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Crop straw incorporation interacts with N fertilizer on N_2O emissions in an intensively cropped farmland

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Abstract

Nitrogen (N) fertilization and straw incorporation strongly influence nitrous oxide (N_2O) emissions from agricultural fields. An in-situ micro-plot experiment on intensively farmed winter wheat (*Triticum aestivum* L.) was conducted to investigate the source and rate of N_2O emissions from soils following labeled ^{15}N fertilization with and without straw incorporation. Four treatments, i.e., no N fertilizer and no straw incorporation (N0S0), straw incorporation only (N0S1), N fertilizer only (N1S0), and N fertilization plus straw incorporation (N1S1), were established in the experiment. The N_2O emissions mainly occurred after N fertilization and lasted for approximately 1–2 weeks, accounting for 60%–67% of the wheat seasonal N_2O emissions. Within the 6 days after basal fertilization and 2–4 days after top-dressing, most of the N_2O fluxes (>50%) were derived from fertilizer. Thereafter, soil-derived N_2O dominated the total N_2O emissions and within 10–20 days after N fertilization, fertilizer-derived N_2O became negligible. Fertilizer N and soil N both accounted for 40%–60% of the seasonal N_2O emissions, which may be explained by the high soil N stock due to long-term high N fertilization in the region. This implies the similar roles of soil N pool and fertilizer N in N_2O generation under intensively farmed soils. The N fertilization had a significant priming effect on the turnover of soil N, which contributed 21.0%–28.6% of the seasonal N_2O emissions. During the basal fertilization/first irrigation event, straw incorporation significantly ($P < 0.05$) stimulated CO_2 fluxes both in N-fertilized and non-N-fertilized plots; however, after the top-dressing/second irrigation event, the significant increase of CO_2 fluxes

induced by straw incorporation was only observed in the N-fertilized treatment. Straw incorporation interacted with N fertilization, and tended to enhance N_2O emissions in the basal fertilization and lower N_2O emissions in the top-dressing period. In N-fertilized plots, the seasonal N_2O emissions from straw-incorporated and straw-removed treatments were similar, indicating that straw incorporation enhanced the N supply without increasing the N_2O emissions. Our study highlights that there are significant benefits of straw incorporation to soil fertility improvement; however, the long-term impacts of straw incorporation on greenhouse gas emissions should be further examined.

Keywords: Nitrous oxide; ^{15}N tracing; Straw incorporation; Nitrogen fertilization; Intensive farming.

1. Introduction

Nitrous oxide (N_2O) is a major greenhouse gas (Ding *et al.*, 2015; Loick *et al.*, 2017), which has 265 times greater global warming potential than CO_2 over a 100-year time horizon (IPCC, 2014). Agricultural soils are the dominant emitters of N_2O, contributing 60% (Smith *et al.*, 2007) and 74% (NCCC, 2012) of global and Chinese N_2O emissions, respectively. A better understanding of the pattern and sources of N_2O emissions from agricultural soils is therefore essential to develop novel and practical strategies to limit climate change (Kim and Giltrap, 2017).

Northern China is a major intensive agricultural region (Tan *et al.*, 2017; Xu *et al.*, 2017), covering about 3 million ha (Ding *et al.*, 2007) and accounting for 67% and 28% of national wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) production (Zhang *et al.*, 2017b), respectively. High productivity in northern China largely relies on excessive utilization of synthetic nitrogen (N) fertilizer at rates of >600 kg N ha^{-1} yr^{-1} (Ju *et al.*, 2009); this high level of N input is likely to result in high N_2O emissions (Zhang *et al.*, 2014b; Omonode *et al.*, 2017) as the N supplied exceeds crop demand (Linquist *et al.*, 2012; Kim *et al.*, 2013; Charles *et al.*, 2017; Song *et al.* 2018) . Reduction in N_2O emissions in northern China could therefore strongly contribute to the mitigation of anthropogenic N_2O emissions at national and global scales (Tan *et al.*, 2017; Xu *et al.*, 2017). Both fertilizer N and soil N pools are responsible for N_2O emissions (Shepherd *et al.*, 2015), so understanding the partitioning of these sources is important both to characterize total emissions and also to allow the precise calculation of emission factors of fertilizer N (IPCC, 2006).

87 Earlier field studies conducted in northern China presumed that the N_2O emitted from
88 fertilizer N applied in the crop season was the predominant source of total N_2O
89 emissions (Liu *et al.*, 2012; Shi *et al.*, 2013; Ying *et al.*, 2017). However, many in-situ
90 studies using ^{15}N tracers carried out in Europe (Linzmeier *et al.*, 2001; Garcia-Ruiz *et*
91 *al.*, 2012), Oceania (Di and Cameron, 2008), and Africa (Gentile *et al.*, 2008) found
92 that soil N could account for as much as 60%–99% of the total N_2O emissions,
93 suggesting that the soil N pool contributed a large proportion of the N_2O emissions.
94 Isotopic analysis using ^{15}N allows the source and amount of N_2O emissions from
95 fertilizer N to be determined directly (Baggs, 2008; Loick *et al.*, 2017), but most in-
96 situ ^{15}N tracing studies in northern China have not measured the $^{15}N_2O$ flux (Cai *et*
97 *al.*, 1998; Xu *et al.*, 2000; Cai *et al.*, 2002; Ju *et al.*, 2009) and thus accurately
98 distinguishing the N_2O derived from fertilizer N and soil N pools has not been
99 achieved. Wan *et al.* (2009) used an incubation method to determine the $^{15}N_2O$
100 derived from different N sources; however, their laboratory experiment was incapable
101 of analyzing the effects of natural field conditions (e.g. temperature, precipitation, and
102 plant growth), which were also essential in affecting $^{15}N_2O$ emissions (Klumpp *et al.*,
103 2011). Thus, it is difficult to draw conclusions from the existing literature that
104 quantify the contribution of fertilizer N vs soil N to N_2O emissions in northern China,
105 where excessive fertilization has been implemented for more than three decades (Gu
106 *et al.*, 2017; Huang *et al.*, 2017; Zhang *et al.*, 2017b).
107 In addition, the high biomass production in northern China has generated vast quantities
108 of crop straw and residues (Zhou *et al.*, 2017). A combination of synthetic N fertilizer

