

Interactive comment on “Contributions of agricultural plants and soils to N₂O emission in a farmland” by J. Li et al.

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Response to review comments of Referee 1 on the manuscript

“Contributions of agricultural plants and soils to N₂O emission in a farmland” by J. Li et al.

General Comments:

1. Direct and indirect Plant N₂O emission is a controversial topic. In this paper, the authors did not provide any direct evidence of the plant N₂O production or transportation. It is suggested that the authors submit the paper to other journals.

[A]: We agree that this is a controversial topic. Field studies on plant N₂O emission

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are scanty, mainly because of difficulty in conducting observations in field conditions without creating measurement artifact. Our study was the first attempt to measure the plant flux with minimal alternation of the measuring environment (light, temperature, N₂O concentration). In this paper, plant and soil N₂O emission and their response to environmental factors were studied together under field conditions. The possible mechanisms underlying the plant N₂O emission were discussed.

It is true that our evidence supporting the transpiration hypothesis was indirect. However we respectfully disagree with the reviewer’s recommendation to decline publication on the basis of this argument alone.

Comments in detail:

2. Page 5512, line 14-15. Do the “plant chambers” and “soil chambers” in the parentheses wrongly labelled?

[A]: These labels are correct. The blank plant chamber is the one without plant. Similarly, the blank soil chamber is the one isolated from the soil.

3. Part 3.1 should be put in the site and method section.

[A]: Done. Thank you.

4. Page 5512 line 13-25. In the first several lines of the paragraph, plant chambers and soil chambers were mentioned, but in the last several lines, the results in transparent and dark chambers were presented, what are the connections of these chambers? Confusing!

[A]: We have adjusted the wording slightly to avoid the confusion. We now state “In the blank tests, the replicate chambers were installed in the field, either containing no plant (in the case of testing blank for the plant chambers) or isolated from the soil (in the case of testing blank for soil chambers).”

5. Page 5512, line 13. Why did the authors write “another performance measure is

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chamber blank”?

[A]: In response to this query, we have added the statement “In measurements of trace gas fluxes at very low levels, it is a common practice to test chamber blanks.” Blank tests are needed for chamber-based flux measurements. If the blank flux is lower than the detect limit, no blank corrections should be applied to the flux data.

6. Page 5513, line 2 to 8. The paragraph should be put in the site and methods section.

[A]: Done.

7. Page 5513, line 2 to 3. What do the authors want to say? In the paper the measurement frequency was two times per week. Even though there are some big diurnal variations, how can you solve it?

[A]: Line 2 to 3 is from Yao et al. (2009). For the limit of manual observations, morning flux (around 09:00 AM) was often used to represent diurnal mean. It was found that N₂O flux exponentially enlarged with the increase of soil temperature. Bias would be occurred in the flux measurements if morning temperature differed to the diurnal mean. The effect of temperature may be corrected based on the relationship between N₂O flux and soil temperature.

8. Page 5527, Table 1. How did the authors get the equations because the authors did not measure diurnal flux? If the equation was get by the whole measurement, how can the authors solve the water and substrate (NH₄⁺ and NO₃⁻) availability problem because during different period the water content and substrate availability is different.

[A]: The equation was obtained from the whole measurement. We agree with the reviewer on this point. We have deleted the relevant parts on temperature effects in the manuscript. Using diurnal data obtained in same fields in 2006, new questions were given in Table A1.

9. Page 5534, Fig.4, and page 5509, line 7. Cotton were sown on April 29, but the measurement began at the beginning of July, how about the flux during May and June?

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How can the results represent the seasonal flux?

[A]: Actually, cotton seedlings were very small until early June (Fig. A1). We started the flux measurement in 1st June. Unfortunately, the flux data in June were in errors because the GC analysis was done incorrectly. So, the available observation was from July to October.

10. Page 5528, Table 2. It is difficult to use dark chamber measurement to represent the nighttime flux.

[A]: We agree with the reviewer on this point because the daytime dark plant flux was observed under different temperature conditions from the night periods. However, because the temperature effects on the dark plant flux were insignificant (Fig. A2), it is acceptable to use the plant flux observed with the dark chambers to approximate the nighttime plant flux.

