake and fertilizer N use efficiency in IA treatment were 9.7%

and 14.4% higher than those in SA treatment (Fig. 4b,c). Moreover, the IA treatment also increased the rice grain yield and straw biomass by 13.9% and 10.3% compared with SA treatment (Fig. 4a). Consequently, the incorporation of N fertilizer into the soil significantly decreased the gas N loss intensity (GNLI) by 24.3% compared with the surface application treatment (Fig. 4d).

3.6 Gaseous N emissions derived from LMM simulations

The LMMs explained 55% of the variation in measured N_2 fluxes, 55% of the variation in NH_3 fluxes and 32% of the variation in N_2O fluxes (Fig. S2). There was no significant difference between the simulated N fluxes and the measured fluxes on most of the sampling days (except for the N_2O fluxes in the control treatment plots) (Fig. S3). The simulated cumulative N_2, NH_3 and total gaseous N emissions were lower than the emissions calculated from field measurements (Fig. 5), but the differences were not significant (Table S4). The simulated N_2O emissions in the N fertilization treatments were significantly higher than those measured in the field, although the N_2O emissions only contributed 0.1–0.4% (LMMs results 0.1–2.5%) of the total gaseous N emissions (Fig. 5).

4 Discussion

4.1 Proportion of gaseous N losses from applied N fertilizer

The N_2 emission from soil denitrification process is considered to be a major pathway for N losses, particularly in flooded paddy fields (Zhu *et al.*, 1989; Zhao *et al.*, 2012). The strict anaerobiosis in flooded paddy soils promotes the reduction of NO_3^- to N_2 through NO_2^-, NO and N_2O intermediates (Butterbach-Bahl *et al.*, 2013). Our results show that the cumulative N_2 emissions from soil denitrification accounted for 10.2–13.5% of the applied N fertilizer (Fig. 3). This is comparable with the 11.1% loss of N fertilizers applied to paddy fields reported by Wang *et al.* (2017), who also used MIMS and the N_2/Ar technique to quantify N_2 emissions. However, our estimate is much lower than denitrification losses reported by Zhao *et al.* (2012) and Ju *et al.* (2009) (22–36% of the N applied as fertilizer), who used ^{15}N tracer methods and a mass balance approach to estimate N_2 emissions from paddy fields in the same study area. The different proportions of N loss in these studies are probably due to the different methods used to quantify N_2 emissions (Groffman *et al.*, 2006).

The proportion of N lost from the applied N fertilizer as NH_3 (15.1–19.1%) was greater than that losses of N_2 (Fig. 3), which refutes our first hypothesis that N_2 losses from applied N fertilizer were greater than NH_3 losses. However, this finding highlights the importance of mitigating NH_3 emissions from paddy fields to increase the N use efficiency of rice production systems, which was only 37% in the SA treatment (Fig. 4). Unlike N_2 emissions, NH_3 emissions from flooded paddy fields in China have been well studied during the past two decades (Ti *et al.*, 2019). Meta-analyses conducted by Xia *et al.* (2016c) and Chen *et al.* (2014) reported that the average proportion of N lost through NH_3 emissions from applied N fertilizer was 17% (n = 78) and 16% (n = 265), respectively, for flooded paddy fields in the same study area (Yangtze River Delta region) and in China as a whole. Their reported proportions are very close to our results (15.1–19.1%).

N_2O emissions from flooded paddy fields are usually negligible because the strong anaerobic environment promotes complete denitrification, which favors further reduction of N_2O to N_2 (Butterbach-Bahl *et al.*, 2013). Our results show that the cumulative N_2O emissions accounted for only 0.09–0.2% of applied N fertilizer (Fig. 3), which is within the range (0.03–2%) reported in regional and global meta-analyses (Xia *et al.*, 2016c; Xia *et al.*, 2018).