with straw incorporation is strongly recommended as an environmentally friendly strategy by researchers (Liao *et al.*, 2015; Zhao *et al.*, 2015; Han *et al.*, 2018) and government agencies (Ministry of Environmental Protection-PRC, 1999; Ministry of Agriculture-PRC, 2015) to improve soil fertility and minimize negative environmental impacts. Incorporation of crop straw is generally believed to have positive effects on soil carbon (C) and N dynamics (Chen *et al.*, 2014; Ghimire *et al.*, 2015; Meng *et al.*, 2017) and on the mitigation of N_2O emissions (Frimpong and Baggs, 2010; Badagliacca *et al.*, 2016). However, results of previous studies on the efficacies of straw incorporation on N_2O emissions were inconsistent, showing either positive (Zhang *et al.*, 2015; Huang *et al.*, 2017), negative (Xia *et al.*, 2014; Yao *et al.*, 2017), or neutral effects (Zhang *et al.*, 2017a). In addition, very few studies have considered the effects of crop straw addition on the source of the generated N_2O (Frimpong *et al.*, 2011; Garcia-Ruiz *et al.*, 2012; Rezaei Rashti *et al.*, 2017; Wu *et al.*, 2017), which would help to quantify total N_2O emissions and inform mitigation actions.

In this study, we used ^{15}N tracing to evaluate the contribution of the soil N and the fertilizer N to the total N_2O emissions in the intensive farming region of northern China. Measurements under straw-incorporated and straw-removed treatments were also performed to investigate the impact of straw incorporation on N_2O emissions and their sources.

2. Materials and methods

2.1 Study site

The experiment was conducted in Huantai county, Shandong province (36°58′N, 117°59′E), a typical intensively farmed region in northern China (Bai *et al.*, 2011). The region has a temperate monsoon climate (Shi *et al.*, 2013). Annual mean precipitation and air temperature in the region is 543 mm and 12.5°C, respectively (Tan *et al.*, 2017). Prior to the experiment, two crops of winter wheat and summer maize per year had been farmed for about 30 years (since the 1980s). The experiment was conducted in the winter wheat season (Oct. 2015 to Jun. 2016; Fig. 1); the cumulative precipitation, mean air temperature, and mean soil temperature (0–10 cm) during the experimental period were 210 mm, 9.8°C, and 8.4°C, respectively. The soil in the experimental site is classified as aquic inceptisol (calcareous, clay loam; Shi *et al.*, 2013). Soil pH, bulk density, total N content, and soil organic matter content of top layer (0-20 cm) were 7.70 (water/soil = 2.5/1), 1.52 g cm^{-3}, 1.00 g kg^{-1}, and 17.4 g kg^{-1}, respectively.

2.2 Experiment design and setup

Four treatments were established in our study: no N fertilizer and no straw incorporated (N0S0), straw incorporation only (N0S1), N fertilizer only (N1S0), and N fertilization plus straw incorporation (N1S1). Each treatment was replicated three times. The resultant 12 microplots (1 × 1 m^2) were randomly established in the

experimental area with a path (1 m) between microplots. Each microplot was enclosed with a PVC board, which was inserted into the soil at 1 m depth and the upper edge was 15 cm above the soil surface. Before winter wheat sowing, phosphorus (P) fertilizer (calcium superphosphate; 140 kg P_2O_5 ha^{-1}) and potassium (K) fertilizer (potassium sulfate; 60 kg K_2O ha^{-1}) were broadcasted in all microplots. The topsoil (0–20 cm) was then plowed with a shovel to mix the P and K fertilizer with soil. In the straw-incorporated treatments (N0S1 and N1S1), straw from the previous maize season (0.96 kg m^{-2}; C/N: 76:1) was chopped at 3-5 cm and incorporated thoroughly with the soil via plowing. Seeding rate for each micro-plot in the study was consistent with that in local conventional farmland (i.e., 150 kg ha^{-1}; about 330 seeds/micro-plot). All microplots were surrounded by guard rows. After sowing, all microplots were irrigated with 75 mm water. In N-fertilized treatments (N1S0 and N1S1), ^{15}N labeled urea (125 kg N ha^{-1}; 10.21% atom % ^{15}N, Shanghai Chem-Industry Institute) was dissolved in the irrigation water and applied uniformly to the microplot as a basal fertilizer. At the jointing stage, irrigation was also applied with 75 mm of water, and an additional 125 kg N (^{15}N labeled urea) ha^{-1} was applied as top-dressing. The detailed dates of field management events are shown in Fig. 1.

2.3 N_2O and CO_2 flux measurements

The closed chamber method was used to simultaneously measure the N_2O and CO_2 fluxes (Shi *et al.*, 2014; Tan *et al.*, 2017). The static chamber consisted of a PVC base frame (20 cm width × 30 cm length × 15 cm height) with a water channel and a

169 removable cover (20 cm width × 30 cm length × 20 cm height). The cover box was
170 equipped with a sampling outlet and a thermometer in the upper plane. The chambers
171 were established in the between-row area of each microplot after plowing, and the
172 base was inserted to a depth of 15 cm in the soil. When collecting gas samples, we
173 filled the water channels with water to keep the chamber airtight.
174 Gas samples were obtained between 9:00 and 11:00 am. Five gas samples were taken
175 at 0, 8, 16, 24, and 32 min after chamber covering for flux measurements, and an
176 additional gas sample was obtained at 60 min closure time for $^{15}N_2O$ analyses. 35 and
177 15 ml gas samples were collected for flux measurements and $^{15}N_2O$ analyses,
178 respectively, using 35-mL polypropylene syringes fitted with 3-way stopcocks. All the
179 gas samples were stored in 12 ml evacuated vials (Labco, UK), and the vials for
180 $^{15}N_2O$ analyses were helium-flushed. It was assumed that N_2O confined in the
181 headspace at the time of chamber closure was equivalent to atmospheric N_2O and
182 contained no excess ^{15}N.
183 The N_2O and CO_2 samples were analyzed within the sampling day using an Agilent
184 7820A gas chromatograph (Agilent Technologies Inc., SCLA, CA, USA), which was
185 equipped with an electron capture detector (ECD) and a flame ionization detector
186 (FID). The carrier gas for N_2O and CO_2 analysis was high-purity N_2, and the buffer
187 gas for ECD was 10% CO_2 in pure N_2. The flow rates of the carrier gas were 25 and
188 30 mL min^{-1} for the ECD and FID, respectively. Temperatures in the column ovens,
189 ECD, and FID were set at 55°C, 330°C, and 250°C, respectively. The N_2O and CO_2
190 fluxes were calculated from the linear or nonlinear changes in gas concentrations