11. Page 5513, line 19. The authors should state it clear that in what period the flux of the “former level” represent.

[A]: “former level” was changed to “level before fertilization”.

12. Page 5515, line 2 to 3, it lacks support to use dark charber mensurement to represent nighttime flux.

[A]: We have given the evidence in query 10. Please see Figure A2

13. Page 5515, line 18, WFPS is water filled pore space.

[A]: We have modified the words according to the referee’s advice.

14. Page 5516, line 5 to 7. The results contradict with the results in Table 1.

[A]: To avoid misunderstanding, “soybean flux” was changed to “plant flux in the soybean field”.

15. Page 5516, delete line 15 to 18.

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[A]: Done.

16. Page 5527, Table 1. N₂O flux is pulse pattern and did not show a fixed temperature response pattern on the field condition. Moreover, soil moisture, substrate availability are other factors to control N₂O emission. From Fig.3 to 5, no relationship between temperature and N₂O flux could be found on a lot of measurements.

[A]: See response to query 8.

17. Page 5517, line 6-7, in Table 3, the readers can not find the exponential relationship between T and N₂O flux.

[A]: In Table 3, all the soil fluxes were log-transformed before the statistical analysis. Please see page 5517, line 17. The relevant parts on temperature effects were deleted in the revised manuscript.

18. Page 5517, line 13, what is base emission. It is suggested that the authors do not change the concept frequently, such as background, base. Or the authors should explain it clearly in the site and method section. As for the soil N₂O emission, there are not any new ideas. The authors are suggested to delete that section.

[A]: According to referee's suggestion, "base emission" has been changed to "background emission". Background emission was needed to show the impact of fertilization on the soil and plant N₂O fluxes. We choose to keep the text on the soil N₂O emission as part of the comparative analysis with the results published in the literature.

19. Page 5518, line 12, how could the authors get the soil-plant system N₂O emission?

[A]: The soil-plant system N₂O emission equals the soil flux plus the plant flux, both in the same units ($\mu\text{g m}^{-2} \text{h}^{-1}$).

20. Page 5518, line 21-23. For which crop?

[A]: For all three crops. Please see Table 2. We added "(Table 2)" in the sentence. The sentence was moved to the last paragraph in page 5519. Please see question 22.

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21. Page 5519, line 1-2. It is difficult to get such conclusion.

[A]: We rewrote this paragraph. Please see question 22.

22. Page 5519, line 3-11. The plant uptake NH₄⁺ or NO₃⁻, and then transformed to protein. No relationship was found between plant N₂O flux and soil NO₃⁻ content, it is difficult to say the N₂O flux from plant comes from the soil.

[A]: In response to this query, we have rewritten this portion of the discussion as follows:

"In the literature, two hypotheses are used to interpret the mechanism of plant N₂O emission: N₂O 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). In the cotton field, both the soil and plant N₂O fluxes were proportional to the soil NH₄⁺ content under low soil moisture conditions (Table 3), implying that the plants may have served as a passive conduit transporting N₂O produced by nitrification in the soil. It was unlikely that N₂O was transformed from NH₄⁺ in the plants because ammonium could not be accumulated in the plants for it is toxic to the plant tissues (Taiz and Zeiger, 2006).

When the soil moisture was high, denitrification becomes the main process of N₂O production (Ruser et al., 2001). It was found that the cotton plant N₂O flux was not correlated with soil nitrate content under wet conditions (Table 3). Even though the plant N₂O flux was related to the soil nitrate content, we could not distinguish if N₂O was produced by nitrate reduction in the plants or by denitrification in the soil. In the dry soybean field, mechanisms underlying the plant N₂O emission were further obscured by the fact that without fertilization the plant N₂O flux was very low and close to the detection limit (Table 2 and Fig. 5).