Overall, the total emission of gaseous N (N_2, NH_3 and N_2O) accounted for 25.4–32.8% of the applied urea N. This refutes our second hypothesis that at least 50% of the N fertilizer applied to flooded paddy fields is lost to the environment through gaseous N pathways. Our results are much lower than the value of 48–52.1% reported by Zhao *et al.* (2012) and Ju *et al.* (2009), which may be attributed to differences in the measurements of N_2 emissions between the MIMS technique and the ^{15}N tracer method (Groffman *et al.*, 2006). In our study, the losses of gaseous N from applied N fertilizer (32.8%) were similar to the proportion of N taken up by plants (37.1%, Fig. 4) in the SA treatment, and much higher than the reported N leaching (3%) and runoff loss (4%) in the same study areas (Zhao *et al.*, 2012). This highlights the need to adopt reasonable N application methods to reduce gaseous N losses, particularly as NH_3 and N_2, to increase the NUE of paddy rice systems in China and elsewhere.

4.2 Effect of incorporating N fertilizer into the soil on gaseous N losses

Incorporating basal N fertilizer into the soil significantly decreased NH_3 emissions by 16.8% compared to surface application (Fig. 3). This proportion is slightly lower than the reduction of 26.4% reported in the meta-analysis of Xia *et al.* (2016a). The difference might be due to the difference in the placement of N fertilizer, which was at 3–5 cm depth in this study compared with 5–15 cm in the meta-analysis. Incorporating N fertilizer into the soil at a deeper depth can more effectively decrease the NH_4^+concentration in floodwater (Li *et al.*, 2014; Gaihre *et al.*, 2015), a key factor in controlling NH_3 volatilization from paddy fields (Wang *et al.*, 2017; Zhang *et al.*, 2017; Yao *et al.*, 2018). We found that the average NH_4^+ concentration in floodwater in the IA treatment was 18.3% lower than the SA treatment during the BF period (Fig. 1d), which resulted in 20.1% lower average NH_3 flux in the IA treatment during this period (Fig. 2). Moreover, incorporating basal N fertilizer into the soil also decreased NH_3 emissions during the TF and PIF periods (Fig. 2 and Table S2), as also observed in previous studies by Xu *et al.* (2013) and Meng *et al.* (2011).

In contrast to NH_4^+ concentrations, NO_3^- concentrations in floodwater were the main factor controlling N_2 emission from soil denitrification in flooded paddy fields in this study (Table S1) (Li *et al.*, 2014; Wang *et al.*, 2017). The NO_3^- concentrations in floodwater in SA treatment were always lower than IA treatment during the BF period (Fig. 1e and Table S2). This coincides with the higher N_2 fluxes in IA treatment during this period relative to SA treatment (Fig. 2). Our findings therefore confirm the trade-offs between NH_3 and N_2 emissions with respect to their response to the fertilizer incorporation during the early stages of rice growth (De Datta *et al.*, 1989; Freney *et al.*, 1990; Li *et al.*, 2014). i.e., reduced NH_3 volatilization was accompanied by increased N_2 losses after basal N fertilization. However, the IA treatment decreased N_2 emissions by 13.3% over the entire rice-growing season, although this decrease was not significant (Fig. 3). This refutes our third hypothesis that incorporating N fertilizer into soils decreases NH_3 emissions but increases N_2 emissions from paddy fields over the entire rice-growing season. Similar results were observed in a maize-cropping system on the North China Plain by Cai *et al.* (2002), where the N fertilizer incorporation decreased N_2 and NH_3 emissions by 6.3 and 56.8%, respectively.

