Nitrous oxide emissions from cultivated black soil: A case study in Northeast China and global estimates using empirical model

Zengming Chen[1,2], Weixin Ding[1], Yiqi Luo[3], Hongyan Yu[1], Yehong Xu[1,2], Christoph Müller[4,5], Xia Xu[3], and Tongbin Zhu[6]

[1]State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing, China, [2]University of Chinese Academy of Sciences, Beijing, China, [3]Department of Botany and Microbiology, University of Oklahoma, Norman, Oklahoma, USA, [4]School of Biology and Environmental Science, University College Dublin, Dublin, Ireland, [5]Department of Plant Ecology, Justus-Liebig University Giessen, Giessen, Germany, [6]School of Geography Science, Nanjing Normal University, Nanjing, China

Abstract Manure application is effective in promoting soil carbon sequestration, but its impact on N_2O emission is not well understood. A field experiment was conducted in a maize-cultivated black soil in Northeast China with six treatments: inorganic fertilizer (NPK), 75% inorganic fertilizer N plus 25% pig (PM1) or chicken (CM1) manure N, 50% inorganic fertilizer N plus 50% pig (PM2) or chicken (CM2) manure N, and no N fertilizer (CK). Annual N_2O emission significantly increased from 0.34 kg N ha^{-1} for CK to 0.86 kg N ha^{-1} for NPK and further to 1.65, 1.02, 1.17, and 0.93 kg N ha^{-1} for PM1, CM1, PM2, and CM2, respectively. A ^{15}N tracing study showed that 71–79% of total N_2O was related to nitrification at 30–70% water-filled pore space (WFPS), and heterotrophic nitrification contributed 49% and 25% to total N_2O at 30% and 70% WFPS, respectively. In an incubation, N_2O emission was only stimulated when nitrate and glucose were applied together at 60% WFPS, indicating that denitrification was carbon limited. PM had a stronger effect on denitrification than CM due to higher decomposability, and the lower N_2O emission at higher manure application rate was associated with decreased mineral N supply. After compiling a worldwide database and establishing an empirical model that related N_2O emissions (kg N ha^{-1}) to precipitation (P_r, m) and fertilizer N application rate (N_r, kg N ha^{-1}) ($N_2O = 1.533P_r + 0.0238P_rN_r$), annual N_2O emission from global-cultivated black soil applied with inorganic fertilizer N was estimated as 347 Gg N. Our results suggested that N_2O emission from cultivated black soils in China was low primarily due to low precipitation and labile organic carbon availability, and would be stimulated by manure application; thus, increased N_2O emission should be taken into consideration as applying manure increases soil organic carbon sequestration.

1. Introduction

Nitrous oxide (N_2O) is involved in global warming and stratospheric ozone destruction, and its concentration in the atmosphere has increased from approximately 273 ppb in 1750 to 325 ppb in 2012 [*United Nations Environment Programme* (*UNEP*), 2013]. N_2O emissions from global agriculture were estimated to be 3.8–6.8 Tg N yr^{-1}, accounting for 25–39% of global total N_2O emissions [*UNEP*, 2013]. Application of inorganic and organic fertilizer nitrogen (N) is the main source of agricultural N_2O emissions [*Davidson*, 2009]. *Bouwman et al.* [2010] estimated that annual direct and indirect N_2O emissions from fertilizer N applied in arable soils globally equaled 4 Tg N, and N fertilizer management is therefore of great concern for N_2O emission mitigation [*Adviento-Borbe et al.*, 2007; *Ma et al.*, 2010].

Organic fertilizer application is an effective measure for increasing soil organic matter (SOM) content and is widely regarded as a win-win strategy that enhances food security and offsets increasing global atmospheric carbon dioxide (CO_2) [*Lal*, 2004]. *Bouwman et al.* [2010] suggested that replacing applied inorganic fertilizer by recycling N in organic materials could be used to decrease N_2O emissions from agricultural field. Results from the field measurements have indeed demonstrated that organic fertilizer could decrease N_2O emissions compared with urea because of its low-mineralization rate [*Alluvione et al.*, 2010] or promotion of N_2O reduction through denitrification [*López-Fernández et al.*, 2007]. However, other studies have shown that organic amendments increase N_2O emissions compared to inorganic fertilizers [*Rochette et al.*, 2000;