191 determined within the 32-min closure period (Hutchinson and Mosier, 1981; Yan *et*
192 *al.*, 2013).
193 Fluxes of N_2O and CO_2 were measured daily for a week after fertilization events. The
194 $^{15}N_2O$ samples were also collected daily during the 7-day continuous sampling period
195 after fertilization events (samples on the 5th day after top-dressing were missing
196 because of rain), and additional $^{15}N_2O$ samples were taken on the 10th day after top-
197 dressing. For the non-fertilization period, only gas fluxes were measured, and the
198 sampling was performed two times a week (samples were taken only once a week
199 over winter).
200 The cumulative N_2O emissions were estimated by summing the daily mean fluxes of
201 measurement and no-measurement days, with daily fluxes of no-measurement days
202 being estimated as the arithmetic average of adjacent data (Huang *et al.*, 2013; Tian *et*
203 *al.*, 2013).

204 2.4 $^{15}N_2O$ analysis and calculation

205 The ^{15}N abundances of N_2O samples were analyzed in the Stable Isotope Facility of
206 the University of California at Davis. Stable isotope ratios of N were measured using
207 a Thermo Scientific GasBench + Precon gas concentration system interfaced to a
208 Thermo Scientific Delta V Plus isotope-ratio mass spectrometer (Thermo Electron
209 Inc., Bremen, Germany).
210 The collected N_2O sample for ^{15}N analysis contained a mixture of atmospheric and
211 emitted N_2O. We used the following equation (Li *et al.*, 2016) to calculate the ^{15}N

abundance (atom fraction ^{15}N) of emitted N_2O (*atom% $^{15}N_{em}$*):

$$atom\%\ {}^{15}N_{em} = (atom\%\ N_{mix} \times C_{mix} - atom\%\ {}^{15}N_{air} \times C_{air})/C_{em} \quad (1)$$

where *atom% $^{15}N_{mix}$* and *atom% $^{15}N_{air}$* are the ^{15}N abundances of headspace samples and ambient air (averaged 0.369% during the experiment), respectively; and C_{mix}, C_{air}, and C_{em} are the N_2O concentration of headspace samples, ambient air, and emitted N_2O respectively, and $C_{mix} = C_{air} + C_{em}$.

The proportion of N_2O flux derived from fertilizer (*% N_2O-N derived from applied N*) was calculated according to the following equation (Nason and Myrold, 1991; Lampe *et al.*, 2006; Vallejo *et al.*, 2014):

$$\%\ N_2O\text{-}N\ \text{derived from applied N} = ({}^{15}N_{ap}\ N_2O_{em}/{}^{15}N_{ap}\ fertilizer) \times 100 \quad (2)$$

where *$^{15}N_{ap}\ N_2O_{em}$* and *$^{15}N_{ape\ fertilizer}$* are the atom% excess of emitted N_2O (*atom% $^{15}N_{em}$* minus *atom% $^{15}N_{air}$*) and ^{15}N labeled urea (10.21% minus *atom% $^{15}N_{air}$*), respectively. The product of the total cumulative N_2O emissions and the *%N_2O-N derived from applied N* was calculated as cumulative fertilizer-derived N_2O emissions. The cumulative fertilizer-derived N_2O emissions after top-dressing may be from the top-dressing fertilizer and also the basal fertilizer, because we used ^{15}N labeled urea in both fertilization events.

2.5 Soil and plant sampling

In all microplots, soil samples were taken six times (i.e., before sowing, the 2nd day after basal fertilization, the 30th day after basal fertilization, the 5th day before top-dressing, the 2nd day after top-dressing, and on harvest). The dates of soil sampling

were shown in Fig. 1. On each soil sampling day, two soil cores (2.5 cm diameter) at 0–20 cm depth were taken within each microplot. Samples from the two soil cores were sieved (2 mm) and mixed well. The boreholes were refilled with PVC columns to avoid a change in gas exchange and water flow in the soil. The soil ammonium-N (NH_4^+-N) and nitrate-N (NO_3^--N) were extracted from the fresh soils (20 g) in 100 mL of 1 M KCl solution and analyzed by a colorimetric continuous flow analyzer (AA3, SEAL Inc., Germany). At harvest, all the grain samples were thoroughly dried in a 65°C oven for the determination of crop yield (dry matter).

2.6 Statistical analysis

Differences in cumulative N_2O emissions, CO_2 emissions, and crop yield were determined by a *t*-test for least significant differences at $P < 0.05$. The values are expressed as arithmetic mean ($n = 3$) and standard error of the replications. The quadratic and linear model was used to estimate relationships between %N_2O-N derived from applied fertilizer N and the day after fertilization. SPSS 22.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analyses.