Two mechanisms of N₂O release via plants are possible: gaseous N₂O may diffuse along the concentration gradient, or be dissolved in the soil solution and released with

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the transpiration stream (Chang et al., 1998; Pihlatie et al., 2005). In the second mechanism, sunlight is needed to provide energy for transpiration. In the cotton field, the correlation between the plant N₂O flux and the soil NH₄⁺ content was significant only under sunlight (Table 3), indicating that N₂O may have been released with the transpiration stream. The responses of the plant N₂O flux to environmental factors (temperature and soil ammonium content) differed under sunlight and darkness, suggesting that stomatal activity might have influenced the release process. However, the impact of sunlight on the plant N₂O emission was statistically insignificant (Table 2). The difference in the plant N₂O flux observed with the transparent and the dark chambers may have been masked by the large variability among the replicates. Large standard deviations on the plant N₂O flux (Figs. 3-5) were indicative of high spatial variability in the soil moisture and nutrient contents. Another possible reason for the lack of correlation with sunlight exposure was that the plant N₂O flux might be dependent on both transpiration rate and the N₂O concentration in the soil solution. The latter experienced large fluctuations during the seasons and was uncorrelated with sunlight.

If the transpiration hypothesis is correct, an order of magnitude estimate for the plant N₂O flux can be made from transpiration and the N₂O concentration in the soil solution. . .”

23. Page 5521. In the conclusion section, the authors only need to write the main important findings in this paper, there is no need to write unrelavent words.

[A]: Done.

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Table A1 Temperature effect on soil N₂O flux when transfer the morning flux to daily mean flux, based on diurnal observations in the crop fields (2006).

| Plot | Equation | r | T _m | T _d | F _m | F _d | F _d /F _m |
|---------------|---------------------------------------|--------|----------------|----------------|----------------|----------------|--------------------------------|
| Cotton field | ln(F _s)=-0.0769*Ts-0.0369 | 0.58** | 20.7 | 21.8 | 4.7 | 5.2 | 1.09 |
| Maize field | ln(F _s)=-0.0563*Ts+1.6329 | 0.52** | 22.4 | 23.5 | 18.1 | 19.2 | 1.06 |
| Soybean field | ln(F _s)=-0.0816*Ts+0.6952 | 0.67** | 22.8 | 23.9 | 12.9 | 14.1 | 1.09 |

r: coefficient of determination;

T_m: mean soil temperature at the depth of 5 cm during the observation period (9:00-9:30 AM);

T_d: daily mean soil temperature at the depth of 5 cm;

F_m: simulated soil N₂O flux around 9:00 AM using the equation in the table;

F_d: simulated soil N₂O flux for daily average using the equation in the table;

F_d/F_m: temperature correction factor.

**; P<0.01.

Based on the field-specific regression equations relating the flux to soil temperature, daily mean N₂O fluxes for all three crop fields were obtained by 10% increase of the morning fluxes.

Fig. 1.

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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 |
|--------------------------------------|--------------|-------------|---------------|
| $F_{p,d}$ | 75±44 a | 15±54 a | -2±27 a |
| $F_{p,n}$ | 33±16 a | 0±8 a | 34±31 a |
| F_p | 54±43 | 7±44 | 16±41 |
| F_s | 444±89 | 34±4 | 38±12 |
| F_s' | 411±82 | 181±58 | 76±12 |
| $F_p/(F_p+F_s)$ | 0.11 | 0.18 | 0.29 |
| WFPS | 66% | 64% | 53% |
| Soil NH ₄ ⁺ -N | 2.46 | 2.13 | 2.63 |
| Soil NO ₃ ⁻ -N | 31.98 | 23.13 | 20.18 |

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

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

F_p : weighting average of plant N₂O flux for the whole day, $F_p=(1.16F_{p,d}+F_{p,n})/2.16$;

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" ($\mu\text{gN}_2\text{O m}^{-2} \text{h}^{-1}$).

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.

*. Different lowercase letters represent significance at the 0.05 level.

Fig. 2.