Compared with the incorporation method, surface applied N requires a longer time to diffuse into soils and participate in nitrification–denitrification processes, thereby decreasing N_2 emissions during the BF period (Xia *et al.*, 2016a). Nevertheless, the incorporation of basal N fertilizer into the soil can gradually enhance root activity during the early stages of rice growth (Zhang *et al.*, 2017). This increased N uptake and plant biomass especially during the rapid growth stages of rice when N requirement was large, such as tillering and panicle initiation stages when TF and PIF were applied (Zhang *et al.*, 2012). As a result, we found that fertilizer N incorporation into the soil improved plant aboveground N uptake and biomass development in IA treatment compared with SA treatment (Fig. 4). This reduced the N availability in the soil (Fig. S6) and therefore reduced various gaseous N losses (N_2 and NH_3) in the IA treatment plots where the N fertilizer was incorporated into the soil, as observed in the TF and PIF periods (Fig. 2a and 2b). Overall, the lower N_2 emissions during the TF and PIF periods gradually outweighed the higher emissions during the BF period (Fig. S7), resulting in lower cumulative N_2 emissions for the IA treatment compared with the SA treatment over the entire rice-growing season (Fig. 3). These results also indicate that reducing the basal N fertilizer proportion but increasing the proportion to the later (tillering and panicle initiation) growth stages of rice, has high potential to further reduce the N_2 and NH_3 emissions from flooded paddy fields as by this crop N demand and N supply is better synchronized (Xia *et al.,* 2016a).

The lower N substrate availability in the IA treatment could also explain the observed 42.5% reduction in N_2O emissions relative to the SA treatment (Fig. 3). This percentage reduction is within the reported range of reduction in N_2O emissions (40.9–56.8% and 30.5–66.4%) observed for fertilizer incorporation in paddy fields in southern China (Yao *et al.*, 2017) and Bangladesh (Gaihre *et al.*, 2015; Gaihre *et al.*, 2018), respectively. The soil–floodwater interface is one of the most biologically active zones in flooded paddy fields (Xing *et al.*, 2009). It is the zone where the most N_2O is produced through coupled nitrification–denitrification processes and this production is stimulated by the supply of N substrates through the surface application of fertilizer (Venterea & Stanenas, 2008; Kessel *et al.*, 2013; Gaihre *et al.*, 2018). Nevertheless, the incorporation of N fertilizer in the deeper soil layers also stimulated N_2O production from nitrification-denitrification processes compared with the control plots (Fig. 3). Water saturation in soils slows or even prevents the diffusion of N_2O produced in deeper soil layers (Yao *et al.*, 2017), which might lead to the further reduction of N_2O to N_2 prior to its release to the atmosphere (Davidson *et al.*, 2000; Butterbach-Bahl *et al.*, 2013).

Compared with surface application, the incorporation of N fertilizer into the soil significantly decreased the total gaseous N losses (Fig. 3), which represents an increase in the fertilizer NUE. Previous studies have reported an exponential increase in NUE with decreasing N losses (Groenigen *et al.*, 2010; Cui *et al.*, 2013). As expected, the fertilizer NUE was increased by 14.4% in the IA treatment compared to SA treatment, which is also reflected by an increased rice yield in the IA treatment (Fig. 4). Through producing higher yield with lower gaseous N losses, IA treatment significantly decreased the gaseous N loss intensity (GNLI, 24.3%) compared to SA treatment. This indicates that the incorporation of N fertilizer into the soil can substantially improve the environmental sustainability of flooded paddy ecosystems. Although N_2 emissions are not detrimental to environmental sustainability itself, the use of N fertilizer (221 Gg N yr^{-1}) in rice cultivation in China could be reduced by about 3% if the fertilizers were incorporated into soils instead of being broadcast over the soil surface (Table S3). The greenhouse gas footprint of paddy rice production would therefore be decreased by about 1.85 Tg CO_2-equivalent yr^{-1} as a result of reduction in the manufacture of synthetic N fertilizer.

Based on a N balance calculation in the SA treatment, we found that about 23% of applied N fertilizer remains in the soil (Table S5), which is comparable with the proportion of 22% observed in a paddy field in the same study area (Yangtze River

Delta) (Ju *et al.*, 2009). This indicates that a reasonable reduction in the current high N fertilization rate would further increase the fertilizer NUE and decrease various N losses from the flooded paddy fields while sustaining the rice yield. A recent meta-analysis of paddy field studies in China demonstrated that decreasing the N fertilizer rate by 24% from the currently used rates would significantly increase NUE by 27% and decrease NH_3 emissions by 28%, N_2O emissions by 41% and hydrological N losses by 29–31% (Xia et *al.,*2016a). Therefore, the combined implementation of incorporating N fertilizer into soils with reasonable reductions in N rates, which should be based on soil N tests, has a huge potential in producing more rice at lower environmental costs.