3. Results

3.1 N_2O and CO_2 fluxes

The peak N_2O emissions were mainly associated with N fertilization and/or irrigation events. The N1S0 and N1S1 treatments exhibited significantly higher N_2O fluxes

(peaking at 0.23–0.66 mg N_2O-N m^{-2} h^{-1}) than the N0S0 and N0S1 treatments (peaking at 0.04–0.07 mg N_2O-N m^{-2} h^{-1}; $P < 0.05$; Fig. 2). This significant ($P < 0.05$) increase of N_2O fluxes induced by fertilization lasted for about 7 days after basal fertilization and 10 days after top-dressing. Thereafter, N_2O fluxes of all treatments remained at <0.02 mg N_2O-N m^{-2} h^{-1}, and no statistically significant differences were found between N-fertilized and non-N-fertilized treatments ($P > 0.05$). In the basal fertilization period, the peak N_2O fluxes tended to be higher in N1S1 treatments (0.66 mg N_2O-N m^{-2} h^{-1}; Fig. 2d) than that in N1S0 treatments (0.51 mg N_2O-N m^{-2} h^{-1}; Fig. 2c). During the top-dressing period, the opposite trend was observed, i.e., 0.23 and 0.48 mg N_2O-N m^{-2} h^{-1} for the N1S1 and N1S0 treatments, respectively (Fig. 2d, c), although no overall significant difference in N_2O fluxes was found ($P > 0.05$).

During the basal fertilization/first irrigation event, straw incorporation strongly stimulated CO_2 fluxes both in non-N-fertilized plots and N-fertilized plots: peak values of CO_2 flux from N0S1 and N1S1 (115–127 mg CO_2-C m^{-2} h^{-1}) were about 2.6-fold higher ($P < 0.05$) than those from N0S0 and N1S0 (39–52 mg CO_2-C m^{-2} h^{-1}; $P < 0.05$; Fig. 3a, b). However, in the top-dressing/second irrigation period, this significant increase of CO_2 fluxes after straw-incorporation was only observed in N-fertilized plots (N1S1; Fig. 3b): the peak CO_2 fluxes in the N1S1 treatment (134.6 ± 7.92 mg CO_2-C m^{-2} h^{-1}) was significantly higher than that in the N0S1 treatment (82.0 ± 7.84 mg CO_2-C m^{-2} h^{-1}; $P < 0.05$).

3.2 Cumulative CO_2 and N_2O emissions and crop yield

In non-N-fertilized plots, the cumulative N_2O emissions of N0S1 treatments (368.20 g N_2O-N ha^{-1}) were 45% higher than those of N0S1 treatments (253.82 g N_2O-N ha^{-1}); however, in the N-fertilized plots, the cumulative N_2O emissions in the straw-incorporated and straw-removed treatments were similar (928.40 and 950.87 g N_2O-N ha^{-1} for N1S1 and N1S0, respectively; Table 1). The N fertilization significantly increased the cumulative N_2O emissions by 152%–274% (928.40–950.87 vs. 253.82–368.20 g N_2O-N ha^{-1}; $P < 0.05$; Table 1).

Seasonal CO_2 emissions increased significantly after straw incorporation, and this was more apparent in non-N-fertilized treatments, i.e., N0S1 exhibited a 43% increase of CO_2 emission compared with N0S0 ($P < 0.05$; Table 1). No significant differences in seasonal CO_2 emissions were found between N-fertilized treatments (N1S0 and N1S1) and their corresponding non-N-fertilized treatments (N0S0 and N0S1; $P > 0.05$).

The crop yield of N1S0 and N1S1 treatments tended to be higher than that of non-N-fertilized treatments (N0S0 and N0S1, respectively), but the differences were not statistically significant ($P > 0.05$; Table 1). In N-fertilized plots, straw incorporation slightly increased the wheat yield, whereas in non-N-fertilized plots, the crop yields of the straw incorporation tended to decline, but no statistical differences were observed ($P > 0.05$; Table 1).

3.3 N_2O derived from the soil and fertilizer N

The proportion of N_2O fluxes derived from the fertilizer N reached maximum (55%–61%) on the 4th or 5th day after basal fertilization and then decreased to <50% on the 7th day after basal fertilization (Fig. 4a). This tendency was well described by a quadratic model ($P < 0.05$; $R^2 = 0.96$ and 0.92 for N1S0 and N1S1, respectively; Fig. 4a). According to this estimation, the percentage of daily N_2O emissions derived from fertilizer N was close to zero on the 10th day after basal fertilization. During the top-dressing period (7 to 17 Apr.), the percentage of fertilizer-derived N_2O reached maximum (56%–59%) on the 2nd day after fertilization and then declined afterwards (Fig. 4b). A linear model estimated that the proportion of fertilizer-derived N_2O was negligible on about the 20th day after top-dressing ($P < 0.05$; $R^2 = 0.60$ and 0.87 for N1S0 and N1S1, respectively; Fig. 4b). Straw incorporation had no significant effect on the ratio of fertilizer-derived N_2O fluxes ($P > 0.05$; Fig. 4). The cumulative fertilizer-derived N_2O emissions after basal fertilization were 209 and 210 g N_2O ha^{-1} for N1S0 and N1S1 treatments (Fig. 5a), respectively, and in the top-dressing period, the corresponding N_2O emissions were 78 and 60 g N_2O-N ha^{-1}, respectively (Fig. 5b).

Fertilizer N-derived N_2O emissions accounted for 41.4%–53.8% of total emissions in the basal fertilization period and 51.8%–51.9% in the top-dressing period (Table 2). The soil-derived N_2O emissions from N-fertilized plots (217–295 g N_2O-N ha^{-1} after basal fertilization and 55–73 g N_2O-N ha^{-1} after top-dressing) were significantly higher than those from non-N-fertilized plots (24-41 g N_2O-N ha^{-1} after basal

316 fertilization and 30–31 g N_2O-N ha^{-1} after top-dressing; $P < 0.05$; Fig. 5). This
317 indicates that the N_2O emissions from the soil N pool were significantly promoted by
318 the N fertilization. Straw incorporation tended to enhance N_2O emissions after the
319 basal-fertilization (Fig. 5a) but decreased after the top-dressing period (Fig. 5b).
320 However, straw incorporation had no significant effect on the cumulative N_2O
321 emissions contributed by the fertilizer and soil N ($P > 0.05$; Fig. 5 and Table 2).

