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Contributions of agricultural plants and soils to N₂O emission in a farmland

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Abstract

The goal of this study was to quantify the roles of plants and soil in the N_2O budget of a cropland in North China. Plant and soil N_2O fluxes were measured with transparent and dark plant chambers and soil chambers, respectively, in three adjacent fields of fertilized cotton, fertilized maize and unfertilized soybean. During the observation period, the soil flux was 448 ± 89 , 230 ± 74 and $90 \pm 14 \mu\text{g } N_2O \text{ m}^{-2} \text{ h}^{-1}$ in cotton, maize and soybean fields, respectively. The plant flux was 54 ± 43 and $16 \pm 41 \mu\text{g } N_2O \text{ m}^{-2} \text{ h}^{-1}$, about 10 % and 26 % to the total ecosystem flux, for the cotton and the soybean field, respectively. Ignoring the contribution of plants would cause an obvious underestimation on the ecosystem N_2O flux. The influence of sunlight on plant N_2O flux was insignificant. However, in the cotton field, the responses of the plant N_2O flux to air temperature and soil ammonium content were significant under sunlight but insignificant under darkness, suggesting that stomatal activity might influence the release process. In the cotton field, temperature sensitivity of plant N_2O emission was 1.13, much lower than the value of soil flux (5.74). No relationship was found between plant N_2O flux and soil nitrate content. It was implied that nitrate reduction in plants might not be the main source of plant N_2O emission under field conditions. The seasonal patterns of the soil and plant N_2O emissions were similarly affected by fertilization, indicating that plants might serve as a passive conduit transporting N_2O produced in the soil.

1 Introduction

In recent years, increasing attention has been paid to nitrous oxide (N_2O) for its contribution to the greenhouse effect and stratospheric ozone destruction. The concentration of atmospheric N_2O has been increasing at a rate of 0.2 %–0.3 % per year (IPCC, 2007). N_2O is mainly derived from microbial nitrification and denitrification in soils. Both processes are controlled by many soil factors including temperature, moisture, nitrate and ammonium content, organic matter content, pH and particle-size. The enhanced N_2O emissions from agricultural and natural ecosystems are caused by increasing soil

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nitrogen availability driven by increased fertilizer use, agricultural N₂ fixation and nitrogen deposition (IPCC, 2001). About 1.25 % of the mineral N fertilizer added to the agricultural soils is emitted as N₂O (Bouwman et al., 1995). The effect of soil moisture on soil N₂O flux is more complex. High soil moisture is conducive to soil denitrification and N₂O production. Excessive amount of soil moisture can promote further reduction of N₂O to N₂, thus suppressing N₂O emission (Stevenson, 1982; Firestone and Davidson, 1989). The regulation of trace N-gas production via nitrification and denitrification has been described by the “hole-in-the-pipe” conceptual model (Firestone and Davidson, 1989).

In comparison to the soil component, much less is known of the contribution of plants to atmospheric N₂O. Plants are thought to affect soil N₂O production indirectly through the influence of root growth on soil microbe processes, including the uptake of NO₃⁻ (Smith and Tiedje, 1979; Bakken, 1988; Mahmood et al., 1997; Simojoki and Jaakkola, 2000; Ghosh et al., 2002; Yang and Cai, 2006). The direct role of plant materials has been investigated mostly under laboratory conditions or using plants growing in pots (Dean and Harper, 1986; Rusch and Rennenberg, 1998; Chen et al., 1999; Yan et al., 2000; Smart and Bloom, 2001; Pihlatie et al., 2005; Zou et al., 2005). These investigations lead to two competing hypotheses on the mechanism of plant N₂O emission: (1) N₂O is actively produced by nitrate reduction in plants (Dean and Harper, 1986; Goshima et al., 1999; Smart and Bloom, 2001; Hakata et al., 2003); (2) plants act as a passive path of N₂O release from soils to the atmosphere (Chang et al., 1998; Rusch and Rennenberg, 1998; Pihlatie et al., 2005). Similar hypotheses can be given on the mechanism of plant N₂O uptake: N₂O may be (1) absorbed and metabolized by plants (Grundman et al., 1993) or (2) conveyed via plants into the soil because soils may act as a sink of N₂O (Blackmer and Bremner, 1976; Ryden, 1981; Henault et al., 1998; Verchot et al., 1999). The study of Lensi and Chalamet (1981) seems to support the second hypothesis as the uptake phenomenon they observed may be a result of passive diffusion caused by their plants being submerged in air of artificially high N₂O concentrations.

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In situ measurements of plant N_2O flux are scanty. The indirect observation, where excited plant parts are used to measure N_2O flux of wheat plants (Zou et al., 2005), may lead to large biases if N_2O produced in the soil is conveyed to the atmosphere via shoots. Measurement on the plant N_2O flux by sealing the soil surface (Chen et al., 2002) may overestimate the plant flux because the release of soil gases is prevented completely and plant shoots become the only path for gas emission from the soil. Furthermore, dark chambers are used in these investigations to avoid overheating the plant. The altered light environment would cause measurement artifact if N_2O is released via the stomatal pathway. There is insufficient field data to test either of the hypotheses put forth by the laboratory investigations cited above. Little is known on how factors such as plant species, soil moisture and nutrient status influence plant N_2O emission.

In this study, we report the measurement of soil and plant N_2O fluxes in fields of cotton, summer maize and soybean in the North China Plain. Three types of chambers were deployed in our experiment, including dark soil chamber, dark plant chamber and transparent plant chamber with temperature control. Our objectives are: (1) to quantify the contribution of the plant and soil to the net ecosystem N_2O exchange in the cropland, (2) to investigate how these fluxes vary with the season and species composition, fertilizer use, and abiotic factors, and (3) to explore mechanisms of N_2O release from or uptake by plants.

