

Organically fertilized
tea plantation
stimulates N₂O
emissions

Z. Yao et al.

This discussion paper is/has been under review for the journal Biogeosciences (BG).
Please refer to the corresponding final paper in BG if available.

Organically fertilized tea plantation stimulates N₂O emissions and lowers NO fluxes in subtropical China

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Received: 16 June 2015 – Accepted: 6 July 2015 – Published: 23 July 2015

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Published by Copernicus Publications on behalf of the European Geosciences Union.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Abstract

Tea plantations are rapidly expanding in China and other countries in the tropical and subtropical zones, but so far there are very few studies including direct measurements on nitrogenous gases fluxes from tea plantations. On the basis of 2 year field measurements from 2012 to 2014, we provided an insight into the assessment of annual nitrous oxide (N_2O) and nitric oxide (NO) fluxes from Chinese subtropical tea plantations under three practices of conventional urea application, alternative oilcake incorporation and no nitrogen fertilization. Clearly, the N_2O and NO fluxes exhibited large intra- and inter-annual variations, and furthermore their temporal variability could be well described by a combination of soil environmental factors including soil mineral N, water-filled pore space and temperature, based on a revised “hole-in-the-pipe” model. Averaged over 2 years, annual background N_2O and NO emissions were approximately 4.0 and 1.6 $\text{kg N ha}^{-1} \text{yr}^{-1}$, respectively. Compared to no nitrogen fertilization, both urea and oilcake application significantly stimulated annual N_2O and NO emissions, amounting to 14.4–32.7 $\text{kg N}_2\text{O-N ha}^{-1} \text{yr}^{-1}$ and at least 12.3–19.4 $\text{kg NO-N ha}^{-1} \text{yr}^{-1}$. In comparison with conventional urea treatment, on average, the application of organic fertilizer significantly increased N_2O emission by 71 % but decreased NO emission by 22 %. Although the magnitude of N_2O and NO fluxes was substantially influenced by N source, the annual direct emission factors of fertilizer N were estimated to be 2.8–5.9, 2.7–4.0 and 6.8–9.1 % for N_2O , NO and $\text{N}_2\text{O} + \text{NO}$, respectively, which are significantly higher than those defaults for global upland croplands. This indicated that the rarely determined N_2O and NO formation appeared to be a significant pathway in the nitrogen cycle of tea plantations, which are a potential source of national nitrogenous gases inventory.

BGD

12, 11625–11659, 2015

Organically fertilized tea plantation stimulates N_2O emissions

Z. Yao et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



1 Introduction

Nitrous oxide (N_2O) and nitric oxide (NO) are two of the most important anthropogenic nitrogen compounds emitted to the atmosphere, which are directly or indirectly involved in global warming and atmospheric chemistry (Williams et al., 1992; IPCC, 2013). It is well accepted that human activities strongly influence the source of N_2O and NO , as nitrogen fertilizer applied in agriculture is now the vital source of inorganic/organic nitrogen substrate for nitrification and denitrification processes, leading to increased N_2O and NO emissions (McElroy and Wang, 2005; Galloway et al., 2008). Recently anthropogenic emissions from the application of nitrogenous fertilizers in agriculture were estimated to be $1.7\text{--}4.8\text{ Tg Nyr}^{-1}$ for N_2O and 3.7 Tg Nyr^{-1} for NO , accounting for approximately 60 and 10 % of the total global estimates, respectively (IPCC, 2013). However, one should admit that a dearth of direct measurements of nitrogenous gases fluxes in some agricultural areas makes these estimates highly uncertain, and it also results in the projection and mitigation of agricultural N_2O and NO emissions posing considerable challenges (Davidson and Kinglerlee, 1997; Reay et al., 2012), although the measurements of these emissions have been made for many decades. Taking as an example, Stehfest and Bouwman (2006) summarized information from 1008 N_2O and 189 NO emission measurements in agricultural fields worldwide, and indicated that the representation of number of measurements in tropical and subtropical climates was only 13–14 and 23–28 % for N_2O and NO , respectively. As suggested by Reay et al. (2012), therefore, a central aim of future study on e.g., N_2O emissions from agricultural systems should be to increase the coverage encompassing various agricultural land-use/cover types and climates as well as management practices.

Tea is one of the three most common beverages (i.e., coffee, tea and cocoa) worldwide, and tea crops are widely planted in the tropical and subtropical regions (Xue et al., 2013). China is the world's largest tea producing country, and its tea plantation area had reached 1.85 million ha in 2009, contributing approximately 52 % to the world total (Han et al., 2013a). Besides, tea is a leaf harvested crop, and nitrogen is the

BGD

12, 11625–11659, 2015

Organically fertilized tea plantation stimulates N_2O emissions

Z. Yao et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



**Organically fertilized
tea plantation
stimulates N₂O
emissions**

Z. Yao et al.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[⏪](#)[⏩](#)[◀](#)[▶](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

most important nutrient for increasing the content of free amino acids, an index of the quality of tea leaves (Tokuda and Hayatsu, 2004). For improving the yield and quality of tea leaves, therefore, large amounts of nitrogen fertilizer are increasingly applied by tea farmers. For instance, the application rates of nitrogen fertilizer to tea plantations have been as high as 450–1200 kg N ha⁻¹ yr⁻¹, which significantly surpasses the recommended rate of 250–375 kg N ha⁻¹ yr⁻¹ for high tea yields (Tokuda and Hayatsu, 2004; Hirono and Nonaka, 2012; Fu et al., 2012; Zhu et al., 2014). Not surprisingly, such high nitrogen inputs can easily induce excess residual nitrogen and acidification of soil, both influence the nitrogen cycle of tea fields in which a great deal of nitrogenous gases are produced (Jumadi et al., 2008; Zhu et al., 2014). It was reported that the N₂O emissions from tea fields were greatly higher than those from other upland fields (Jumadi et al., 2005; Han et al., 2013a). Akiyama et al. (2006) analyzed data on N₂O emissions from 36 sites with 246 measurements in Japanese agricultural fields and reported that the mean fertilizer-induced emission factor of N₂O in tea fields was much higher as compared to other upland fields and paddy fields. Nevertheless, there are still very little data available on N₂O emissions from Chinese tea plantations (Fu et al., 2012; Li et al., 2013; Han et al., 2013a). Meanwhile, tea plantations to which large amounts of nitrogen fertilizer have been added are also probably one of the important sources of NO. So far, however, no study is available for NO fluxes from tea fields worldwide, which hinders the development of the sound NO emission inventory (Huang and Li, 2014).

As tea production in China has intensified to meet market demands over the past decades, public concerns over the negative impacts of conventional synthetic nitrogen fertilizers application in tea plantations on human health and environmental quality have also increased (Pimentel et al., 2005; Han et al., 2013b). These concerns have led to increased grower interest in organically fertilized tea plantations, and by 2011 approximately 45 000 ha of tea fields were under organic fertilization in China (Han et al., 2013b). Furthermore, the conversion of conventional synthetic nitrogen fertilization to organic fertilizer practice in tea plantations has been identified as a feasible measure in

**Organically fertilized
tea plantation
stimulates N₂O
emissions**

Z. Yao et al.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

the aspects of promoting soil carbon sequestration and ameliorating soil pH (Han et al., 2013b; Wang et al., 2014). On the other hand, organic fertilization systems have been shown to substantially affect N₂O emissions compared with conventional management practices, but the influence can be either stimulatory (Akiyama and Tsuruta, 2003a, b; Syväsalo et al., 2006) or marginal and even inhibitory (Akiyama and Tsuruta, 2003b; Burger et al., 2005; Petersen et al., 2006; Kramer et al., 2006). Although these studies have demonstrated organic fertilizer practices may improve soil quality and influence nitrogenous gases fluxes in some agricultural systems, no study has specifically compared N₂O and NO emissions in response to organic and synthetic nitrogen fertilizer application in tea plantations to our knowledge.