322 3.4 Soil N

323 Before the wheat was sown, soil NO_3^--N concentrations under the four treatments
324 were all <10 mg kg^{-1} (Fig. 6a). Application of N fertilizer significantly ($P < 0.05$)
325 increased the NO_3^--N concentrations to 45.4–48.2 mg N kg^{-1} during the basal
326 fertilization period, and 25.8–32.7 mg N kg^{-1} during the top-dressing period (Fig. 6a).
327 On the 2nd day after top-dressing (9 Apr.), N1S1 was observed to have a remarkable
328 effect of reducing soil NO_3^--N concentrations compared with N1S0; however, in other
329 periods, no apparent differences of soil NO_3^- ($P > 0.05$) were detected between N1S1
330 and N1S0 treatments (Fig. 6a). Soil NH_4^+-N concentrations always remained at a low
331 level (<3.5 mg kg^{-1}), and there were no significant differences among treatments (Fig.
332 6b).
333

4. Discussion

4.1 Duration of N_2O emissions

The N_2O emission peaks occurred mainly after N fertilization events and lasted for approximately 1–2 weeks (Fig. 2), which is consistent with a number of recent studies (Bell *et al.*, 2015; Hinton *et al.*, 2015; Tan *et al.*, 2017; Yao *et al.*, 2017). This was mainly attributed to the high soil mineral N content after fertilization events (Ju *et al.*, 2011; Luo *et al.*, 2017; Zhang *et al.*, 2019; Fig. 6). In our study, the N_2O emission peaks occurring during the fertilization period (7 days after basal fertilization and 10 days after top-dressing), i.e., 578–620 g N_2O-N ha^{-1}, accounting for 59.6%–67.2% of the seasonal N_2O emissions (Fig. 2). Likewise, Ding *et al.* (2013) reported that up to 82%–98% of the fertilizer-induced N_2O emissions were emitted within the two weeks following fertilization. That is, although the growth period of winter wheat lasted for more than 240 days in the northern China, most N_2O was emitted in the initial 1–2 weeks following each fertilization event. This finding highlights that N_2O mitigation measures in the wheat season should mainly target the fertilization periods.

The proportion of fertilizer-derived N_2O fluxes declined to <50% since the 7^{th} day after basal fertilization (Fig. 4a) and the 2^{nd}–4^{th} day after top-dressing (Fig. 4b). Within 10–20 days after fertilization, fertilizer-derived N_2O became negligible (Fig. 4a, b). This could be explained by the reduced fertilizer-derived reactive N in soil due to microbial immobilization (Cai *et al.*, 2017), plant uptake (Omonode *et al.*, 2017), and losses through NH_3 volatilization (Xia *et al.*, 2017) and nitrate leaching (Huang *et*

355 *al.*, 2017), etc. Similar findings were reported by a previous ^{15}N tracing study
356 conducted in Europe (Linzmeier *et al.*, 2001). These results suggest that the duration
357 of N_2O measurement to assess the fertilizer contribution is shorter than previously
358 assumed. Intergovernmental Panel on Climate Change (IPCC) guidelines for
359 estimating N_2O emission factors recommend that emission measurements are made
360 for one year following fertilizer application (IPCC, 2006). Our research suggests that
361 direct fertilizer emissions may occur over a period of weeks, and it may be
362 appropriate to reassess the period over which emission factors are calculated for
363 greenhouse gas inventory purposes.

364 4.2 Sources of N_2O emissions

365 Fertilizer-derived N_2O accounted for 41.4%–53.8% of the cumulative N_2O emissions
366 in the fertilization period (Table 2), which was higher than regions in Europe (10%–
367 40%; Linzmeier *et al.*, 2001) and Oceania (<4%; Di and Cameron, 2008). This was
368 most likely to be related to the significantly higher N application rate in the
369 intensively farmed region of northern China (250 kg N ha^{-1} $season^{-1}$ in our study)
370 compared with Linzmeier *et al.* (2001) (160 kg N ha^{-1} $season^{-1}$) and Di and Cameron
371 (2008) (200 kg N ha^{-1} yr^{-1}). Our finding highlights that there is a great potential for
372 lowering fertilizer-derived N_2O emissions by optimizing the N application rate in the
373 study region.
374 Despite the high N application level in northern China, a large proportion (46.2%–
375 58.6%) of soil-derived seasonal N_2O emissions was detected (Fig. 5), indicating a

high risk of N_2O generation from soil N. Background cumulative N_2O emissions (N_2O emissions from non-N-fertilized treatments) in our study were 0.25–0.37 kg N_2O-N ha^{-1} $season^{-1}$ (Table 1), comparable to the values (0.22–0.47 kg N_2O-N ha^{-1} $season^{-1}$) reported in previous site-specific studies (Cui *et al.*, 2012; Hu *et al.*, 2013; Huang *et al.*, 2013) and a meta-analysis conducted in the same region (Xu *et al.*, 2017). The results of this study showed that the soil-derived N_2O emissions in the fertilized plots were significantly higher than the background N_2O emissions ($P < 0.05$; Fig. 5), which could be attributed to the priming effect of N fertilizer on the soil N pool (Linzmeier *et al.*, 2001; Lampe *et al.*, 2006; Di and Cameron, 2008). This priming effect was most likely to have resulted from enhanced native soil N turnover induced by the increased microbial activity and root exudation (Kuzyakov *et al*., 2000; Pearce, 2016; Liu *et al*., 2017). Quantifying the contribution of fertilizer-derived N to the N_2O released by background emissions is a challenging task, but is important because background emissions are used in the calculation of emission factors (IPCC, 2006). However, these emission sources are difficult to separate in the field studies. Our findings indicate that the overall N_2O flux needs to be understood in the context of an interaction between fertilizer and soil N pools.