2 Site and methods

2.1 Site description

The study was carried out in the crop fields at the Yucheng Comprehensive Experimental Station ($36^{\circ}57' N$, $116^{\circ}36' E$, 22 m elevation), Chinese Academy of Sciences, in 2007. The station is located in the North China Plain, with a temperate monsoon climate. The annual average air temperature is 13.2° . The warmest and coldest months

are July (26.9°) and January (−2.4°), respectively. Annual precipitation is 585 mm, 70 % of which occurs from June to August. Soil type is alluvium deposited by Yellow River and soil texture of the root zone is sandy loam. Soil organic content and total nitrogen in the tillage layer are 1.2 % and 0.14 %, respectively. Soil pH value ranges from 7.1 to 8.5.

Farmland management methods were typical of the North China Plain. Cotton, summer maize and summer soybean were sowed on 29 April, 14 June and 5 July, respectively in three adjacent fields roughly 6 ha in size. The previous crop in the summer maize and soybean fields was winter wheat, and the cotton field was fallow before cotton sowing. For cotton, summer maize and summer soybean, the plant density was 4.7, 6.1 and 18.4 plants m^{−2}, respectively and the maximum LAI was 2.2, 4.0 and 5.5, respectively. On 23 July, synthetic fertilizer was applied to the cotton and summer maize fields at a rate of 78 kg N ha^{−1}. No fertilizer was applied to the soybean field. The harvesting date was 21 September for maize, from 4 September to 16 November for cotton. The soybean plants were cut and left in the field on 17 September before harvest.

2.2 Field observation

N₂O flux was measured with the closed chamber method in a cotton, maize and soybean field from July to October, 2007. Whole of season emissions were measured for cotton plants, cotton soil and maize soil. The observation was stopped for maize plants when they were grown up (higher than 1 m). In the soybean field, flux measurements for plants and soil were interrupted in the filling stage because the field was suddenly turned to another use. To quantify plant and soil N₂O fluxes, and the influence of sunlight, measurement in each field was carried out with three kinds of chambers (Fig. 1): (A) transparent plant chambers with active temperature control (Fig. 2), (B) dark plant chambers, and (C) dark soil chambers, with three replicates for each kind of chamber. All dark chambers were covered with quilts to keep a constant temperature inside. The plant chambers would enclose one plant inside in each

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measurement. These chambers consisted of a plexiglass or stainless steel body in the size of $50 \times 50 \times 50 \text{ cm}^3$ and a stainless steel base frame either inserted in the soil for the soil flux measurement or elevated 3 cm above the soil surface for the plant measurement. An extension made of plexiglass or stainless steel was added after the plant grew taller so that the total volume of chamber was $50 \times 50 \times 100 \text{ cm}^3$. The temperature inside the transparent chamber was controlled manually with ice bottles so that it matched the ambient temperature to within 2° during the measurement. Two small fans were installed in each chamber to mix the air inside. For the plant chambers (chambers A and B), two detachable plexiglass baseboards were carefully placed on the base frame with a small hole in the middle to accommodate the plant stem. This hole and the gap between the base plates and the frame were sealed with clay putty and glue tape, respectively. The chamber body was fit into a narrow groove on the base frame sealed with water (Fig. 1).

Gas samples were collected by airtight syringes (100 ml in volume) 0, 10, 20 and 30 min after closing of the chamber. All measurements were carried out in the morning (around 09:00 AM), at a frequency of two measurements per week for each site. Together with the flux measurements, air temperature inside and outside the chamber, soil temperature (0 and 5 cm depth) and soil water content (0–5 cm depth) were measured. Three soil samples were taken biweekly at each site at depths of 0–20 and 20–40 cm. Inorganic NH_4^+ and NO_3^- were measured by a flow-injection analyzer (Tecator, Aquatec 5400 analyzer) with the extractant of 100 ml of 2 M KCl solution to 10 g fresh soil.

2.3 Gas chromatography analysis

Gas samples were analyzed by gas chromatography (GC) at the experimental station, within 10 h after collection. The gas chromatography (Agilent 4890D) was equipped with a ^{63}Ni electron capture detector and a stainless steel separation cylinder (3 mm in diameter, 2 mm in length) with a Porapak Q (80/100 mesh) inside. N_2 gas with high purity (99.999 %) was used as the carrier gas. Working temperatures of the cylinder

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and the detector were 55° and 330° respectively (Wang and Wang, 2003). Standard gas with a N₂O concentration of 320 ppbv mixed in N₂ was supplied by the State Standard Material Center of China. The GC had a good linear response within the range of N₂O concentrations from 250 to 1000 ppbv. Alkali asbestos was used to scrub CO₂ from the gas samples before the GC analysis to avoid CO₂ cross-contamination on the N₂O detection spectrum (Scheer et al., 2008).

2.4 Flux calculation

N₂O flux was calculated as follows

$$F = h \frac{MP}{RT} \frac{\partial C}{\partial t} \quad (1)$$

where F is the N₂O flux, with positive value denoting N₂O emission, h the height of the chamber, M molar mass of N₂O, P air pressure, R gas constant for air, T air temperature inside the chamber, C N₂O molar mixing ratio inside the chamber, t the time after closing the chamber. Based on Eq. (1), soil flux was expressed in the unit of μg N₂O per m² ground area per hour. Plant flux expressed as μg N₂O per m² ground area per hour was calculated as follows:

$$F_p = F \frac{D}{4} \quad (2)$$

where D is plant density in number of plants per m² and the factor 4 recognizes that the chamber base area is 0.25 m².