In this paper, we present the results of a 2 year field study in which N₂O and NO fluxes were measured simultaneously in Chinese subtropical tea plantations under three practices of conventional urea application, alternative organic fertilizer incorporation and no nitrogen fertilization. The main objectives of the present study were to characterize and quantify annual N₂O and NO fluxes and their direct emission factors across different years, and to evaluate the effect of organic fertilizer management on N₂O and NO fluxes as well as to clarify the underlying mechanisms and factors regulating these fluxes from tea plantations.

2 Materials and methods

2.1 Site description and field treatments

Field measurements were carried out in a tea planting farm (32°07′22″N, 110°43′11″E, approx. 441 m above sea level) of the Agricultural Bureau of Fangxian, Hubei Province, China. The region is characterized by a northern subtropical monsoon climate with cool and dry winters as well as warm and humid summers. From 2003 to 2011, the mean annual precipitation and air temperature for this site were 914 mm and 14.2 °C, respectively. Before the campaign of tea cultivations, all fields in this area had been

Organically fertilized tea plantation stimulates N₂O emissions

Z. Yao et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

cultivated with rice-fallow or rice-oilseed rape rotation cropping system. The tea plants in the experimental field were transplanted in March 2008, thereafter it has been continuously cultivated with regular synthetic nitrogen fertilizers and irrigation additions according to common regional management practice. The topsoil (0–15 cm) of the experimental site is of a loamy texture with (mean \pm SE, $n = 12$) $12.7 \pm 0.1\%$ clay (< 0.002 mm), $39.3 \pm 0.5\%$ silt (0.002–0.02 mm), and $48.0 \pm 0.6\%$ sand (0.02–2 mm). Other important soil physiochemical properties include organic carbon content of 13.6 ± 0.2 g kg⁻¹, total nitrogen content of 1.5 ± 0.1 g kg⁻¹, pH of 5.0 ± 0.1 , and bulk density of 1.25 ± 0.03 g cm⁻³.

Our field study was performed over the course of two consecutive years from September 2012 to October 2014. As shown in Table 1, three experimental treatments were set up on the tea (T) field with approximately 4 year-old plantation: one with additions of urea (UN) that is the common practice for this region, another with applications of organic fertilizer (OM) that is likely to be used alternative practice in the future for this region, and the final treatment with no synthetic nitrogen fertilizers or organic fertilizers (NN) application. These fertilizer treatments were arranged in a randomized complete block design with four replicates, resulting in a total of 12 plots (each with an area of 8 m \times 8 m). For the TUN plots, urea was applied at the common rate of 450 kg N ha⁻¹ yr⁻¹ in two splits (one third of annual nitrogen inputs as basal fertilization in the autumn time, two thirds as topdressing in the spring time). With respect to TOM, organic fertilizer was applied at rates and times in accordance with TUN (Table 1). The form of fertilizer applied in TOM was oilcake, which is a typical organic fertilizer in tea cultivations of China and other countries like Japan. This organic fertilizer contained 7.1 % N and had a C : N ratio of 6.1. In addition, all treatments received equal amounts of phosphorous and potassium (i.e., 225 kg P₂O₅ ha⁻¹ yr⁻¹ and 225 kg K₂O ha⁻¹ yr⁻¹) in terms of fertilizer recommendations by local farmers. On each replicated plot, the width of the canopy of tea plants was approximately 0.5 m, and the distance of inter-row space between the canopies was about 0.4 m. All of the fertilizers were applied as band application in the inter-row space between canopies with widths of approximately

0.2 m, and then incorporated into soils with a depth of approximately 0.1 m, which is the conventional practice in tea cultivations. Due to the young plantation age, the present tea plants did not receive any trimming during the experimental period.

2.2 Measurements of N₂O and NO fluxes

5 The fluxes of N₂O and NO were in situ measured simultaneously using manually closed chamber-based techniques (Zheng et al., 2008; Yao et al., 2009). As mentioned above, all fertilizers were incorporated in the form of bands between the rows of tea plants, and the remaining area was covered by canopy under which no fertilizer was applied. To better evaluate gas fluxes from the tea field, a size of rectangular stainless-steel frame
10 of 0.70 m × 0.90 m (width × length) was set up in each replicated plot, which covered four tea plants and parts of spaces between rows. That is, the frame covered the whole canopy area (i.e., 0.5 m in length) and two-half of the fertilized inter-row spaces on both sides of tea canopy (i.e., 0.2 m in length each side), representing the whole tea field landscape. To eliminate the possibility of influence on N₂O and NO fluxes from the temporary installation of chamber bases (Matson et al., 1990), the frames were inserted
15 into the soil to a depth of 0.15 m one month before the start of flux measurements, and they were maintained in place throughout the entire observation period, except when it was removed for necessary farming practices (e.g., band fertilization). Besides, the sampling locations were connected with boardwalks to prevent soil disturbance during the sampling period. In general, flux measurements were conducted five times per week during the first week after each fertilization event, and three times per week during the rest of time. Almost all of the gas sampling was taken between 09:00 and 11:00 local standard time on each measuring day to minimize the influence of diurnal temperature variation. Based on the size of frames and the height of tea plants, insulated chambers with a bottom area of 0.70 m × 0.90 m and a height of 1.0 m were designed for
20 gas samplings. These chambers were wrapped with a layer of styrofoam and aluminum foil to minimize temperature changes during the sampling period. Also, two circulating fans driven by 12 V DC were installed inside the sampling chamber to facilitate mixing

Organically fertilized tea plantation stimulates N₂O emissions

Z. Yao et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



**Organically fertilized
tea plantation
stimulates N₂O
emissions**

Z. Yao et al.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[⏪](#)[⏩](#)[◀](#)[▶](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

of chamber air and thus inhibiting the formation of gas concentration gradients, and a hole of 2 cm diameter was fitted in the top panel for equilibrating the pressure during the placement of them on the base frames. This hole was embedded during the gas sampling using a pressure balance tube whose diameter and length were determined according to the recommendation of Hutchinson and Mosier (1981). To acquire the N₂O flux, five gas samples were withdrawn from the chamber headspace using 60 mL polypropylene syringes fitted with three-way stopcocks at fixed intervals of 0, 10, 20, 30 and 40 min after covering. Within 3 h after collection, the N₂O concentrations of gas samples stored in airtight syringes were directly analyzed in the laboratory established beside the experimental field, using a gas chromatograph (GC, Agilent 7890A, Agilent Technologies, CA, USA) equipped with an electron capture detector at 330 °C on the basis of DN-CO₂ method, as described in detail by Zheng et al. (2008). The N₂O was separated by two stainless steel columns (both with an inner diameter of 2 mm, one with a length of 1 m and the other with a length of 2 m) packed with Porapak Q, 80/100 mesh at 55 °C isothermally. To ensure quality assurance, the GC system was calibrated by inserting five standard N₂O samples with concentrations of 350 ppbv (the national center for standard matters, Beijing, China) between every 10 unknown samples. Results of GC analyses were accepted when five standard gas calibrations produced coefficient of variation lower than 1 %. The N₂O flux was determined by the linear or nonlinear change of gas concentrations during the time of chamber closure (Wang et al., 2013). In this study, the minimum detection limit of N₂O flux was approximately 2.6 µg N m⁻² h⁻¹.