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

| Item | Crop | Coefficient | | | | | | |
|------------------------|---------|-------------|--------------------------------------|----------|----------|--------------------------------------|----------|----------|
| | | WFPS | Soil NH ₄ ⁺ -N | | | Soil NO ₃ ⁻ -N | | |
| | | | Total | WFPS<67% | WFPS≥67% | Total | WFPS<67% | WFPS≥67% |
| Light plant flux | Cotton | 0.33 | 0.04 | 0.62* | -0.32 | 0.24 | -0.43 | 0.45 |
| | Soybean | 0.15 | -0.12 | -0.17 | ND | -0.48 | -0.67 | ND |
| Dark plant flux | Cotton | 0.42 | -0.05 | -0.09 | -0.17 | 0.27 | 0.34 | 0.30 |
| | Soybean | -0.29 | 0.47 | 0.40 | ND | -0.46 | -0.38 | ND |
| Soil flux [#] | Cotton | 0.41* | 0.15 | 0.73* | -0.14 | 0.09 | -0.51 | 0.19 |
| | Maize | 0.59** | 0.24 | 0.29 | 0.49 | 0.71*** | 0.46 | 0.87*** |
| | Soybean | 0.20 | -0.51 | -0.70* | ND | -0.75** | -0.74** | ND |

WFPS is the percentage of soil water filled pore space 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.

Fig. 3.

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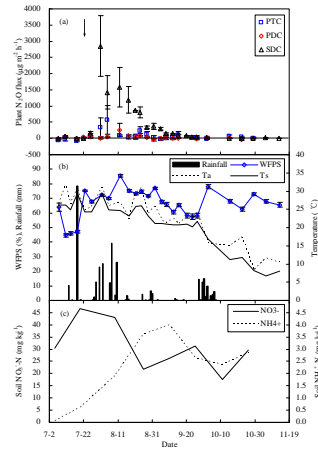


Fig. 3 Seasonal variation of plant and soil N_2O fluxes, air temperature (T_a), soil temperature at depth of 5 cm (T_s), the percentage of soil pore volume filled with water at the depth of 0-5 cm (WFPS), soil NO_3^- -N and NH_4^+ -N content (0-40 cm) in a cotton field. PTC: plant flux observed with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

Fig. 4.

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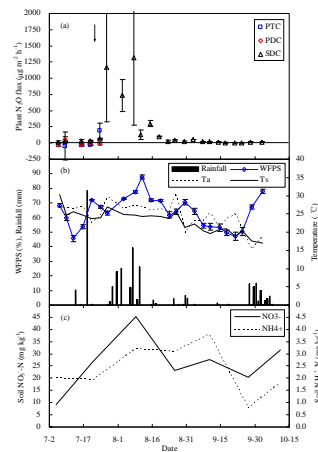


Fig. 4 Seasonal variation of plant and soil N_2O fluxes, air temperature (T_a), soil temperature at depth of 5 cm (T_s), the percentage of soil pore volume filled with water at the depth of 0-5 cm (WFPS), soil NO_3^- -N and NH_4^+ -N content (0-40 cm) in a maize field. The meaning of PTC, PDC and SDC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

Fig. 5.

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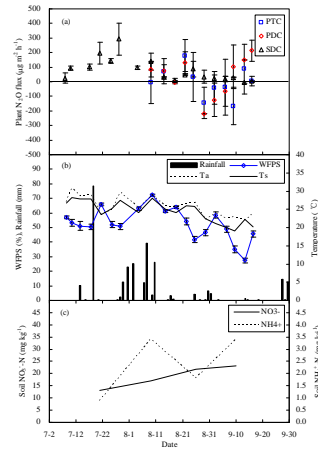


Fig. 5 Seasonal variation of plant and soil N_2O fluxes, air temperature (T_a), soil temperature at depth of 5 cm (T_s), the percentage of soil pore volume filled with water at the depth of 0-5 cm (WFPS), soil NO_3^- -N and NH_4^+ -N content (0-40 cm) in a soybean field. The meaning of PTC, PDC and SDC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

Fig. 6.

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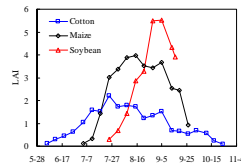


Fig. A1 Seasonal variation of leaf area index (LAI) of cotton, maize and soybean in 2007.

Fig. 7.

C2700

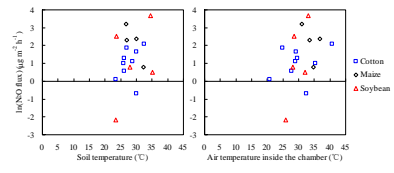


Fig. A2 The relationship between plant N₂O flux and temperature under dark conditions, based on diurnal observations in the crop fields (2006).

Fig. 8.

C2701