van Groenigen et al., 2004; *Hayakawa et al.*, 2009]. Meanwhile, *Akiyama and Tsuruta* [2003], *Vallejo et al.* [2006], and *Meijide et al.* [2009] found no significant differences in N_2O emissions between inorganic and organic fertilizers. *Cayuela et al.* [2010] and *Aguilera et al.* [2013] attributed these controversial results to differences in the type of added organic materials, and the composition of organic C and N might be the controlling factors [*Velthof et al.*, 2003; *Chen et al.*, 2013]. Application rate of organic fertilizer can also influence its impact on soil N_2O emissions; similarly, results in the literature are inconsistent [*Rochette et al.*, 2000; *van Groenigen et al.*, 2004]. It is necessary to connect the quantity and quality of organic materials with other factors for predicting soil N_2O emissions; however, the knowledge of their effects on N_2O production is still not clear [*Aguilera et al.*, 2013; *Chen et al.*, 2013].

In soil, N_2O production is strongly regulated by soil temperature, oxygen, moisture, N, and C substrate availability, as well as other factors [*Dobbie and Smith*, 2003; *Vasconcelos et al.*, 2004; *Zhu et al.*, 2013]. Numerous field studies found that N_2O emissions increase with soil water-filled pore space (WFPS) [*Dobbie and Smith*, 2003; *Barton et al.*, 2008; *Wang et al.*, 2011], and 60–90% WFPS generally promotes high N_2O emissions via denitrification [*Davidson*, 1991]. However, this value may vary in different soils; thus, for a certain soil the effect of soil moisture on N_2O emissions needs to be explored to make its production mechanism clear. As a by-product, N_2O production from autotrophic nitrification under aerobic conditions is minor relative to denitrification. However, recent research reported that the ammonia oxidation pathway could be a significant source of N_2O under high WFPS or low-oxygen availability through nitrifier denitrification [*Kool et al.*, 2011; *Zhu et al.*, 2013]. Additionally, our understanding of heterotrophic nitrification is mainly confined in grassland and forest soils that have high organic C concentration and low pH, but some studies demonstrated that heterotrophic nitrification should not be ignored for agricultural soils [*Bateman and Baggs*, 2005; *Cai et al.*, 2010]. Undoubtedly, it is essential to understand the contribution of different N turnover processes to N_2O production in order to improve the current models and develop management practices that reduce N_2O emissions [*Bateman and Baggs*, 2005; *Barton et al.*, 2008; *Zhu et al.*, 2013].

Black soil, namely Mollisol, is widely recognized as inherently productive and fertile and covers approximately 916 million ha across the world, mainly in the midlatitudes of North America, Eurasia, and South America [*Liu et al.*, 2012]. The cultivated black soil in Northeast China is vital for crop production; however, a loss of soil organic carbon (SOC) has occurred in the past three decades as a result of erosion and low input of organic materials [*Xie et al.*, 2007]. Crop residues are traditionally removed from the field and used by farmers as cooking and heating fuels during winter, and organic manure has been used to increase the SOC levels in order to maintain soil fertility in this region [*Jiang et al.*, 2014]. However, organic manure application alone does not usually supply enough N for crop growth. Combined application of inorganic and organic fertilizers is recommended because this increases N use efficiency by improving the synchrony between soil N supply and crop demand [*Garcia-Ruiz and Baggs*, 2007]. However, this practice may induce large N_2O emissions from soil since mineral N and organic C are simultaneously present [*van Groenigen et al.*, 2004; *Vallejo et al.*, 2006]. Thus, it is crucial to assess the impact of this C sequestration strategy on soil N_2O emissions [*Li et al.*, 2005; *Qiu et al.*, 2009]. Many field measurements of N_2O emissions have been conducted in black soil across the world (Table S5 in the supporting information); however, to date studies focusing on comparison and estimation of N_2O emissions from cultivated black soil in different regions are scarce. In this study, a field experiment was established in a cultivated black soil in Northeast China and two laboratory experiments were set up. We compiled available field measurement data on N_2O emissions from global-cultivated black soil and established an empirical model. The objectives were to (i) assess the effects of application of inorganic fertilizer and manure on N_2O emissions, (ii) investigate the primary processes of N_2O production, and (iii) estimate N_2O emissions from global-cultivated black soil applied with inorganic fertilizer N.

2. Materials and Methods

2.1. Field Study

2.1.1. Study Site

The field experiment was conducted in a rainfed cultivated field at the Hailun National Agro-ecological Experimental Station, Heilongjiang Province, China (47°26′N, 126°38′E). The area has a temperate continental monsoon climate, with a short hot summer and long cold winter. The 30 year mean annual air temperature is 1.5°C, and the mean monthly temperature varies from −23°C in January to 21°C in July. Mean annual precipitation is 550 mm, more than 80% of which falls during the crop growing season from May to September.