For each NO flux measurement, gas samples were collected from the same chamber that was used for N₂O flux measurements (Yao et al., 2009). Before closing the chamber, approximately 2.5–3 L gas sample from the headspace of each chamber was extracted into an evacuated bag made of inert aluminum-coated plastic, and this measurement was regarded as time 0 min for NO analysis. After 40 min under chamber enclosure conditions (i.e., after finishing N₂O sample collections), another headspace gas sample with the same volume was extracted from each chamber into another evacuated



BGD

12, 11625–11659, 2015

Organically fertilized tea plantation stimulates N₂O emissions

Z. Yao et al.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[⏪](#)[⏩](#)[◀](#)[▶](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

uated bag. From these bag samples, NO concentrations were analyzed within 1 h by using a model 42i chemiluminescence NO-NO₂-NO_x analyzer (Thermo Environmental Instruments Inc., USA). The NO_x analyzer instrument was calibrated monthly in the laboratory using a TE-146i dilution-titration instrument (dynamic gas calibrator). A cylinder of standard gas of 50 ppmv NO in N₂ (the national center for standard matters, Beijing, China) and a zero gas generator (Model 111 Zero Air Supply) were used for multipoint calibrating, spanning, and zeroing of the NO_x analyzer. The NO flux was determined from the concentration at the end of the chamber enclosure period by subtracting the concentration at time 0 min. It should be noted that although some studies deriving N₂O and NO fluxes by employing either a simple linear regression method (e.g., Williams and Davidson, 1993; Kim and Kim, 2002; Zheng et al., 2003; Venterea et al., 2003; Li and Wang et al., 2007; Pang et al., 2009; Zhao et al., 2015) or a nonlinear regression model (e.g., Valente et al., 1995; Kroon et al., 2008; Yao et al., 2010a; Wang et al., 2013) have been widely adopted, it is clear that inappropriate application of a linear model to nonlinear data may seriously underestimate the trace gas flux (Hutchinson and Livingston, 1993; Kutzbach et al., 2007). For example, Kroon et al. (2008) suggested that on average, the N₂O emission estimates with the linear regression method were 46 % lower than the estimates with the exponential regression method. Similarly, Mei et al. (2009) conducted a field intercomparison of NO flux measurements with linear and nonlinear regression methods, and observed that the linear estimates of NO flux were 26 % lower on average relative to the nonlinear method. However, to date there has been limited field comparison of these two methods to assess comparability of N₂O or NO fluxes calculated by them. Based on our data sets of NO measured in wheat fields using the automatically static translucent chamber-based system (the raw data from the case studies of Zheng et al., 2003 and Yao et al., 2010a), the NO fluxes were re-estimated using linear and nonlinear regression methods. In order to better compare the two regression methods, a subset of data collected in the evening time was used that satisfied the present conditions of static opaque chamber technique. Finally, approximately 3489 pairs of observations were used for comparing the difference

between the two regression methods; and the results showed that the linear model underestimated the NO fluxes by 3 to 59 % (mean: 31 %) at the 95 % confidence interval, as compared to the nonlinear method. Overall, these findings indicate that data sets of N₂O collected in this study are relatively reliable, but the present method of linear accumulation assumption inevitably introduce an extent of underestimation into the NO fluxes for cases with nonlinear accumulations. Therefore, please keep in mind that the NO fluxes reported in this study represent the minimum magnitude for the present tea plantations.

2.3 Auxiliary measurements

The air temperature inside the chamber headspace during the flux measurements was recorded with a manual thermocouple thermometer (JM624, Tianjin, China). Air pressure and temperature as well as daily precipitation were obtained from an automatic meteorological station set up on the experimental farm. The air temperature measured in the chamber enclosures and air pressure obtained from the meteorological station were directly utilized in the flux computations to calculate the gas density during the sampling conditions. Soil (5 cm) temperature was automatically measured in 30 min intervals from the direct vicinity of the chamber frames using a HOBO temperature sensor (Onset, USA). Soil water content (0–6 cm) was recorded daily using a portable frequency domain reflectometry (FDR) probe (MPM-160, China). Three replicate soil samples (0–10 cm) in each plot were collected at 1–2 week intervals using a 3 cm diameter gauge auger. Following the collection, the fresh samples were bulked into one composite sample for each treatment, and then immediately extracted with 1 M KCl and 0.05 M K₂SO₄ to determine the concentrations of soil mineral N (NH₄⁺ and NO₃⁻) and dissolved organic carbon (DOC), respectively, both with a soil: solution ratio of 1 : 5. The NH₄⁺, NO₃⁻ and DOC concentrations were measured simultaneously with a continuous flow colorimetric analysis instrument (San++, Skalar Analytical B.V., Netherlands).

BGD

12, 11625–11659, 2015

Organically fertilized tea plantation stimulates N₂O emissions

Z. Yao et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

⏪

⏩

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



2.4 Statistical analysis

Statistical analysis was conducted using the SPSS19.0 (SPSS China, Beijing, China). Before variance component analysis, all data were tested for normal distribution using the Nonparametric Tests approach, and the original data that failed the test were log transformed ($P = 0.01-0.42$). To determine differences in nitrogenous gases fluxes and soil environmental variables among treatments during the given pronounced flux-related event (e.g., fertilization events, growing period), Linear Mixed Models for randomized complete block design were used with least significant difference tests at $P < 0.05$ level. Differences in N_2O and NO emissions due to main effects like fertilizer treatment, year, treatment \times year and block \times treatment as random effect were analyzed using Linear Mixed Models, and the model was fitted using the restricted maximum likelihood procedure. Multiple linear or non-linear regression analysis was applied to examine the correlations between N_2O and NO fluxes and soil environmental factors.

3 Results

3.1 Environmental variables

Annual precipitation was 804 mm from mid September 2012 to the end of September 2013, 890 mm from the beginning of October 2013 to mid October 2014 (Fig. 1a); both values were smaller than the multiyear average precipitation (914 mm). Apart from the precipitation, sprinkling irrigation was applied four times per year depending on climatic conditions, amounting to 150 and 135 mm for the two years, respectively. Soil temperature showed comparable fluctuations with the air temperature, ranging from -0.1 to 28.3°C . The mean annual soil temperature was 14.9 and 14.6°C for the 2012/2013 and 2013/2014, respectively (Fig. 1a), with no treatment impacts. Soil water content expressed as WFPS (water-filled pore space) ranged from 20 to 80 % during

BGD

12, 11625–11659, 2015

Organically fertilized tea plantation stimulates N_2O emissions

Z. Yao et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



the study period, which was mainly influenced by rainfall and irrigation events. The mean WFPS across 2012 to 2014 were 49.1, 49.7 and 48.6 % for TNN, TUN and TOM, respectively, with no significant difference among them (Fig. 1b).