3 Results

3.1 Flux detection limits

Since the background N₂O fluxes for soils and plants are generally low, it is necessary to understand the resolution of the GC analysis for N₂O concentration. The average

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5 resolution of GC analysis was 1.1 % for N₂O concentration and 2.2 % for the variation of N₂O concentration. In other words, any change in the N₂O concentration below 7 ppbv after chamber closure would be within measurement noise. Under 20° and standard atmospheric pressure and with a measurement duration of 30 min, the detection limit of the soil N₂O flux was around 13 μg N₂O m⁻² h⁻¹. The detection limits of plant N₂O flux, when expressed on the basis of unit ground area, depends on plant density, and were about 15, 20 and 60 μg N₂O m⁻² h⁻¹ for cotton, maize and soybean, respectively. About 25 % of the observations were below these detection limits. There are two approaches to handling the data below the detection limit. The first approach advocates using all
10 the data and the second approach sets the flux to zero if the concentration variation falls below the detection limit (Sjögersten and Wookey, 2002). In this study, the first approach was used to calculate the mean N₂O flux.

15 Another performance measure is chamber blank. In the blank test, the replicate chambers were installed in the field, either containing no plant (plant chambers) or isolated from the soil (soil chambers). N₂O concentration was measured in the same manner as in the regular field observations and was used to compute the blank flux value. Six blank tests were done for the transparent and dark chambers, all with three replications, at various times of the day. The blank fluxes were $-1.44 \pm 0.87 \mu\text{g N}_2\text{O m}^{-2} \text{h}^{-1}$ for transparent chamber and $-3.49 \pm 2.09 \mu\text{g N}_2\text{O m}^{-2} \text{h}^{-1}$ for the dark chamber. Con-
20 sidering the plant density, the blank fluxes of transparent plant chambers were -2, -2 and $-7 \mu\text{g N}_2\text{O m}^{-2} \text{h}^{-1}$ for cotton, maize and soybean, respectively. The blank fluxes of dark plant chambers were -4, -5 and $-16 \mu\text{g N}_2\text{O m}^{-2} \text{h}^{-1}$ for cotton, maize and soybean, respectively. These blank values were lower than the flux detection limits, indicating that the chambers themselves did not interfere with the N₂O concentration
25 variations. Therefore, no blank correction was applied to the flux data.

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3.2 Soil N₂O flux

Low-frequency field measurements (once-daily or less) may cause bias errors in seasonal mean soil N₂O flux when pronounced diurnal variations occur (Yao et al., 2009). In our study, the morning flux may be lower than the daily average because the flux showed exponential dependence on the increase of soil temperature and soil temperature in the morning (around 09:00 AM) was on average 1° lower than the daily mean (Table 1). In the following, the temperature effect was corrected using the field-specific regression equations relating the flux to soil temperature (Table 1).

Soil N₂O fluxes showed strong seasonal variations (Figs. 3–5). The background flux, or flux observed during periods not impacted by fertilization, was around 27 μg N₂O m⁻² h⁻¹ for cotton and maize which was roughly one third of that of soybean (Table 2). In the maize and cotton fields, soil flux increased rapidly in response to fertilization on 23 July, peaking on 27 July in the maize field and on 1 August in the cotton field. After a heavy rain event, a second peak appeared on August 8 in the maize field. The spatial variation as indicated by the standard deviation was also greatly enhanced by fertilization. The maximum flux, with 3113 μg N₂O m⁻² h⁻¹ in the cotton field and 1271 μg N₂O m⁻² h⁻¹ in the maize field, was observed 9 and 16 days after the fertilizer application, respectively. Afterwards, the soil N₂O emission decreased gradually with time. One or two month after fertilization, it dropped to the former level (Figs. 3 and 4). A maximum flux of 346 μg N₂O m⁻² h⁻¹ in the soybean field was observed on 28 July (Fig. 5). Seasonal mean soil fluxes were 448, 230 and 90 μg N₂O m⁻² h⁻¹ for the cotton, maize and soybean fields, respectively (Table 2). N₂O released from cotton and maize soils accounted for 2.2 % and 0.5 % of the applied fertilizer nitrogen.

3.3 Plant N₂O flux

In the cotton field, the seasonal pattern of plant N₂O flux measured with transparent chambers shows the influence of fertilization (Fig. 3). Before fertilization, cotton plants released N₂O at very low rates (<10 μg N₂O m⁻² h⁻¹). On three occasions, the

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flux was negative, indicating uptake of N_2O from the atmosphere. A maximum uptake rate of $-73 \mu\text{g N}_2\text{O m}^{-2} \text{h}^{-1}$ was observed on 18 July. Plant N_2O emission was enhanced by fertilization on 23 July, showing a maximum value of $573 \mu\text{g N}_2\text{O m}^{-2} \text{h}^{-1}$ two weeks later than the fertilizer application. The peak time (5 August) was slightly delayed than the peak time of the soil flux (1 August). The second peak of plant N_2O flux ($227 \mu\text{g N}_2\text{O m}^{-2} \text{h}^{-1}$) appeared one month after fertilization. Thereafter, plant N_2O emission decreased with time (Fig. 3). Plant flux measured with the dark chamber can be considered as light exclusion treatment. N_2O flux of cotton plants under darkness was generally lower and less variable than the flux measured with the transparent chamber, except for an episodic large value of $264 \mu\text{g N}_2\text{O m}^{-2} \text{h}^{-1}$ on 12 August (Fig. 3). The response of the dark flux to fertilization was weak. Averaged over the whole season, the dark flux was $33 \mu\text{g N}_2\text{O m}^{-2} \text{h}^{-1}$, about half of the value observed with the transparent chamber ($75 \mu\text{g N}_2\text{O m}^{-2} \text{h}^{-1}$, Table 2).