4.3 Uncertainties

Previous studies have shown that a low sampling frequency might introduce uncertainties into the cumulative emission of gaseous N from soils (Barton *et al.*, 2015). We used LMMs to simulate three types of gaseous N emissions to examine whether a relatively low sampling frequency affected our main conclusions (Table S1). Our results showed that the emissions of gaseous N (except for the N_2O emissions) based on field measurements was not significantly different from the simulated emissions derived from the LMMs (Fig. 5 and Table S4). Both datasets support our main conclusion that the incorporation of N fertilizer into the soil is effective in decreasing the losses of gaseous N (N_2, NH_3 and N_2O) from flooded paddy fields.

The MIMS technique has its limitations. For example, this application of MIMS requires the incubation of intact soil cores (10 cm depth), which may underestimate the N_2 emissions from deeper soil layers. In addition, rice plants can transport O_2 into the rhizosphere, which may affect soil denitrification processes and associated N_2 emissions (Zhao *et al.*, 2012), but as rice plants are excluded in the cores analyzed for N_2 fluxes this effect was not considered in our study. Nevertheless, MIMS is a promising method that can be used to directly measure N_2 fluxes from flooded paddy fields.

5 Conclusions

This is the first study to simultaneously quantify N_2, NH_3 and N_2O emissions from a flooded paddy field over an entire rice-growing season under two different fertilization methods. Our study shows that about one-third of the applied fertilizer N is lost to the environment via gaseous pathways if the fertilizer is applied to the soil surface. The incorporation of N fertilizer into the soil decreased gaseous N emissions by 13.3–42.5% relative to surface application, while simultaneously improving rice yield and decreasing gaseous N loss intensity. Our results clearly show that the incorporation of N fertilizer into the soil is effective in increasing NUE and reducing environmental N losses from paddy rice ecosystems, and should therefore be widely adopted.

Acknowledgments

This study was financially supported by the National Science Foundation of China (41425005, 41977097, 41401279 and 31600392), the Research Fund of the State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences (Y412201422), the Natural Science Foundation of Guangdong Province (2016A030310013) and the Humboldt Postdoctoral Research Fellowship and the R&D program of Guangdong Provincial Department of Science and Technology (2018B030324003). We gratefully acknowledge the technical assistance provided by Changshu Agroecological Experimental Station of the Chinese Academy of Sciences.

Data Sharing and Data Accessibility

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Supplementary data

Supporting Information associated with this paper is available online.

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Table 1 Dates of agricultural activities performed during the rice-growing season

Agricultural activities	Date
Basal N fertilization (50%)[a]	June 17
Rice seedlings transplantation	June 17
Tillering N fertilization (30%)	July 9
Midseason drainage	July 27–July 31
Panicle initiation N fertilization (20%)	August 11
Final drainge	October 14
Harvesting	October 26

[a] 50% denotes that 50% of N fertilizer was applied as a basal fertilizer.

Figure legends

Fig. 1. Seasonal variations in (a) N_2, (b) NH_3 and (c) N_2O fluxes and (d) floodwater NH_4^+ and (e) NO_3^- concentrations in different treatment plots over the rice-growing season in 2012. CK, no N addition; SA, surface application of N fertilizer; IA, incorporation of N fertilizer into the soil. The vertical bars denote the standard deviation (n = 3). The arrows indicate a N fertilizer application event. The one-way ANOVA was performed for the three treatments and '*' denotes a significant difference between the IA and SA treatments at $P < 0.05$.