Farmland in northern China has received continuously high synthetic N applications (600 kg N ha^{-1} yr^{-1}) over a long period (> 30 years). Consequently, large amounts of residual N have accumulated in soil (Cui *et al.*, 2013), which represents a large source of N_2O emissions in the subsequent crop season (Grant *et al.*, 2006). Our findings show that N_2O emissions induced by the priming effect accounted for 43.7%–87.6%

of soil-derived N_2O emissions (Fig. 5) and 21.0%–50.5% of the total emissions (Table 2), indicating that the risk of N_2O loss from the accumulated soil N could be intensified by N fertilization. In this case, optimum fertilization on the basis of soil residual N testing could be implemented in the region (Ju *et al.*, 2004; Wu *et al.*, 2014; Zhang *et al.*, 2014a) to increase N use efficiency and reduce the risk of N_2O loss from both fertilizer N and the soil N pools.

4.3 Impacts of straw incorporation on N_2O emissions

In N-fertilized treatments, straw incorporation tended to increase N_2O emissions in the basal fertilization period (Fig. 5a) but the opposite tendency was observed in the top-dressing period (Fig. 5b). For maize straw with a high C/N ratio (76:1 in our study), microbes would immobilize the N within soil to decompose the maize straw (Abalos *et al.*, 2013; Lin *et al.*, 2013; Lehtinen *et al.*, 2014). In the basal fertilization period, N uptake by plants was negligible, and soil N may be adequate for the decomposition of straw, as indicated by the similar CO_2 fluxes between N0S1 (Fig. 3a) and N1S1 (Fig. 3b) treatments (Esther *et al.*, 2014). Therefore, microbial N immobilization had no apparent effect on soil mineral N content after basal fertilization (Fig. 6). The increased N_2O emissions under straw-incorporated treatments (Fig. 5a) were probably derived from straw decomposition (Vigil *et al.*, 1991; Frimpong *et al.*, 2010). However, in the top-dressing period (at the jointing stage), significant increases of CO_2 fluxes after straw incorporation were only observed in N-fertilized plots (N1S1; Fig. 3b), suggesting that soil available N was the limiting factor of straw decomposition (López-

Bellido *et al.*, 2005; Song *et al.*, 2011; Chen *et al.*, 2014; Li *et al.*, 2017). The competition for available N between microorganisms and plants in straw-incorporated plots could have resulted in a decreased NO_3^--N concentration (Fig. 6a) and lower N_2O emissions (Fig. 5b). Our results demonstrate that crop straw interacts with N fertilization to control N_2O emissions in intensively farmed soils.

At the seasonal scale, when no N fertilizer was applied, 45% higher N_2O emissions were observed under the straw-incorporated (368 g N_2O-N ha^{-1} $season^{-1}$) treatments relative to the treatments without straw (254 g N_2O-N ha^{-1} $season^{-1}$); however, in N-fertilized plots, the N_2O emissions from straw-incorporated and straw-removed treatments were similar (928 vs. 951 g N_2O-N ha^{-1} $season^{-1}$; Table 1). Similar observations were reported by previous meta-analyses (Shan and Yan, 2013; Xu *et al.*, 2017). In the non-N-fertilized soils where N_2O production was relatively constrained by the limited available N (Kim and Giltrap, 2017), straw input supplied about 60 kg N ha^{-1} in our study (N% = 0.69), nearly the same level as soil mineral N quantity (77 kg N ha^{-1}, 0–100 cm; data not shown), which provided an important substrate for N_2O generation (Kumar and Goh, 1999; Chen *et al.*, 2013; Huang *et al.*, 2017). However, in the N-fertilized plots, N_2O emissions induced by straw N addition were probably overwhelmed by the intensive N fertilization (Yao *et al.*, 2017), although straw-incorporated treatments recieved about 24% higher total N input than straw-removed treatments. Our results suggest that straw incorporation could enhance the N supply without increasing the N_2O emissions in intensively managed soils.

It should be mentioned that the soil temperature during the wheat season in northern

China (10.8°C) is relatively low, which resulted in a moderate microbial activity and slow straw decomposition rate (Hartmann *et al.*, 2014; Warren Raffa *et al.*, 2015). Thus, it is probably not possible to critically examine significant effects of straw incorporation in just one cropping season. Further in-situ ^{15}N tracer studies should be conducted to assess the long-term effect of straw incorporation on the rate and source of N_2O emissions.

Conclusions

This in-situ ^{15}N tracing study provided an insight into the rate and source of N_2O emissions and the effect of straw incorporation on N_2O emissions in the intensively farmed soils of northern China. About 60%–67% of the wheat seasonal N_2O emissions were lost in the one to two weeks following fertilization events. Within 10–20 days after fertilization, fertilizer-derived N_2O became negligible, suggesting that it may be appropriate to reassess the period over which emission factors are calculated for greenhouse gas inventory purposes. Because of the long duration of high N input in this region, fertilizer N and soil N both accounted for about 40%–60% of the seasonal N_2O emissions in the fertilization period, which implies equivalent roles of the soil N pool and fertilizer N in N_2O generation in long-term intensively farmed soils. During the basal fertilization/first irrigation events, straw incorporation significantly stimulated CO_2 fluxes both in N-fertilized and non-N-fertilized plots; however, after the top-dressing/second irrigation events, the significant increase of CO_2 fluxes induced by straw incorporation was only observed in the N-fertilized

462 treatment. Application of N fertilizer had a significant priming effect on the soil N
463 pool, which may increase the risk of N_2O loss from N accumulated in the soil. Straw
464 incorporation interacted with N fertilization, and exhibited a tendency of enhancing
465 N_2O emissions in the basal fertilization and lowering N_2O emissions in the top-
466 dressing period. In N-fertilized plots, the seasonal N_2O emissions from straw-
467 incorporated and straw-removed treatments were similar, indicating straw
468 incorporation enhanced N supply without increasing the N_2O emissions. Our study
469 highlights the necessity of examining the long-term impacts of N fertilization and
470 straw incorporation on greenhouse gas emissions.
471

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825

826 **Figure captions**

827 **Figure 1** Dates of field management practices and sampling, and amounts of
828 irrigation and N fertilizer applications during the experimental period.

829 **Figure 2** Fluxes of N_2O under (a) N0S0, (b) N0S1, (c) N1S0, and (d) N1S1
830 treatments. Error bars represent standard error (n = 3). The solid arrows indicate ^{15}N
831 fertilizer application, and the dotted arrows indicate irrigation events.