Table 1. Chemical Properties of Pig Manure (PM) and Chicken Manure (CM)[a]

Manure Type	pH (H_2O)	Moisture (%)	Total N (g kg^{-1})	Organic C (g kg^{-1})	C:N Ratio	NH_4^+ (mg N kg^{-1})	NO_3^- (mg N kg^{-1})	DOC (g C kg^{-1})	NDF (%)	ADL (%)
PM	7.7a	4.0b	10.2b	87.9b	8.6a	85.2b	5.0b	8.4a	23.0a	3.1b
CM	8.0a	13.2a	14.8a	119.3a	8.1a	397.6a	163.5a	5.5b	20.4b	5.5a

[a]Different letters following values within the same column indicate significant differences between manures at $P < 0.05$. DOC, dissolved organic carbon; NDF, neutral detergent fiber; and ADL, acid detergent lignin.

Prior to our field experiment, the field had been cultivated under a maize-soybean rotation with fertilizer application rates of 200–250 kg N ha^{-1} for maize and 100–150 kg N ha^{-1} for soybean. The soil, derived from loamy loess and characterized with a deep mollic epipedon, is classified as black soil according to the genetic classification and Typic Hapludolls based on the U.S. Department of Agriculture (USDA) soil taxonomy. The surface soil (0–20 cm) prior to the experiment had a pH of 5.9, bulk density of 1.03 g cm^{-3}, a clay loam texture with 8% sand, 72% silt, and 20% clay, and contained 27.5 g kg^{-1} organic C, 0.67 g kg^{-1} labile organic carbon (LOC), 2.2 g kg^{-1} total N, 30.7 mg kg^{-1} NO_3^--N, and 6.7 mg kg^{-1} NH_4^+-N.

2.1.2. Field Experiment Design

The field experiment included six treatments: inorganic fertilizer (NPK), 75% inorganic fertilizer N plus 25% pig (PM1) or chicken (CM1) manure N, 50% inorganic fertilizer N plus 50% pig (PM2) or chicken (CM2) manure N, and no N fertilizer as control (CK). The treatments were arranged in a randomized complete block design with three replicate plots (each 12 × 4 m). Manure (37.5 kg N ha^{-1} for PM1 and CM1, 75 kg N ha^{-1} for PM2 and CM2) was applied as a basal fertilizer on 13 May 2011. Urea was added with two splits: 75 kg N ha^{-1} for NPK and 37.5 kg N ha^{-1} for PM1 and CM1 as the basal fertilizer, and 75 kg N ha^{-1} as the supplemental fertilizer for all fertilized treatments on 15 June 2011. Consequently, the N application rate for fertilized treatments was 150 kg N ha^{-1}, and the amount of organic C added in PM1, CM1, PM2, and CM2 treatments was 323, 303, 647, and 606 kg C ha^{-1}, respectively. Organic fertilizers were commercially available, with a mean pellet size of 5 mm in diameter. The chemical properties are listed in Table 1.

In Northeast China, fields were split into ridges and furrows by rotary tillage in autumn following harvest each year to maintain higher-soil temperature for seed germination in the following early spring. On 14 May 2011, basal fertilizers were evenly applied in bands at the ridges and covered with soil. Maize seeds were immediately sown on the ridges with a plant spacing of 25 cm and row spacing of 70 cm. A cylindrical polyvinyl chloride (PVC) tube (10 cm in height and 10 cm inner diameter) was inserted 5 cm into the soil at the center of one ridge in each plot, and three maize seeds were sown in the center of the tube. An area (subplot) of 1.4 × 2.0 m in each plot was left unplanted for measurement of CO_2 emissions resulting from the decomposition of SOM or manure. Three weeks after emergence, seedlings were thinned to a planting density of 57,000 plants ha^{-1}, and one seedling was left in each tube. During the maize growing season, visible weeds were removed manually. The mature maize was harvested on 27 September 2011. Grain and straw were oven dried at 60°C until a constant weight was achieved, and the weight was used to calculate the grain yield and aboveground biomass.