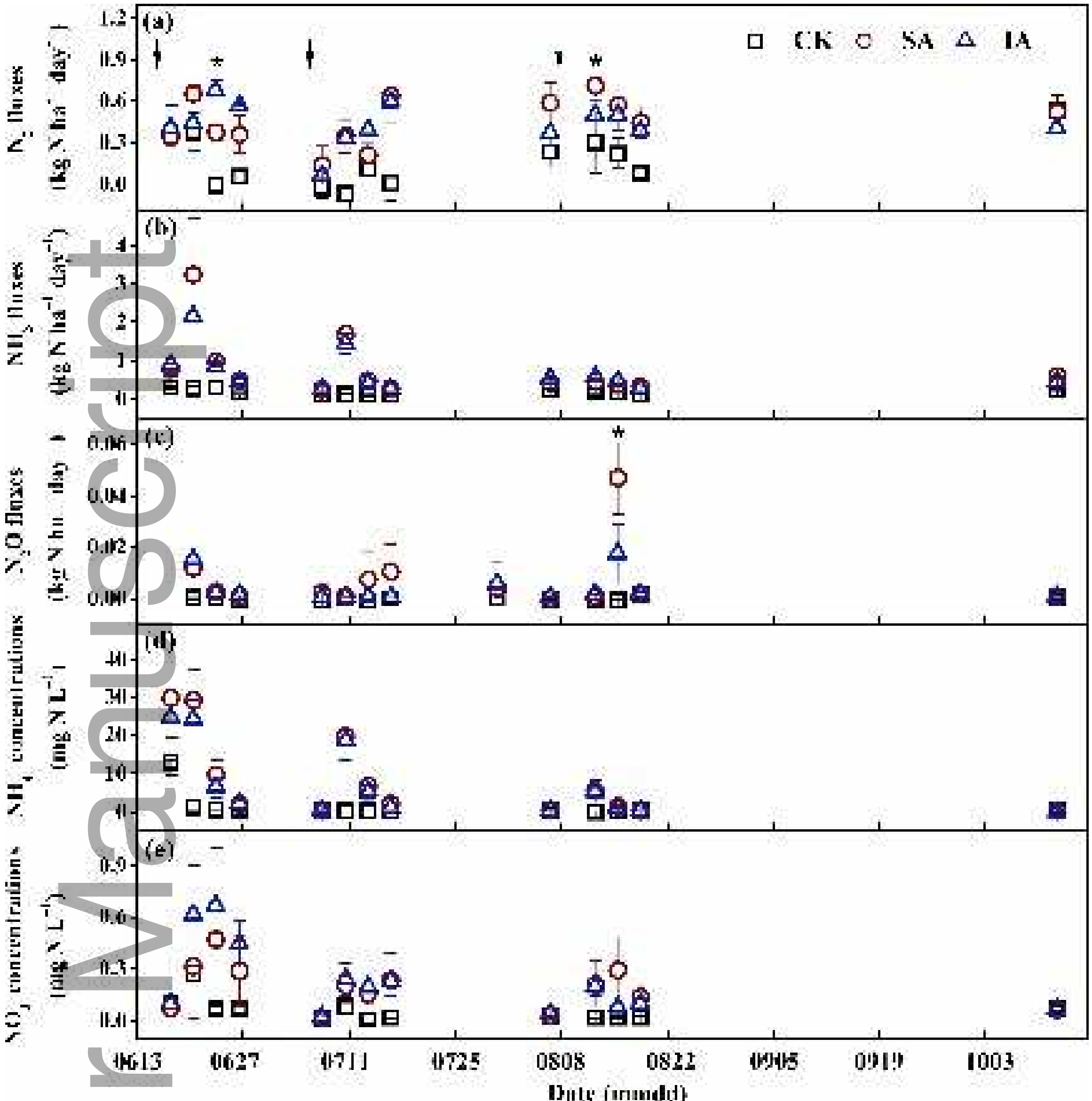
Fig. 2. Average (a) N_2, (b) NH_3 and (c) N_2O fluxes during different N fertilization periods. BF, basal N fertilization period (18 June–8 July); TF, tillering N fertilization period (9 July–10 August); PIF, panicle initiation N fertilization period (11 August–13 October). CK, no N addition; SA, surface application of N fertilizer; IA, incorporation of N fertilizer into the soil. Different letters indicate significant differences among different treatments during the same N fertilization period ($P < 0.05$).