Measurement of plant flux in the maize field was limited to the early part of the growing season when the plants were small enough to fit inside the plant chambers. Most of measurements showed negative flux, suggesting a small plant uptake of N_2O from the atmosphere. The observed uptake rate was higher for the plant in transparent chamber than in dark chamber. After fertilization, plant flux increased rapidly under sunlight, resulting in a positive mean flux in the early growing stage. However, dark plant flux remained a low level and close to zero in average (Fig. 4 and Table 2). Estimate of seasonal mean flux for maize plants was not possible for lack of data in the later growing season.

Compared with soil flux, soybean plant N_2O flux changed in a large range of $\pm 200 \mu\text{g N}_2\text{O m}^{-2} \text{h}^{-1}$ (Fig. 5). Seasonal mean flux of soybean plants was -2 and $34 \mu\text{g N}_2\text{O m}^{-2} \text{h}^{-1}$ observed with the transparent and dark chambers, respectively (Table 2). Because one chamber covered one plant, high density of soybean plants enlarged the observational errors. As a result, nearly half of plant flux data were below the detection limit (Fig. 5).

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The total seasonal plant flux was estimated from the data obtained with the transparent and the dark chambers, which were considered to represent the status of daytime and nighttime, respectively. A local day to night length ratio of 1.16 was used as a weighting factor to calculate seasonal mean plant N₂O flux. The seasonal average N₂O flux for the cotton and soybean plants was 54 and 16 μg N₂O m⁻² h⁻¹, respectively (Table 2). Ignoring the plant contribution would cause an underestimation of the ecosystem flux by 10 % and 26 % for the cotton and soybean fields, respectively (Table 2).

3.4 Factors influencing soil and plant N₂O fluxes

Soil N₂O flux showed exponential increase with the increase of soil temperature at the depth of 5 cm for all three crop fields ($P < 0.01$). The temperature sensitivity (Q_{10}) was 5.74, 21.43 and 11.08 for the cotton, maize and soybean soils, respectively. Soil N₂O flux displayed exponential dependence on soil moisture at the depth of 0–5 cm in the cotton and maize fields ($P < 0.05$ and $P < 0.01$, respectively) (Table 3). However, the correlation was insignificant in the soybean field due to its low soil moisture (Tables 2 and 4). The three fields responded differently to soil nitrogen status. Natural log transformed soil N₂O flux was linearly correlated with soil ammonium content ($P < 0.05$) in the cotton field when soil pore volume filled with water (WFPS) was less than 67 %. It was linearly correlated with soil nitrate content ($P < 0.001$) in the maize field, especially when WFPS was higher than 67 %. In the soybean field, soil water content was low (Table 2). Natural log transformed soil N₂O flux was linearly correlated with soil nitrate and ammonium content ($P < 0.05$ and $P < 0.01$ respectively) but the correlations were negative (Table 3).

The difference between plant N₂O flux under sunlight and darkness was not statistically significant on account of large spatial variability. However, in the cotton field, the responses of plant N₂O flux to environmental factors were different under sunlight and darkness. Under sunlight, a significant correlation existed between plant N₂O flux and soil ammonium content at the depth of 0–40 cm ($P < 0.05$) when soil moisture was low

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(WFPS < 67%). The cotton plant N₂O flux was positively correlated with air temperature ($P < 0.05$) but had no relationship with soil temperature, moisture or soil nitrate content. The temperature sensitivity of the plant N₂O emission was 1.13, much lower than the value of the soil N₂O emission. Under darkness, the correlations between the cotton plant N₂O flux and environmental factors were insignificant. Without fertilization, soybean N₂O flux was so low that it had no relationship with any environmental factors under light or dark conditions (Table 3).

3.5 Relationship between soil and plant N₂O emissions

The seasonal patterns of soil and plant N₂O emissions were similarly affected by fertilization (Figs. 2 and 3). The cotton plant N₂O flux was positively correlated with soil N₂O flux under both light and dark conditions ($P < 0.01$ and $P < 0.05$ respectively). During the early growing season of maize, the correlation between soil and plant N₂O fluxes was positive and significant under sunlight ($P < 0.01$). However, in the soybean field without fertilization, both plant and soil N₂O fluxes were low and did not correlate with each other (Table 4). In the cotton field, soil and plant N₂O fluxes showed an increasing trend with the increase of temperature, and both were positively correlated with soil ammonium content under low soil moisture. However, no consistent relationship was found for the soybean field (Table 3).

4 Discussion

4.1 Soil N₂O emission

Compared with other reports (e.g. Cates and Keeney, 1987; Mummey et al., 1998; Xing et al., 1998; Choudhary et al., 2001; Ruser et al., 2001), the observed soil N₂O fluxes in our experiment were averagely higher by 90 % in the cotton field and 60 % in the maize field, but less by 50 % in the soybean field. Seasonal fluxes of cotton and

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maize fields were enhanced due to fertilization. The extreme high emission following the fertilizer application emphasizes the importance of measuring the flux at high temporal frequency. In the cotton field, suitable soil moisture favored both nitrification and denitrification processes. Therefore, high soil ammonium and nitrate contents led to a much higher N_2O flux over there than in the soybean and maize fields (Table 2).