Soil NH_4^+ concentrations in TUN and TOM remarkably increased following the fertilizer applications in March and October, and varied from 4.1 to 654 mg N kg^{-1} SDW (soil dry weight) (Fig. 2a). The temporal patterns of NO_3^- concentrations were also affected by nitrogen applications, ranging from 2.4 to 188 mg N kg^{-1} SDW, but the elevated peaks were observed slightly later than the peaks for NH_4^+ (Fig. 2b), reflecting the occurrence of nitrification. In contrast, both NH_4^+ and NO_3^- concentrations in TNN were relatively stable and always below 50 mg N kg^{-1} SDW. Over the 2012–2014, soil NH_4^+ averaged 10, 158 and 109 mg N kg^{-1} SDW for TNN, TUN and TOM, respectively. The mean NO_3^- from 2012 to 2014 in TNN, TUN and TOM were 5.7, 44 and 49 mg N kg^{-1} SDW, respectively. Clearly, TUN and TOM significantly enhanced soil mineral N concentrations, compared to TNN ($P < 0.05$). Among the fertilized treatments, TUN showed higher NH_4^+ concentrations and slightly lower NO_3^- concentrations, compared to TOM (Fig. 2a and b).

Over the whole study period, soil DOC concentrations ranged from 17 to 317 mg C kg^{-1} SDW in TNN, from 10 to 488 mg C kg^{-1} SDW in TUN and from 20 to 559 mg C kg^{-1} SDW in TOM (Fig. 2c). Mean DOC concentrations for TNN, TUN and TOM were 142, 146 and 179 mg C kg^{-1} SDW, respectively. Obviously, TOM increased soil DOC concentration compared to TNN and TUN ($P < 0.05$), but there was no significant difference between TUN and TNN.

3.2 Annual N_2O and NO fluxes and their direct emission factors

Seasonal pattern of N_2O fluxes was generally driven by temporal variation in air and soil temperatures, which was relatively higher during the tea growing season from March to September than in winter. The cumulative N_2O release from all treatments across the tea growing season accounted for 54–86 % of the annual emission. Meanwhile,

BGD

12, 11625–11659, 2015

Organically fertilized tea plantation stimulates N_2O emissions

Z. Yao et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Organically fertilized tea plantation stimulates N₂O emissions

Z. Yao et al.

[Title Page](#)

[Abstract](#)

[Introduction](#)

[Conclusions](#)

[References](#)

[Tables](#)

[Figures](#)



[Back](#)

[Close](#)

[Full Screen / Esc](#)

[Printer-friendly Version](#)

[Interactive Discussion](#)



the seasonal variability of N₂O fluxes were also influenced by fertilization and rainfall/irrigation events (Fig. 3a). The N₂O fluxes in TUN and TOM increased after each of the fertilizer applications, and then gradually decreased to the levels comparable to those from TNN. Obviously, the N₂O emissions varied significantly with fertilizer treatment and year. Across the investigated two years, annual N₂O emissions ranged from 1.9 kg N ha⁻¹ yr⁻¹ for TNN to 32.7 kg N ha⁻¹ yr⁻¹ for TOM (Table 2). Compared to TNN, the 2 year mean N₂O emissions were remarkably increased by 345 and 660 % for TUN and TOM, respectively ($P < 0.05$). In comparison with TUN, TOM significantly increased annual N₂O emission by 71 % on average ($P < 0.05$). On the annual scale, the direct emission factors of N₂O were an average of 3.1 and 5.9 % for tea plantations under urea and organic fertilizer treatment, respectively.

Clearly, the NO fluxes demonstrated a seasonal variability that was similar to the N₂O fluxes. That is, they were higher from March to September and lower from December to March, and also affected by fertilization and rainfall/irrigation events (Fig. 3b). Similar to N₂O, the NO emissions were greatly influenced by fertilizer treatment and year. The annual NO emissions from all treatments ranged from 0.4 to 19.4 kg N ha⁻¹ yr⁻¹ (Table 2), of which 53–77 % was released during the tea growing season. Compared to TNN, the fertilizer applications (TUN and TOM) significantly increased annual NO emission by 8–11 times on average ($P < 0.05$). In contrast to N₂O, TOM significantly decreased annual NO emission by 22 % relative to TUN ($P < 0.05$). Averaging across the two years, the direct emission factors of NO were 3.8 and 2.9 % for TUN and TOM, respectively. In addition, the N₂O+NO emissions were, on average, 5.6, 36.7 and 45.1 kg N ha⁻¹ yr⁻¹ for TNN, TUN and TOM, respectively, indicating that alternative organic fertilization significantly enhanced nitrogen oxide emissions (Table 2).

3.3 Relationships of N₂O and NO fluxes with soil environmental factors

Across the 2 year study period, stepwise multiple regression analysis showed that WFPS was the key factor controlling N₂O and NO fluxes for both TUN and TOM.

**Organically fertilized
tea plantation
stimulates N₂O
emissions**

Z. Yao et al.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[◀](#)[▶](#)[◀](#)[▶](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

Furthermore, a nonlinear response curve best described the decreases in molar ratios of NO to N₂O fluxes with increasing WFPS (Fig. 4). However, variations in WFPS could explain only 22–30% of the variance in the ratios, suggesting the importance of some other factors (e.g., soil mineral N and temperature) on regulating these fluxes. To better evaluate the combined effects of soil environmental factors on N₂O and NO fluxes, therefore, the revised “hole-in-the-pipe” model as described by Yao et al. (2015) and Yan et al. (2015) was tested in this study. Over the entire study period, the analysis results displayed that the temporal variations of N₂O and NO fluxes in TUN and TOM could be well described by a combination of soil environmental factors, including soil mineral N, WFPS and temperature. That is, for TUN: $\ln(\text{N}_2\text{O} + \text{NO}) = 0.30 \ln(\text{NH}_4^+ + \text{NO}_3^-) + 2.53 \ln(\text{WFPS}) - \frac{13.9}{RT_k}$, $R^2 = 0.97$, $P < 0.01$; and $\ln(\text{N}_2\text{O} + \text{NO}) = 0.17 \ln(\text{NH}_4^+ + \text{NO}_3^-) + 2.68 \ln(\text{WFPS}) - \frac{13.6}{RT_k}$, $R^2 = 0.96$, $P < 0.01$ for TOM; in which R and T_k are the molar gas constant ($8.31 \text{ J mol}^{-1} \text{ K}^{-1}$) and soil temperature in Kelvin, respectively.

4 Discussion

4.1 Intra- and inter-annual variations of N₂O and NO fluxes and related environmental factors

Currently, the existing studies on tea fields only focused on N₂O fluxes (Jumadi et al., 2005; Akiyama et al., 2006; Gogoi and Baruah, 2011; Fu et al., 2012; Han et al., 2013a; Yamamoto et al., 2014), and therefore they are not directly comparable to our present study. Our results demonstrated annual characteristics of N₂O and NO fluxes simultaneously, which is important for better understanding of how the climatic and environmental factors affecting soil nitrogen turnover processes in tea plantations. Generally, the subtropical climate is characterized by the hot-humid season from April through September and the cool-dry season from October through March every year, leading

**Organically fertilized
tea plantation
stimulates N₂O
emissions**

Z. Yao et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

to significant seasonal variations in soil environmental factors (Lin et al., 2010). Driven by the seasonality of soil temperature, WFPS, NH_4^+ and NO_3^- contents, the N_2O and NO fluxes showed large temporal variations (Skiba et al., 1998; Williams et al., 1999; Yan et al., 2015), characterizing by significantly higher during the tea growing season than in winter in this study (Fig. 3). The present result is in agreement with previous studies conducted in other agricultural systems under the subtropical climate, such as in vegetable fields (Min et al., 2012; Yao et al., 2015), paddy rice-upland crop rotation ecosystems (Yao et al., 2013) and orchard plantations (Lin et al., 2010), highlighting the climatic controls on N_2O and NO fluxes. Furthermore, up to 97 % of the variance in N_2O and NO fluxes could be explained by the combined effects of soil temperature, WFPS and mineral N content, indicating an essential role of the environmental factors on N_2O and NO fluxes. Overall, the knowledge of temporal variations in N_2O and NO fluxes and their related driving forces plays an important role for up-scaling nitrogenous gas fluxes to the regional and global scale.