Fig. 3. Cumulative (a) N_2, (b) NH_3 and (c) N_2O emissions and (d) the total emissions for the different treatments based on field measurements over the rice-growing season in 2012. CK, no N addition; SA, surface application of N fertilizer; IA, incorporation of N fertilizer into the soil. The vertical bars denote the standard deviation (n = 3). Different letters indicate significant differences at $P < 0.05$.

Fig. 4. (a) Rice aboveground biomass, (b) N uptake, (c) fertilizer N use efficiency and (d) gaseous N loss intensity in different treatment plots during the rice-growing season in 2012. CK, no N addition; SA, surface application of N fertilizer; IA,

incorporation of N fertilizer into the soil. The vertical bars denote the standard deviation (n = 3). Different letters indicate significant differences at $P < 0.05$.

Fig. 5. Gaseous N emissions derived from field measurement (blue dash line) and LMMs simulation (soild line) in different treatments over the rice-growing season in 2012. CK, no N addition; SA, surface application of N fertilizer; IA, incorporation of N fertilizer into the soil. Different letters indicate significant differences among different treatments at $P < 0.05$. The blue asterisk indicates a significant difference ($P < 0.05$) between the N_2O emissions derived from field measurment and LMMs simulation.



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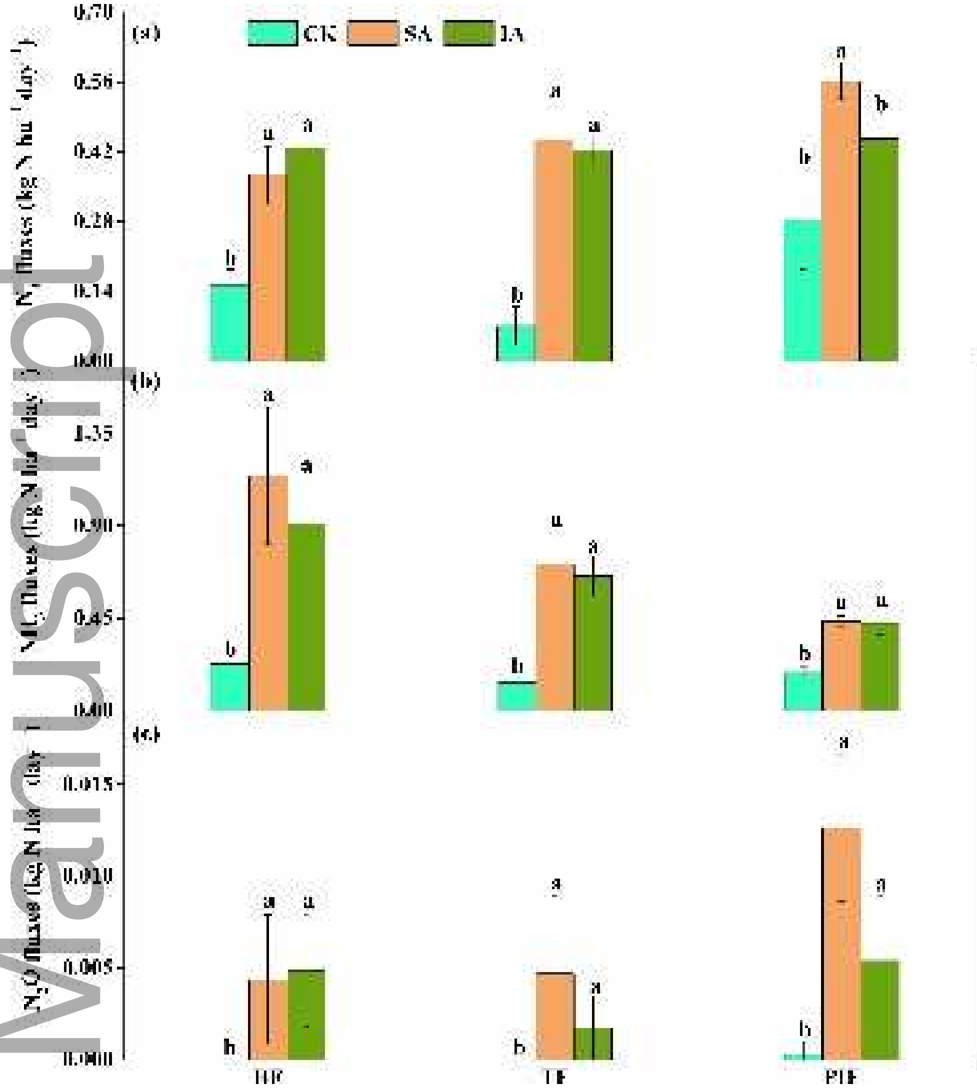