In all the three crop fields, soil N_2O release increased exponentially with the increase of soil temperature (Table 3), in agreement with the results for grasslands and other farmlands (Denmead et al., 1979; Blackmer et al., 1982; Clayton et al., 1994; Flessa et al., 2002; Wang, 2005). In a wide temperature range, the activity of nitrifier and denitrifier appeared to be enhanced with the increase of temperature. So did N_2O production by nitrification and denitrification. In our experiment, the values of temperature sensitivity for soil N_2O emission in the three croplands were less or within the values reported for arable soils (8.9–50.0) (Dobbie and Smith, 2001). A large base emission rate ($2.27 \mu g N_2O m^{-2} h^{-1}$) led to a small Q_{10} in the cotton field (5.74). In the maize and soybean fields, the base emission rates were very low (0.03 and $0.12 \mu g N_2O m^{-2} h^{-1}$, respectively). The unusually large Q_{10} in the maize field (21.43) may have been caused by the timing of the fertilizer application which occurred at high temperature and may also have been confounded by the seasonal change in soil moisture. In the cotton and maize fields, soil N_2O release rose exponentially with the increase of soil moisture (Table 3), in agreement with the experiment results from croplands and forests (Skiba et al., 1998; Keller and Reiners, 1994; Metay et al., 2007). In the soybean field, the correlation was not significant with soil moisture which was generally low.

Under aerobic conditions, nitrification is the dominant N_2O producing process in the soil (Pihlatie et al., 2004). The availability of ammonium is a key factor limiting the nitrification rate. In the cotton field, the soil N_2O release was positively correlated with soil ammonium content under low moisture (Table 3), agreeing with the results obtained by Macdonald et al. (1997). On the other hand, denitrification benefits from high soil moist content. N_2O reduction rate declines with the increase of soil nitrate and nitrite content under anaerobic conditions because nitrate and nitrite are better

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electronic receivers than N₂O (Stevenson, 1982). This may be the reason why N₂O emission from the moist soil was proportional to the soil nitrate content (Table 3). Our result in the maize field was consistent with the reports by Mahmood et al. (1998) and Ruser et al. (2001). In the soybean field, correlations between soil N₂O flux and soil ammonium and nitrate content (Table 3) were negative, contrary to many reports in crop fields (e.g. Smith et al., 1998; Mahmood et al., 1998; Ruser et al., 2001). The disparity may be attributed to small soil N₂O emission caused by low soil moisture and available nitrogen content (Table 2 and Fig. 5).

4.2 Plant N₂O emission

In our experiment, the soybean N₂O flux was about two times of the values obtained by Chen et al. (2002) for their soybean field. N₂O emissions from the cotton and soybean plants accounted for 12 % and 31 % of the total fluxes from soil-plant systems, respectively. The high ratio in the soybean field resulted from low soil N₂O release. Pot experiments showed that without flooding in the rice paddy, 17.5 % of the produced N₂O convey from soil via plants to the atmosphere (Yan et al., 2000), which is in the range of our values.

Evidence of stomatal control on the plant flux was not consistent. In the three crop fields, large standard deviations on plant N₂O flux (Figs. 2–4) were indicative of high spatial variability in soil moisture and nutrient contents. The difference in plant N₂O flux observed with the transparent and the dark chambers may have been masked by the large variability among the replicates. Consequently, the influence of light on plant N₂O flux was not statistically significant, implying no stomatal control on plant N₂O emission. On the other hand, in the cotton field, the responses of the plant flux to air temperature and soil ammonium content were significant under light conditions but insignificant under dark conditions (Table 3), suggesting that stomatal activity might influence the release process.

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After fertilization, the significant correlation between soil and plant N_2O fluxes (Table 4) implied that N_2O -N released by plants came from the soil. Two hypotheses are used to interpret the mechanism of plant N_2O emission: N_2O may be produced by nitrate reduction in plants (Dean and Harper, 1986; Goshima et al., 1999; Smart and Bloom, 2001; Hakata et al., 2003), or produced in the soil and conveyed via plants to the atmosphere (Chang et al., 1998; Rusch and Rennenberg, 1998; Pihlatie et al., 2005). According to the first hypothesis, plant N_2O flux should be proportional to the nitrate content. However, in the cotton field, both the soil and plant N_2O fluxes were positively correlated with soil ammonium content but had no relationships with soil nitrate content (Table 3). Our results do not support first hypothesis, especially in the field with fertilization.

In the upland, it is possible that N_2O is released to the atmosphere via shoots, as it does in rice paddy and wetland (Mosier et al., 1990; Rusch and Rennenberg, 1998). A potential mechanism is N_2O conveyance with the transpiration stream in the plants (Chang et al., 1998; Pihlatie et al., 2005). Soil solution dissolved with N_2O might be taken up by the roots and conveyed to the shoots. After fertilization, N_2O dissolved in the soil solution was often close to saturation due to high N_2O concentration in the soil air. Along the concentration gradient, N_2O might be diffused from the soil to the shoots, from the sap to the air within shoots, and released to the atmosphere through the stoma and the epidermis. Similar to oxygen (Sharkey, 1991), gaseous N_2O exchange between the leaves and the atmosphere might not be totally controlled by the stoma. The significant influence of air temperature on the plant N_2O release (Table 3) may have resulted from the temperature effect on the solubility of N_2O in the sap. If the transpiration hypotheses were correct, an order of magnitude estimate for the plant N_2O flux can be made from transpiration and N_2O concentration in the soil solution. However, we do not expect that plant N_2O flux to be simply correlated with the transpiration rate or the soil water content (Table 3) because in the fertilized soils, N_2O production varied greatly resulting in the shifts of N_2O concentration in the soil solution.