On the other hand, our study clearly demonstrated that annual N_2O and NO emissions were significantly affected by the factor of year (Fig. 3), even though the field management and soil temperature were comparable across the two study years. A presumable reason for the pronounced inter-annual variations of N_2O and NO fluxes was the difference in precipitation, particularly rainfall distribution throughout a year. For example, the cumulative rainfall of 94 mm over a period from 20 to 26 June 2013 was received that brought soil water content changing from 25 to 64 % WFPS on average (Fig. 1). As was also observed by our auxiliary measurements that soil NH_4^+ and NO_3^- increased after rainfall events during this period (Fig. 2a and b), the drying-rewetting event could enhance the availability of nitrogen substrate and stimulate microbial activity (Davidson, 1992; Williams et al., 1992; Yao et al., 2010b), and thus, resulting in the following elevated fluxes of N_2O and NO (Fig. 3a and b). Similarly, a number of studies also reported that the large inter-annual variability in N_2O and NO fluxes were mainly influenced by the difference in annual distribution of the precipitation (e.g., Akiyama and Tsuruta, 2003b; Yao et al., 2013).

4.2 Organic fertilization influencing annual N₂O and NO emissions

As tea plantations displayed high N₂O production activities, they might be a major source of nitrogenous gases in agricultural systems (Tokuda and Hayatsu, 2001, 2004; Zhu et al., 2014). Our observations confirmed earlier findings, with annual N₂O emissions ranging from 14.4 to 32.7 kg N ha⁻¹ yr⁻¹ and NO emissions from 12.3 to 19.4 kg N ha⁻¹ yr⁻¹ for the fertilized tea plantations (Table 2). Generally, our annual N₂O emissions were within the range of the reported magnitudes of 4.3–30.9 kg N ha⁻¹ yr⁻¹ for Chinese subtropical tea fields (Fu et al., 2012; Han et al., 2013a). Based on the thorough review of Akiyama et al. (2006), annual N₂O emissions were presented from 0.6 to 61.0 kg N ha⁻¹ yr⁻¹ for Japanese tea plantations, with a mean value of 24.3 kg N ha⁻¹ yr⁻¹. Obviously, the mean annual N₂O emission in our study (mean: 24.1 ± 4.0 kg N ha⁻¹ yr⁻¹) was well consistent with the Japanese estimated value. In contrast, the magnitude of N₂O emissions from the present tea plantations was much higher than that from the paddy rice-fallow cropping systems in the same region (0.8–6.6 kg N ha⁻¹ yr⁻¹, Yao et al., 2014). With respect to NO, this is the first time reporting annual NO emission for tea plantations to our knowledge. On average, tea plantations released at least 16.8 kg N ha⁻¹ yr⁻¹ NO into the atmosphere, which fell within the range of 1.1–47.1 kg N ha⁻¹ yr⁻¹ for Chinese conventional vegetable fields under the subtropical climate (e.g., Li and Wang, 2007; Mei et al., 2009; Deng et al., 2012; Yao et al., 2015). As these authors acknowledged, their high NO emissions for vegetable fields were mainly attributed to quite high nitrogen inputs, ranging from 317 to 1464 kg N ha⁻¹ yr⁻¹. Nevertheless, our observed annual NO emissions were relatively higher as compared to those estimates of 0.5–6.5 kg N ha⁻¹ yr⁻¹ for rice-wheat cropping systems with nitrogen application rates of 150–375 kg N ha⁻¹ yr⁻¹ (Zheng et al., 2003; Yao et al., 2013; Zhao et al., 2015) and of 4.0–6.9 kg N ha⁻¹ yr⁻¹ for forest ecosystems (Li et al., 2007) in Chinese subtropical regions.

Although the fertilized tea plantations emitted large amounts of N₂O and NO, the magnitude of these emissions was significantly influenced by the applied fertilizer type.

Organically fertilized tea plantation stimulates N₂O emissions

Z. Yao et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



That is, organically fertilized tea plantation increased N₂O emission by 71 % but decreased NO emission by 22 %, compared to conventional urea application (Table 2). Our stimulatory effect of organic fertilization on N₂O emission and simultaneously inhibitory impact on NO emission supports the findings of some previous studies (Thorn-ton et al., 1998; Akiyama and Tsuruta, 2003a, b; Hayakawa et al., 2009). However, other studies showed that organic fertilization may reduce N₂O emissions or that emis-sions of N₂O and NO were not affected at all (Harrison et al., 1995; Akiyama and Tsuruta, 2003b; Vallejo et al., 2006; Yao et al., 2009). It was generally accepted that the NO to N₂O emission ratio was used as a potential indicator for distinguishing be-tween nitrification and denitrification process (Anderson and Levine, 1986; Skiba et al., 1992; Harrison et al., 1995; Williams et al., 1998). As calculated from the results of Ta-ble 2, the molar ratios of NO to N₂O emissions were in the range of 1.8–2.5 for the TUN plots but < 1.0 in the TOM plots. This may indicate that nitrification was probably the dominant process for N₂O and NO production in the conventional urea treatment, while denitrification would be more dominant process in organic fertilization, although both nitrification and denitrification could occur under the present soil moisture conditions (i.e., 20–80 % WFPS) according to a conceptual model proposed by Davidson (1991). Denitrifiers have a very high affinity for NO and tend to utilize it in preference to N₂O as a substrate even in well-aerated soils (Conrad, 2002; Yamulki and Jarvis, 2002). It is therefore to be expected the differences in N₂O and NO emission response between urea and organic fertilizer treatment. This view was further supported by our observa-tions on soil NH₄⁺ and DOC. It is well recognized that NH₄⁺ enhanced NO fluxes since it affected nitrification, whereas the addition of DOC generally diminished these fluxes by enhancing soil respiration and thereby inducing the anaerobic conditions that favored the production of N₂O and the consumption of NO through denitrification (Granli and Bockman, 1994; Vallejo et al., 2006; Meijide et al., 2007). In this study, therefore, TOM with lower NH₄⁺ and higher DOC, emitted more N₂O and less NO than those of TUN. Alternatively, opposite trends observed for N₂O and NO emissions between TUN and TOM was probably regulated by soil heterotrophic nitrification, the direct oxidation of