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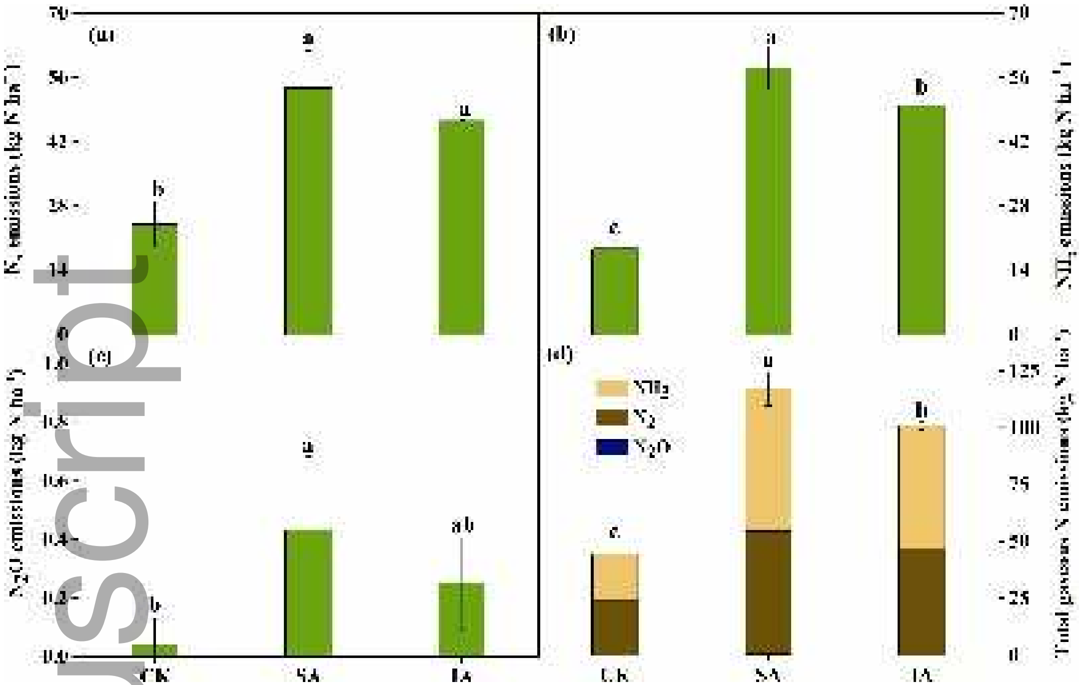