On the global scale, evapotranspiration was estimated to be $7.6 \times 10^{18} \text{ g y}^{-1}$ from the farmland (Oki and Kanae, 2006). The crop coefficient – the ratio of transpiration to evaporation – is close to 1 when LAI is larger than 2.5 (Kang et al., 2003). According to the reported average N_2O concentration in the soil solution ($96.6 \mu\text{g N}_2\text{O l}^{-1}$) in the agricultural fields of Dowdell et al. (1979), the estimated global N_2O emission from agricultural plants would be $0.73 \times 10^{12} \text{ g N}_2\text{O y}^{-1}$, or 17.5 % of the global N_2O emission from the arable soil (IPCC, 2001). This estimate and our field observations suggest that neglect of plant N_2O release may be an important reason for the unbalance of the global atmospheric N_2O sources and sinks.

4.3 Plant N_2O uptake

Using the flux-gradient micrometeorological method, Li et al. (2008) found N_2O can be absorbed by their maize ecosystem under dry soil conditions. In this study, N_2O uptake by cotton, maize and soybean plants were observed using the chamber method, especially when soil moisture was low (Figs. 2–4). Lensi and Chalamet (1981) first demonstrated that plant leaves are able to absorb N_2O . Using the ^{15}N isotope method, Grundman et al. (1993) reported that N_2O can be absorbed and metabolized by maize leaves, but the N metabolized is much less than the N absorbed. Another school of thought argues that plants act as a passive path of N_2O from the atmosphere to the soils. Many studies found that N_2O can be absorbed by both wet and dry soils (Blackmer and Bremner, 1976; Ryden, 1981; Henault et al., 1998; Verchot et al., 1999; Flechard et al., 2005). In our experiment, N_2O absorption by the cotton plants usually occurred under dry soil conditions (Figs. 2–4). Regardless of the mechanisms involved, the amount of uptake was smaller than the plant emission when averaged over the growing seasons.

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During observation period, the soil flux was 448 ± 89 , 230 ± 74 and $90 \pm 14 \mu\text{g N}_2\text{O m}^{-2} \text{h}^{-1}$ in cotton, maize and soybean fields, respectively. The plant flux was $54 \pm 43 \mu\text{g N}_2\text{O m}^{-2} \text{h}^{-1}$ in the cotton field and $16 \pm 41 \mu\text{g N}_2\text{O m}^{-2} \text{h}^{-1}$ in the soybean field. Ecosystem N_2O flux would be underestimated by 10% and 26% in the cotton and soybean field, respectively, if plants were neglected and only soils were involved in the measurements. Furthermore, occasional N_2O uptake was observed for all the three plants usually when soil moisture was low.

~~The contribution of plants to ecosystem N_2O flux varied with species composition, plant density, fertilizer use, and abiotic factors.~~ The influence of sunlight on plant flux was insignificant. However, in the cotton field, the responses of plant N_2O flux to some environmental factors were different under sunlight and darkness, suggesting that stomatal activity might influence the release process. Under sunlight, plant efflux enlarged exponentially with the increase of air temperature ($P < 0.05$), coinciding with the relationship between soil N_2O emission and soil temperature ($P < 0.01$). Temperature sensitivity of cotton N_2O emission was 1.13, much lower than the value of soil flux (5.74). Both soil and plant N_2O fluxes were positively correlated with soil ammonium content under low soil moisture ($P < 0.05$). Nevertheless, the consistent relationship was not found under darkness, or in the fields without fertilization. In fertilized fields, plant and soil N_2O fluxes correlated with each other and their seasonal patterns were consistent. Further study showed that plant N_2O flux had no relationships with soil nitrate content. It was implied that N_2O might not be produced by nitrate reduction in plants but primarily produced in the soil and released to the atmosphere via shoots.

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Table 1. Temperature effect on soil N₂O flux when transfer the morning flux to daily mean flux.

Plot	Equation	r	T_s	T'_s	F_s	F'_s	F'_s/F_s
Cotton field	$\ln(F_s) = 0.1747 T_s + 0.8189$	0.59**	20.7	21.8	84.7	102.7	1.2
Maize field	$\ln(F_s) = 0.3065 T_s - 3.396$	0.54**	22.4	23.5	31.7	44.4	1.4
Soybean field	$\ln(F_s) = 0.2405 T_s - 2.0852$	0.62**	22.8	23.9	30.3	39.4	1.3

r : coefficient of determination;

T_s : mean soil temperature at the depth of 5 cm during the observation period (09:00–09:30 AM);

T'_s : daily mean soil temperature at the depth of 5 cm;

F_s : simulated soil N₂O flux around 09:00 AM using the equation in the table;

F'_s : simulated soil N₂O flux for daily average using the equation in the table;

F'_s/F_s , temperature correction factor.

Daily mean flux equals to the measured morning flux times the temperature correction factor.

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Table 2. Seasonal mean plant and soil N₂O fluxes, soil moisture and available nitrogen content in crop fields.

Item	Cotton field	Maize field	Soybean field
F_{pt}	75 ± 44 a*	15 ± 54 a*	-2 ± 27 a*
F_{pd}	33 ± 16 a*	0 ± 8 a*	34 ± 31 a*
F_p	54 ± 43	7 ± 44	16 ± 41
F'_s	484 ± 97	43 ± 5	45 ± 14
F_s	448 ± 89	230 ± 74	90 ± 14
$F_p/(F_s + F_p)$	0.10	0.15	0.26
WFPS	66 %	64 %	53 %
Soil NH ₄ ⁺ -N	2.46	2.13	2.63
Soil NO ₃ ⁻ -N	31.98	23.13	20.18

F_{pt} : mean plant N₂O flux observed by transparent chambers, representing the daytime flux;

F_{pd} : mean plant N₂O flux observed by dark chambers, representing the nighttime flux;

F_p : weighting average of plant N₂O flux for the whole day,

$F_p = (1.16 F_{pt} + F_{pd}) / 2.16$;

F'_s : mean soil N₂O flux in the period corresponding to plant flux;

F_s : mean soil flux over the whole observation period;

All fluxes were “average ± spatial standard error” (μg N₂O m⁻² h⁻¹).