organic N to NO_3^- without passing through mineralization (Müller et al., 2004; Islam et al., 2007). It has been identified that heterotrophic nitrification, especially for acidic soils with organic amendments, plays an important role in soil nitrogen transformations, including the production and consumption processes of NH_4^+ and NO_3^- as well as N_2O and NO (Dunfield and Knowles, 1998; Zhu et al., 2011, 2014; Medinets et al., 2015). Hence, one can assume that given WFPS being comparable in all treatments, heterotrophic nitrification was the most important process for consumption of NO and production of N_2O in the organic fertilizer treatment, whereas autotrophic nitrification dominated in urea application. Besides, it has been validated that soils receiving organic amendments significantly reduce NO fluxes as a result of increased NO consumption via aerobic co-oxidation reactions in heterotrophic bacteria (Baumgärtner et al., 1996; Dunfield and Knowles, 1998; Conrad, 2002). This assumption could be also supported by our measurements on soil NH_4^+ and NO_3^- . That is, TOM showed comparable even slightly higher NO_3^- relative to TUN, although TUN demonstrated relatively high NH_4^+ due to the rapid release of urea hydrolysis (Fig. 2a and b). This indicated that heterotrophic nitrification contributed substantially to the production of NO_3^- in TOM, because the application of organic matter can enhance the direct oxidation of organic N to NO_3^- via soil heterotrophic nitrification (Zhu et al., 2011, 2014). Overall, although our data supported the above mentioned views, the exact reaction mechanisms were not determined directly in the present study. Therefore, further detailed investigations are needed to provide a complete assessment on the relative contribution of autotrophic nitrification, heterotrophic nitrification and denitrification to N_2O and NO fluxes from tea plantations, based on new approaches and techniques, e.g., ^{15}N tracing techniques (Müller et al., 2007).

BGD

12, 11625–11659, 2015

**Organically fertilized
tea plantation
stimulates N_2O
emissions**

Z. Yao et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

⏪

⏩

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



4.3 Background N₂O and NO emissions and direct emission factors of fertilizer N

Although background N₂O and NO emissions occurring in the zero-N control have been recognized as a major component for developing national emission inventory of nitrogenous gases (Zheng et al., 2004; Huang and Li, 2014), direct measurements on background emissions, especially measurements covering an entire year for tea plantations have been rare (Akiyama et al., 2006). In our study, the mean annual background emissions were 4.0 kg N ha⁻¹ yr⁻¹ for N₂O and at least 1.6 kg N ha⁻¹ yr⁻¹ for NO, respectively (Table 2). Our background N₂O emission is comparable to the preliminary estimate of 3.66–4.24 kg N ha⁻¹ yr⁻¹ for Japanese tea fields (Akiyama et al., 2006), but it is relatively lower than the reported value of 7.1 kg N ha⁻¹ yr⁻¹ for another tea field in the Chinese subtropical region (Fu et al., 2012). Nevertheless, these background N₂O emissions of present and previous studies in tea plantations are generally higher than those estimates for cereal grain croplands (ranging from 0.1 to 3.67 kg N ha⁻¹ yr⁻¹, with a mean of 1.35 kg N ha⁻¹ yr⁻¹, Gu et al., 2007) and vegetable fields (1.1–2.7 kg N ha⁻¹ yr⁻¹, Wang et al., 2011; Liu et al., 2013) in China, or the recommended default value of 1 kg N ha⁻¹ yr⁻¹ by IPCC (IPCC, 2006). Similarly, our mean background NO emission from tea plantations is greater relative to cereal grain croplands (0.2–0.9 kg N ha⁻¹ yr⁻¹, Yao et al., 2013; Yan et al., 2015) and vegetable fields (0.2–0.8 kg N ha⁻¹ yr⁻¹, Yao et al., 2015) in China. These comparisons highlight the characteristic of high background N₂O and NO emissions from tea plantations, which is probably due to long-term heavy nitrogen fertilization and subsequent soil acidification (Tokuda and Hayatsu, 2004; Yamamoto et al., 2014). Soil acidity appears to be an important factor in affecting biotic and abiotic processes and consequently promoting nitrogen losses, such as enhancing N₂O production ratios from nitrification and depressing the conversion of N₂O to N₂ in denitrification (Zhu et al., 2011) as well as inducing chemodenitrification for NO production (Venterea et al., 2003; Medinets et al., 2015).

Organically fertilized tea plantation stimulates N₂O emissions

Z. Yao et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



**Organically fertilized
tea plantation
stimulates N₂O
emissions**

Z. Yao et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

In this study, the mean annual emission factor of NO for TUN was 3.8 %, which was substantially higher than those estimated for Chinese rice fields (0.04 %) and uplands (0.67 %) (Huang and Li, 2014), or the average value of 0.7 % for global upland croplands (Bouwman et al., 2002; Yan et al., 2005). The NO emissions from TUN were greatly reduced by practicing TOM, giving emission factor of 2.9 % (Table 2). Although the NO emission factors were lower for TOM relative to TUN, TOM could not be proposed as a preferred management option for tea plantations because it emitted much higher N₂O or N₂O+NO. The N₂O emission factors obtained on this study site (i.e., 3.1 % for TUN and 5.9 % for TOM) were considerably higher than those estimated for Japanese tea fields (2.8 %, Akiyama et al., 2006) and another Chinese subtropical tea field (1.9–2.2 %, Fu et al., 2012), or the IPCC default value of 1 % for global upland croplands (IPCC, 2006). These results corroborated the assertion that tea plantations were an important source of atmospheric N₂O in tropical and subtropical regions, and furthermore they extended the earlier findings by demonstrating the characteristic of high NO and N₂O+NO emissions from tea plantations.

It is noteworthy that although our investigated tea plantations represent the major and typical tea-planting types in Chinese subtropical regions, the obtained background and direct emission factors of N₂O and NO could not be simply extrapolated to a regional scale due to the limited site results (e.g., only four chamber-spatial measurements for each treatment) and the characteristics of high spatial variability of nitrogenous gases fluxes (e.g., Li et al., 2013). A more holistic approach for regional estimates of N₂O and NO emissions from tea plantations should be based on meta-analysis of published nitrogenous gases fluxes to obtain representative background and direct emission factors or on the basis of biogeochemical modeling validated by regional field data, as suggested methodologies by IPCC (IPCC, 2006).

5 Conclusions

On the basis of 2 year field measurements, this study provided an integrated evaluation on N₂O and NO emissions in response to no nitrogen fertilization, conventional urea and alternative oilcake application in Chinese subtropical tea plantations. Clearly, both N₂O and NO emissions varied substantially within a year and between different years. Soil water-filled pore space, temperature and mineral nitrogen content appeared to be the major factors regulating the seasonality of N₂O and NO fluxes, and their correlation could be well presented by a revised “hole-in-the-pipe” model. Compared to no nitrogen fertilization, the application of urea and organic fertilizer to tea plantations stimulated annual N₂O and NO emissions. On average, the organic fertilizer induced emission factor of N₂O (i.e., 5.9%) was significantly higher than the urea-induced emission factor of 3.1%; however, the urea-induced emission factor of NO (i.e., 3.8%) was significantly higher than the organic fertilizer induced emission factor of 2.9%. Although the magnitude of N₂O and NO emissions was significantly influenced by the applied fertilizer type, annual emission factors of N₂O and NO induced by either urea or organic fertilizer application were all substantially higher than those defaults for global upland croplands, indicating tea plantations may contribute substantially to total N₂O and NO emissions from croplands in China.