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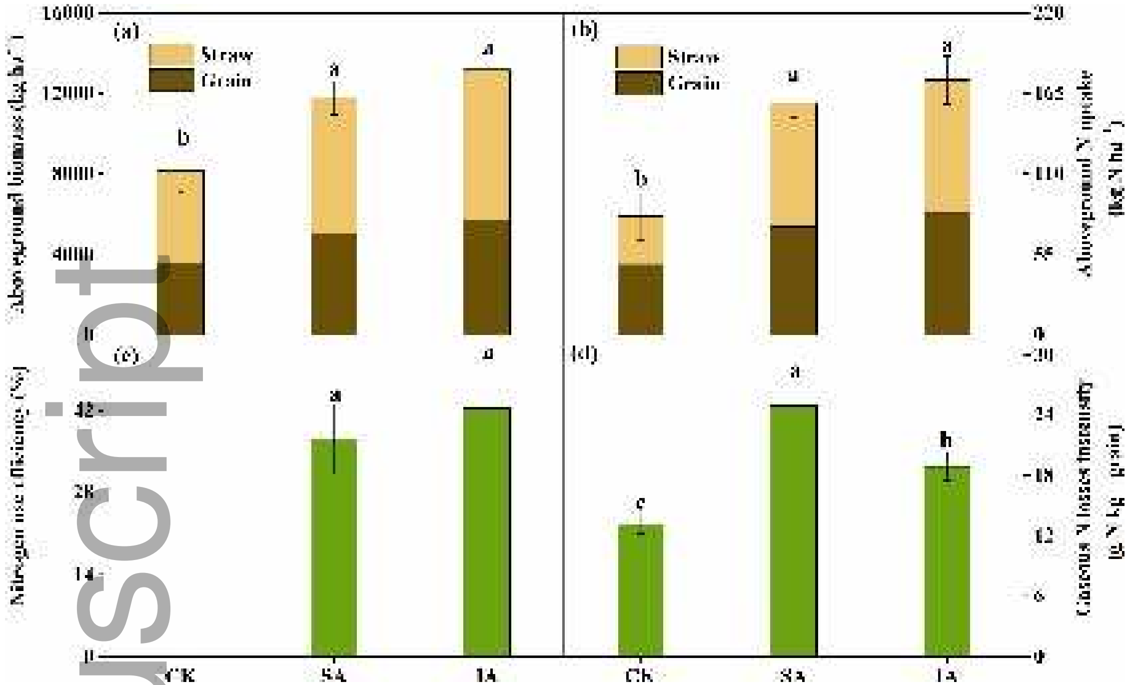

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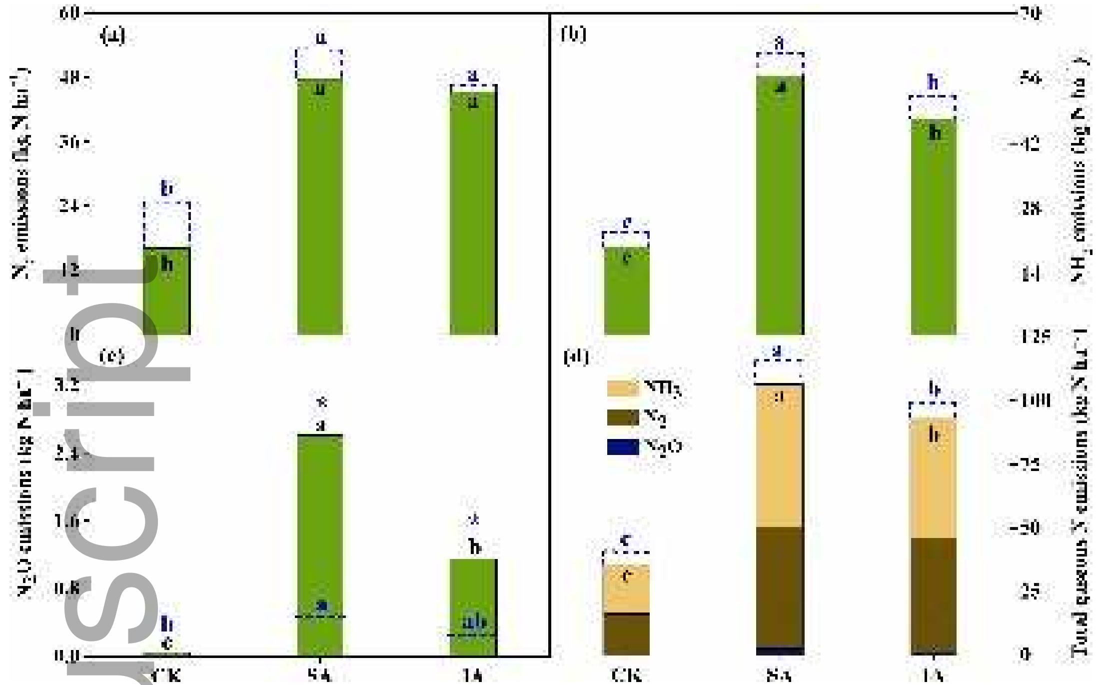

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