WFPS was the percentage of soil pore volume filled with water at the depth of 0–5 cm.

Soil NO₃⁻-N and NH₄⁺-N content was the average at the depth of 0–40 cm (mg kg⁻¹).

In the maize field, plant flux measurement was limited in the early growing stage.

* Lowercase represents $P < 0.05$.

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Table 3. Coefficients of linear correlation between N₂O flux and bio-environmental factors in crop fields.

Item	Crop	Coefficient								
		T _a	T _s	WFPS	Soil NH ₄ ⁺ -N			Soil NO ₃ ⁻ -N		
					Total	WFPS < 67 %	WFPS ≥ 67 %	Total	WFPS < 67 %	WFPS ≥ 67 %
Light plant flux	Cotton	0.45*	0.25	0.33	0.04	0.62*	-0.32	0.24	-0.43	0.45
	Soybean	-0.29	0.52	0.15	-0.12	-0.17	ND	-0.48	-0.67	ND
Dark plant flux	Cotton	0.18	0.21	0.42	-70.05	-0.09	-0.17	0.27	0.34	0.30
	Soybean	0.02	0.15	-0.29	0.47	0.40	ND	-0.46	-0.38	ND
Soil flux	Cotton		0.59**	0.41*	0.15	0.73*	-0.14	0.09	-0.51	0.19
	Maize		0.54**	0.59**	0.24	0.29	0.49	0.71***	0.46	0.87***
	Soybean		0.62**	0.20	-0.51	-0.70*	ND	-0.75**	-0.74**	ND

T_a is air temperature inside the chamber;
 T_s is the soil temperature at the depth of 5 cm;
 WFPS is the percentage of soil pore volume filled with water at the depth of 0–5 cm;
 Soil ammonia and nitrate content is the average at the depth of 0–40 cm.
 All soil fluxes were log transform before statistic.

* means *P* < 0.05;
 ** *P* < 0.01;
 *** *P* < 0.001.
 ND means no data.

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Table 4. Coefficients of linear correlation between soil and plant N₂O flux, between plant flux under sunlight and darkness.

Crop	Item	Coefficient	
		Light plant flux	Dark plant flux
Cotton	Dark plant flux	0.20	
	Soil flux	0.70**	0.44*
Maize	Dark plant flux	−0.35	
	Soil flux	0.99	−0.07
Soybean	Dark plant flux	0.53	
	Soil flux	0.04	0.00

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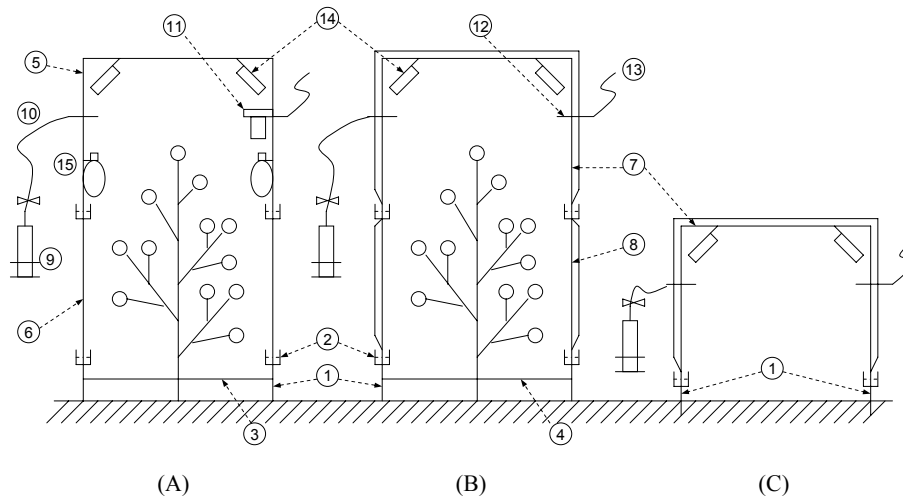


Fig. 1. The schematic diagrams of transparent plant chambers **(A)**, dark plant chambers **(B)**, and dark soil chambers **(C)**. The chambers consist of parts as follow: (1) Stainless steel base frame; (2) Groove on the base frame sealed with water; (3) Transparent plexiglass baseboard; (4) Opaque plexiglass baseboard; (5) Transparent plexiglass top chamber; (6) Transparent plexiglass extension chamber; (7) Stainless steel top chamber covered with quilt; (8) Stainless steel extension chamber covered with quilt; (9) Airtight syringe; (10) Gas pipe with switch; (11) Ventilated thermometer with radiation shield; (12) Thermometer; (13) Signal wire and Power cord; (14) Electric fan; (15) Ice bottle.

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Fig. 2. Photos of measurements using transparent plant chambers in cotton (left), maize (up right) and soybean (down right) fields.