Acknowledgements. This study was financially supported by the Ministry of Science and Technology of China (2012CB417106), the National Nature Science Foundation of China (41305129, 41321064) and the Youth Innovation Promotion Association CAS (2013055). We are also grateful to Y. Du, P. Li, and S. Li for their assistance in field and laboratory measurements.

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BGD

12, 11625–11659, 2015

Organically fertilized tea plantation stimulates N₂O emissions

Z. Yao et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Organically fertilized tea plantation stimulates N₂O emissions

Z. Yao et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

⏪

⏩

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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**Organically fertilized
tea plantation
stimulates N₂O
emissions**

Z. Yao et al.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[◀](#)[▶](#)[◀](#)[▶](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

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Organically fertilized tea plantation stimulates N₂O emissions

Z. Yao et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

⏪

⏩

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Hutchinson, G. L. and Livingston, G. P.: Use of chamber systems to measure trace gas fluxes, in: *Agricultural Ecosystem Effects on Trace Gases and Global Climate*, edited by: Harper, L. A., Am. Soc. Agron., Madison, Wis., 63–78, 1993.

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Organically fertilized tea plantation stimulates N₂O emissions

Z. Yao et al.

[Title Page](#)

[Abstract](#)

[Introduction](#)

[Conclusions](#)

[References](#)

[Tables](#)

[Figures](#)

[⏪](#)

[⏩](#)

[◀](#)

[▶](#)

[Back](#)

[Close](#)

[Full Screen / Esc](#)

[Printer-friendly Version](#)

[Interactive Discussion](#)



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Organically fertilized tea plantation stimulates N₂O emissions

Z. Yao et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

⏪

⏩

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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Organically fertilized tea plantation stimulates N₂O emissions

Z. Yao et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

⏪

⏩

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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Organically fertilized tea plantation stimulates N₂O emissions

Z. Yao et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

⏪

⏩

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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**Organically fertilized
tea plantation
stimulates N₂O
emissions**

Z. Yao et al.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[◀](#)[▶](#)[◀](#)[▶](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

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Organically fertilized tea plantation stimulates N₂O emissions

Z. Yao et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

⏪

⏩

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Table 1. Field management of synthetic and organic nitrogen fertilizers for tea plantations under different treatments during the period of 2012–2014.

	Nitrogen application rate (kg N ha ⁻¹)			Application date
	TNN	TUN	TOM*	
Basal fertilization	0	Urea (150)	Oilcake (150)	08 October (2012) 06 October (2013)
Topdressing	0	Urea (300)	Oilcake (300)	18 February (2013) 01 March (2014)
Total	0	450	450	

* The fertilizer of oilcake contained 7.1 % N and had a C : N ratio of 6.1

Organically fertilized tea plantation stimulates N₂O emissions

Z. Yao et al.

Table 2. Annual cumulative emissions of nitrous oxide (N₂O, in kg N ha⁻¹ yr⁻¹), nitric oxide (NO, in kg N ha⁻¹ yr⁻¹) and N₂O plus NO (in kg N ha⁻¹ yr⁻¹) as well as their respective direct emission factors (EF_d, in %) for tea plantations under different fertilizer treatments during the period of 2012–2014.

Year	Treatment ^b	N ₂ O ^c	EF _{d-N₂O}	NO ^c	EF _{d-NO}	N ₂ O+NO ^c	EF _{d-N₂O+NO}
2012–2013	TNN	6.2 ± 0.3a		2.8 ± 0.5a		9.0 ± 0.4a	
	TUN	21.1 ± 2.5b	3.3 ± 0.5	19.4 ± 0.3b	3.7 ± 0.1	40.6 ± 2.6b	7.0 ± 0.6
	TOM	32.7 ± 0.7c	5.9 ± 0.2	17.0 ± 0.4c	3.2 ± 0.1	49.8 ± 1.0c	9.1 ± 0.2
2013–2014	TNN	1.9 ± 0.1a		0.4 ± 0.1a		2.3 ± 0.2a	
	TUN	14.4 ± 2.6b	2.8 ± 0.6	18.3 ± 0.5b	4.0 ± 0.1	32.8 ± 2.2b	6.8 ± 0.5
	TOM	28.1 ± 1.3c	5.8 ± 0.3	12.3 ± 1.1c	2.7 ± 0.3	40.5 ± 2.3c	8.5 ± 0.5
2012–2014 ^a	TNN	4.0 ± 0.1a		1.6 ± 0.2a		5.6 ± 0.2a	
	TUN	17.8 ± 2.5b	3.1 ± 0.6	18.9 ± 0.4b	3.8 ± 0.1	36.7 ± 2.4b	6.9 ± 0.5
	TOM	30.4 ± 0.9c	5.9 ± 0.2	14.7 ± 0.6c	2.9 ± 0.1	45.1 ± 1.4c	8.8 ± 0.3

Data shown are means ± standard errors of 4-spatial replicates. ^a Mean values of the two investigated years. ^b TNN, no nitrogen fertilizer application; TUN, the common practice with urea application rate of 450 kg N ha⁻¹ yr⁻¹; and TOM, the alternative practice with organic fertilizer application rate of 450 kg N ha⁻¹ yr⁻¹. ^c Different letters within the same column indicate significant differences among treatments in each year at *P* < 0.05 level.

Organically fertilized tea plantation stimulates N₂O emissions

Z. Yao et al.

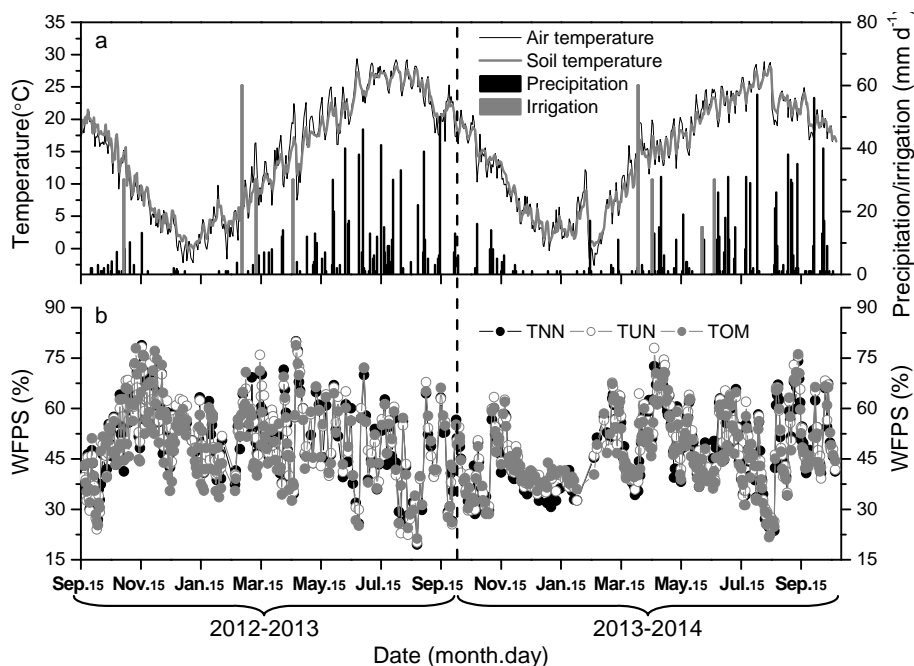


Figure 1. The temporal changes of (a) air and soil (5 cm) temperatures, daily precipitation and irrigation, and (b) soil water content expressed as WFPS (water-filled pore space) at a depth of 0–6 cm for all the fertilizer treatments (i.e., the common practice with urea application (TUN), the alternative practice with organic fertilizer application (TOM), and no nitrogen fertilizer application (TNN)) in tea plantations during the period from September 2012 to October 2014.