832 **Figure 3** Fluxes of CO_2 for (a) non-N-fertilized and (b) N-fertilized treatments. Error
833 bars represent standard error (n = 3). Dotted and solid arrows indicate irrigation
834 events and N fertilizer application, respectively.

835 **Figure 4** Percentage of applied N-derived daily N_2O emissions after (a) basal
836 fertilization (23 Oct.) and (b) top-dressing (7 Apr.) for the N1S0 and N1S1 treatments.
837 ** represents 0.01 significance level. Error bars represent standard error (n = 3).

838 **Figure 5** Cumulative N_2O emissions after (a) basal fertilization (23 Oct.) and (b) top-
839 dressing (7 Apr.), which are divided into fertilizer-derived and soil-derived. Different
840 capital and lowercase letters indicate significant differences of fertilizer-derived and
841 soil-derived N_2O emissions, respectively, at $P < 0.05$. Error bars represent standard
842 error (n = 3). Dashed lines and braces are used to indicate the additional N_2O release
843 from the soil N pool after N fertilization.

844 **Figure 6** (a) NO_3^--N and (b) NH_4^+-N content from different sampling dates. Error
845 bars represent standard error (n = 3). Arrows indicate irrigation events and/or N
846 fertilizer application.

847 **Table 1** Cumulative N_2O and CO_2 emissions and crop yield (dry matter). Data are
848 expressed as mean ± standard error (n = 3). Different letters indicate significant
849 differences among the treatments at $P < 0.05$.

Treatment	N_2O emission ($g\ N_2O\text{-}N\ ha^{-1}$)	CO_2 emission ($Mg\ CO_2\text{-}C\ ha^{-1}$)	Yield ($g\ m^{-2}$)
N0S0	253.82 ± 51.57 b	1.46 ± 0.08 c	700.15 ± 54.11 a
N0S1	368.20 ± 32.50 b	2.09 ± 0.16 a	637.21 ± 49.20 a
N1S0	950.87 ± 150.67 a	1.64 ± 0.12 bc	770.51 ± 48.46 a
N1S1	928.40 ± 79.89 a	1.96 ± 0.03 ab	817.38 ± 93.04 a

850

851 **Table 2** Proportion of N_2O emissions derived from background, priming effect, and
852 fertilizer. Data are expressed as mean ± standard error (n = 3). Different letters
853 indicate significant differences between different treatments at $P < 0.05$.

Event	Treatment	Background	Priming effect	Fertilizer
Basal fertilization	N1S0	5.74 ± 0.36% a	40.47 ± 8.33% a	53.8 ± 8.18% a
	N1S1	8.17 ± 1.54% a	50.47 ± 6.53% a	41.38 ± 800% a
Top-dressing	N1S0	19.60 ± 0.89% a	28.61 ± 4.59% a	51.81 ± 3.74% a
	N1S1	27.07 ± 2.97% a	21.02 ± 1.54% a	51.93 ± 2.35% a

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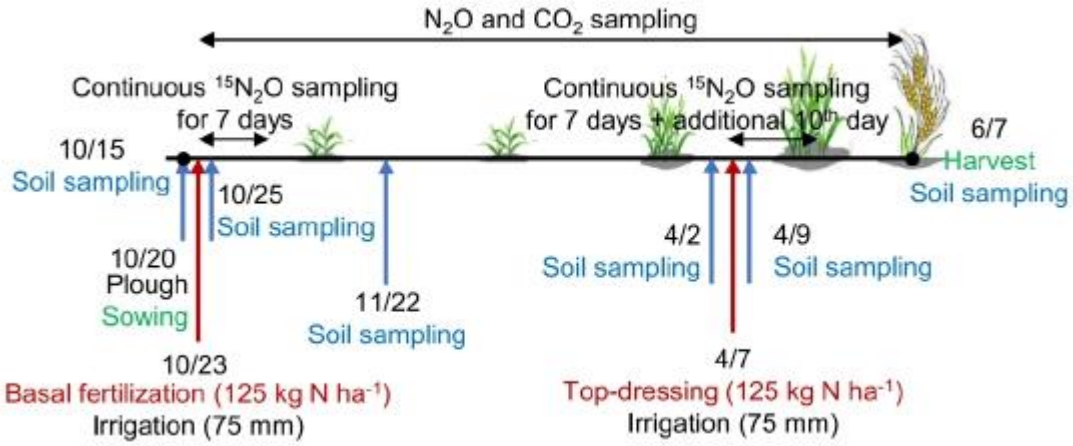
858 **1**


N_2O and CO_2 sampling
Continuous $^{15}N_2O$ sampling for 7 days
Continuous $^{15}N_2O$ sampling for 7 days + additional 10th day
10/15
Soil sampling
10/25
Soil sampling
10/20
Plough
Sowing
11/22
Soil sampling
10/23
Basal fertilization (125 kg N ha^{-1})
Irrigation (75 mm)
4/2
Soil sampling
4/9
Soil sampling
4/7
Top-dressing (125 kg N ha^{-1})
Irrigation (75 mm)
6/7
Harvest
Soil sampling