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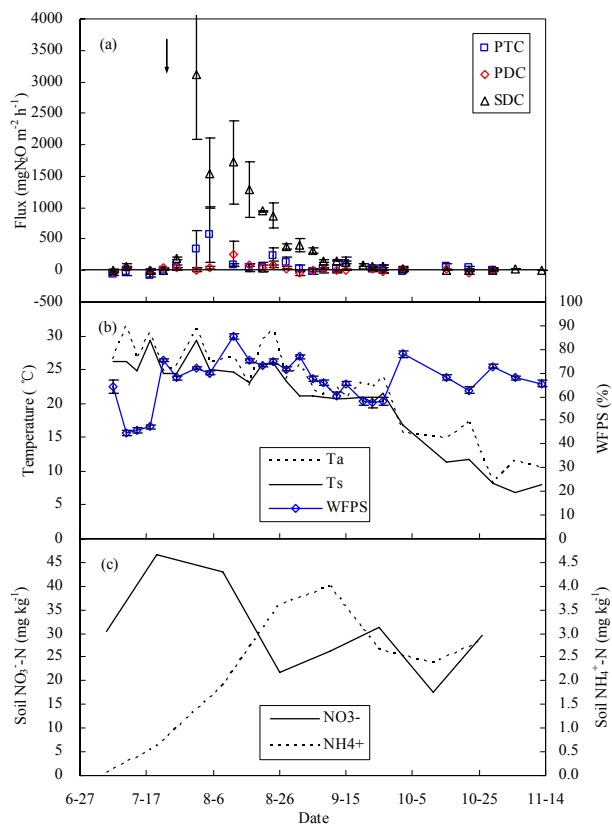


Fig. 3. Seasonal variation of plant and soil N_2O fluxes, air temperature (T_a), soil temperature at depth of 5 cm (T_s), the percentage of soil pore volume filled with water at the depth of 0–5 cm (WFPS), soil NO_3^- -N and NH_4^+ -N content (0–40 cm) in a cotton field. PTC: plant flux observed with transparent chambers; PDC: plant flux observed with dark chambers; SDC: soil flux observed with dark chambers. Arrow indicates fertilizer application.

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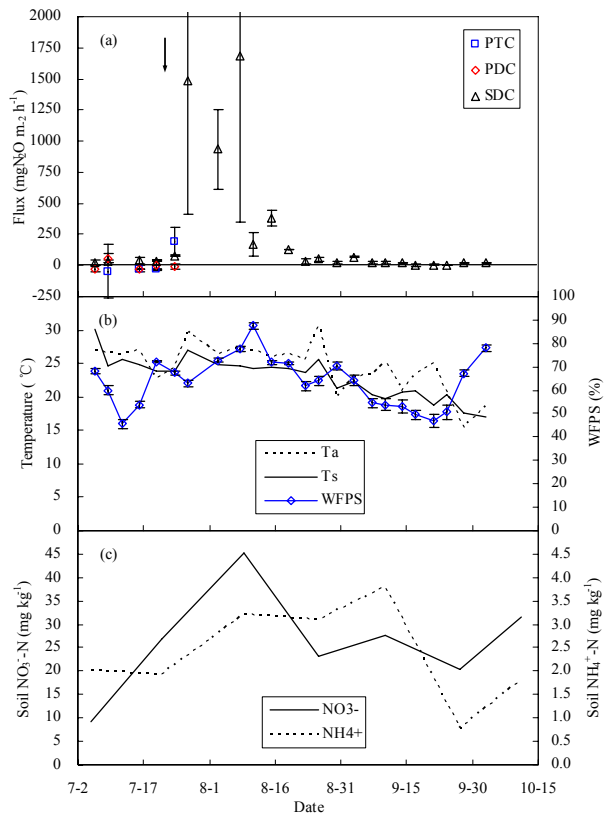


Fig. 4. Seasonal variation of plant and soil N_2O fluxes, air temperature (T_a), soil temperature at depth of 5 cm (T_s), the percentage of soil pore volume filled with water at the depth of 0–5 cm (WFPS), soil NO_3^- -N and NH_4^+ -N content (0–40 cm) in a maize field. The meaning of PTC, PDC and SDC is the same as Fig. 3. Arrow indicates fertilizer application.

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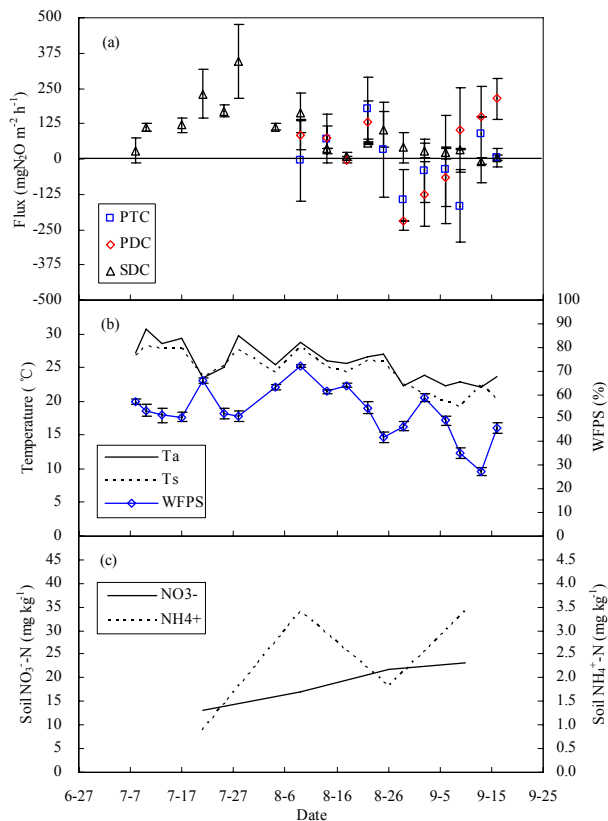


Fig. 5. Seasonal variation of plant and soil N_2O fluxes, air temperature (T_a), soil temperature at depth of 5 cm (T_s), the percentage of soil pore volume filled with water at the depth of 0–5 cm (WFPS), soil $\text{NO}_3^- \text{-N}$ and $\text{NH}_4^+ \text{-N}$ content (0–40 cm) in a soybean field. The meaning of PTC, PDC and SDC is the same as Fig. 3.