[Title Page](#)
[Abstract](#)
[Introduction](#)
[Conclusions](#)
[References](#)
[Tables](#)
[Figures](#)
[⏪](#)
[⏩](#)
[◀](#)
[▶](#)
[Back](#)
[Close](#)
[Full Screen / Esc](#)
[Printer-friendly Version](#)
[Interactive Discussion](#)

Organically fertilized tea plantation stimulates N_2O emissions

Z. Yao et al.

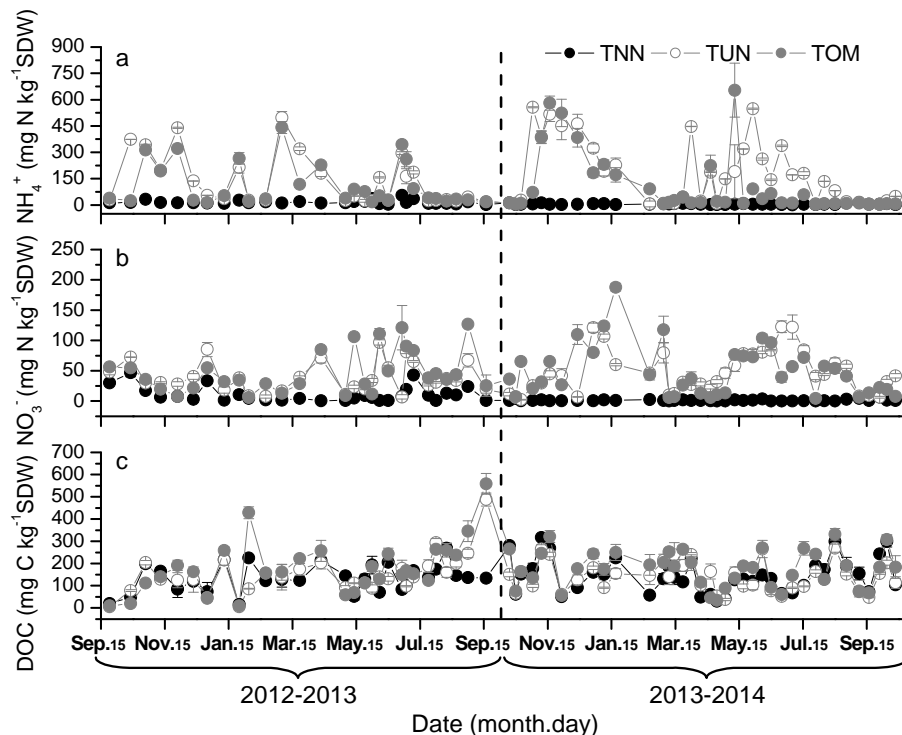


Figure 2. Seasonal changes of the soil **(a)** ammonium (NH_4^+), **(b)** nitrate (NO_3^-), and **(c)** dissolved organic carbon (DOC) concentrations (mean \pm standard error) for all the fertilizer treatments (i.e., the common practice with urea application (TUN), the alternative practice with organic fertilizer application (TOM), and no nitrogen fertilizer application (TNN)) in tea plantations during the period from September 2012 to October 2014. SDW is the abbreviation of soil dry weight.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Organically fertilized tea plantation stimulates N₂O emissions

Z. Yao et al.

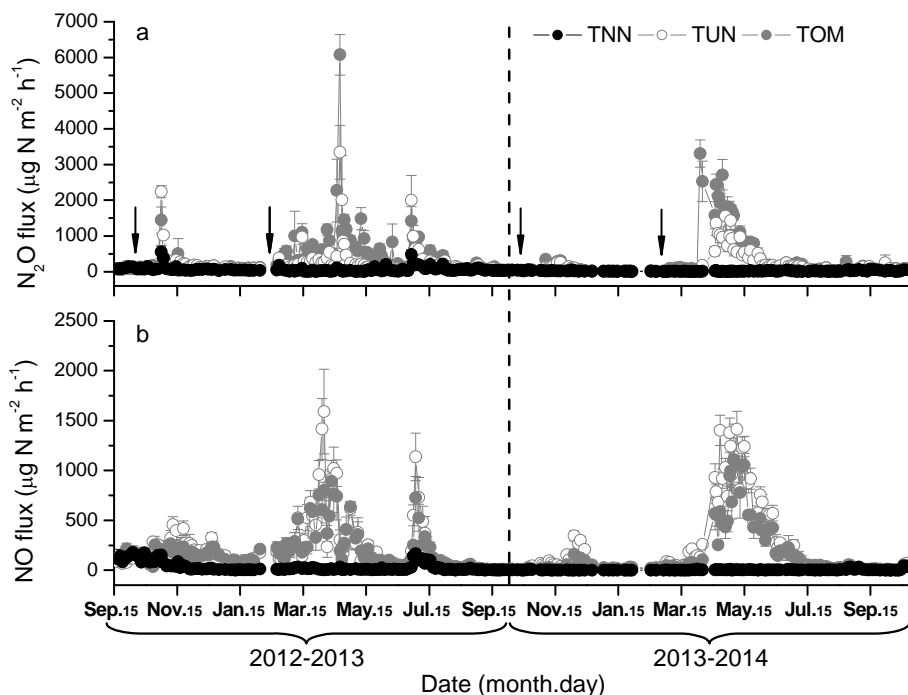


Figure 3. Seasonal changes of (a) nitrous oxide (N₂O), and (b) nitric oxide (NO) fluxes (mean ± standard error) for all the fertilizer treatments (i.e., the common practice with urea application (TUN), the alternative practice with organic fertilizer application (TOM), and no nitrogen fertilizer application (TNN)) in tea plantations during the period from September 2012 to October 2014. The downward arrows denote the time of fertilization.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Organically fertilized tea plantation stimulates N₂O emissions

Z. Yao et al.

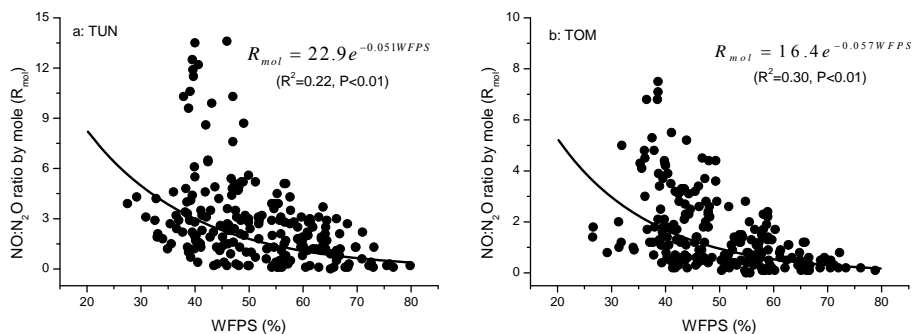


Figure 4. Effect of soil water content (expressed as WFPS, water-filled pore space) on the molar ratios of nitric oxide (NO) to nitrous oxide (N₂O) fluxes in the fertilized treatments (i.e., the common practice with urea application (TUN), and the alternative practice with organic fertilizer application (TOM)) across the 2 year study period.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