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861 **Fig. 2**

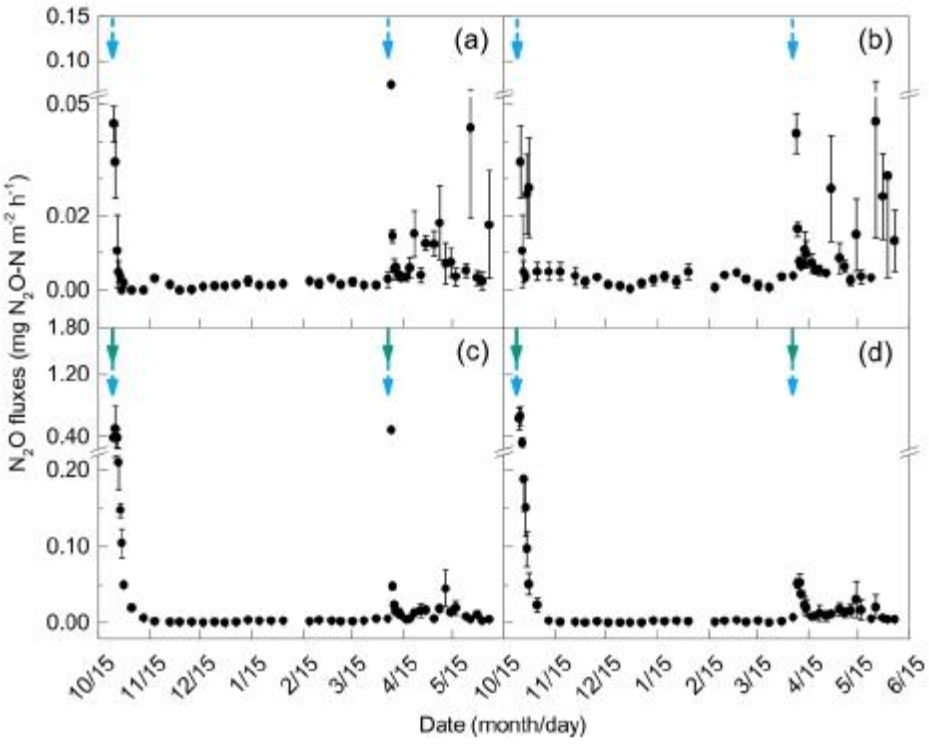

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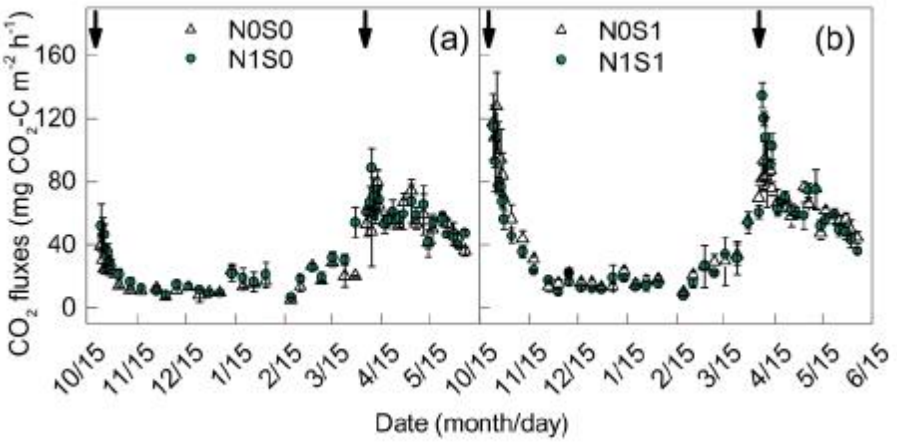
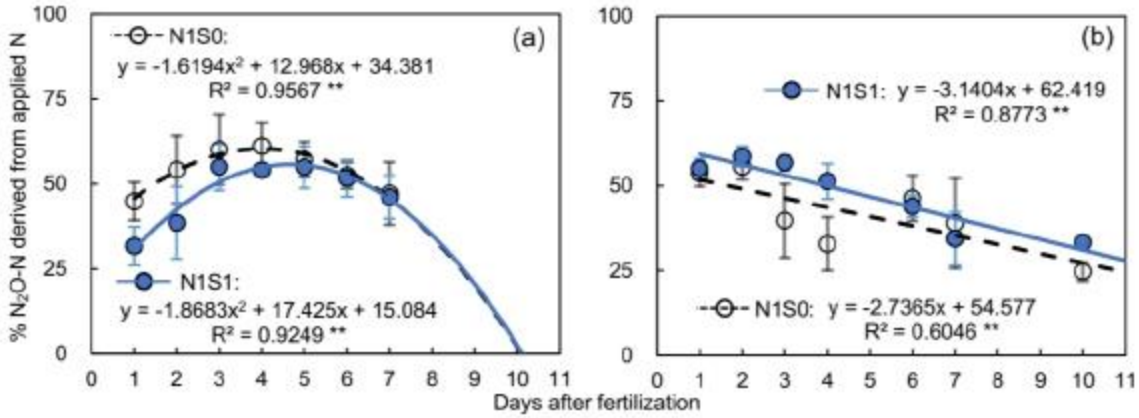
Fig. 3


N0S0
N1S0
(a)
N0S1
N1S1
(b)
CO2 fluxes (mg CO2-C m-2 h-1)
0
40
80
120
160
10/15
11/15
12/15
1/15
2/15
3/15
4/15
5/15
6/15
Date (month/day)

867 **Fig. 4**



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870 **Fig. 5**

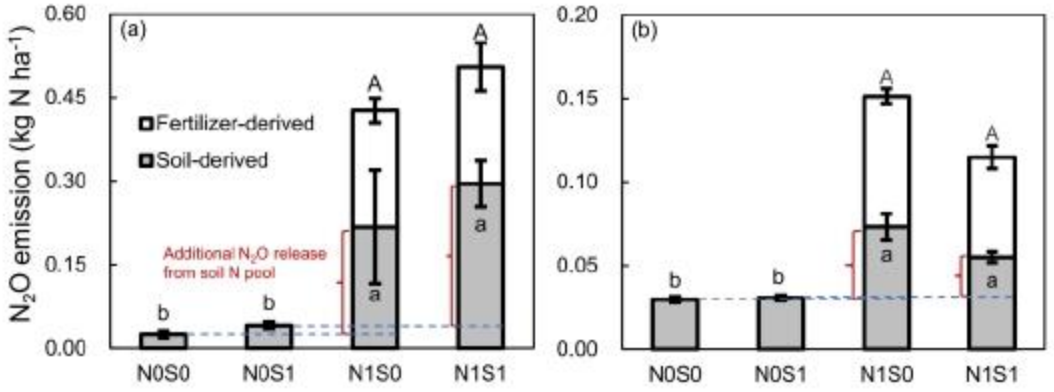

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Fig. 6