2.1.3. Field Measurement of N_2O and CO_2 Fluxes

Soil N_2O and CO_2 fluxes were measured using the static closed chamber method from 14 May 2011 to 10 May 2012. A rectangular PVC base frame (70 cm × 30 cm × 20 cm) with a 5 cm groove around the upper edge was permanently inserted 10 cm into the soil around the above mentioned PVC tube, making it in the center of the base frame. At the time of gas sampling, a PVC pipe (30 cm in height, 10 cm outer diameter) was inserted into the exiting PVC tube. A stainless steel rectangular chamber (70 cm × 25 cm × 20 cm) was fitted to the base frame by inserting into the groove, which was filled with water for airtightness. The chamber consisted of two separate parts combined by two hinges and airtight rubber seal, covered with white plastic foam to minimize temperature change inside the chamber during sampling process. In the middle of the chamber top, a 10 cm diameter opening was made for inserting the PVC pipe and it was sealed with airtight rubber. Two vents were punched on the chamber, one on the side for connecting to a 3 mm inner diameter silicone rubber tube for gas sampling, and another on the top for measuring the air temperature inside the chamber. Two circulating fans were positioned inside the top chamber to ensure adequate gas mixing. In the unplanted area, an integral PVC chamber was used to collect gases. Further details have been described by *Ding et al.* [2007b].

Gas fluxes were measured twice weekly during the growing season and weekly or biweekly during the nongrowing season. Sampling was conducted at the same time between 09:00 and 12:00 to minimize the diurnal variation. Four gas samples were collected from the chamber at 0, 10, 20, and 30 min after chamber closure using an airtight plastic syringe fitted with a three-way stopcock connected to the chamber through the abovementioned silicone rubber tube. Samples were immediately transferred into preevacuated 20 mL glass vials sealed with butyl rubber stoppers. The chamber temperature was measured during gas collection. Gas samples were analyzed using a gas chromatograph (GC; Agilent 7890, Agilent Technologies, Santa Clara, CA, USA) equipped with an electron capture detector and a thermal conductivity detector. N_2O and CO_2 fluxes were calculated from the slope of the linear increase in concentration during the chamber closure period.

2.1.4. Auxiliary Field Measurement

Precipitation and air temperature were monitored at a meteorological station in the vicinity of the study field. Soil temperature at 5 and 10 cm depth was measured with a digital thermometer or geothermometer (in winter), and soil water content at 5 cm depth was measured using a time domain reflectometry probe while the soil was not frozen and was expressed as water-filled pore space (WFPS). Soil samples (0–20 cm) were taken weekly until the soil became frozen (26 times in total) using a 5 cm diameter gouge auger. Three separate soil cores in each plot were combined and taken to the laboratory for analysis. Mineral N was extracted by adding 100 mL of 2 M KCl solution to 10 g of fresh soil (on an oven-dried basis) and shaken for 1 h. The concentration of NH_4^+ and NO_3^- was measured with a continuous-flow autoanalyzer (San^{++} System, Skalar Analytical BV, Breda, the Netherlands). LOC was determined biweekly (14 times in total) using a modified potassium permanganate ($KMnO_4$) oxidation method [*Mirsky et al.,* 2008]. Briefly, 10 mL of 0.02 M $KMnO_4$-0.1 M $CaCl_2$ solution was added to 2.5 g of soil (on an oven-dried basis), shaken for 2 min, settled for 10 min, and the absorbance at 550 nm was measured.

2.1.5. Soil and Manure Analysis

Organic C and total N concentrations in soil and manure were determined by the wet oxidation redox titration method and micro-Kjeldahl method, respectively; and the concentrations of NH_4^+ and NO_3^- in manure were measured using the MgO-Devarda alloy distillation method. Dissolved organic carbon (DOC) was extracted from manure with deionized water, shaken for 30 min, centrifuged for 10 min at 10000 rpm, and filtered through a 0.45 mm polyethersulfone membrane filter. DOC concentration in extracts was measured with a TOC analyzer (vario TOC Cube, Elementar, Hanau, Germany). Manure ash-free neutral detergent fiber (NDF) was analyzed by adding sodium sulfite and a heat stable amylase [*van Soest et al.,* 1991], and acid detergent lignin (ADL) was determined by solubilization of cellulose with sulphuric acid [*Robertson and van Soest*, 1981].

2.2. ^{15}N Tracing Incubation Experiment

The incubation experiment was conducted at three soil moisture levels (30%, 50%, and 70% WFPS) and included two different ^{15}N treatments with three replicates. Fresh soil of 30 g (on an oven-dried basis) was weighed into each of 90 250 mL flasks. After a 24 h preincubation, $^{15}NH_4^{14}NO_3$ (10.23 atom%) was added to half of the flasks, and $^{14}NH_4^{15}NO_3$ (10.28 atom%) was added to the other half, at a rate of 50 mg NH_4^+-N kg^{-1} and 50 mg NO_3^--N kg^{-1}. Flasks were divided into five groups, each containing nine flasks with $^{15}NH_4^{14}NO_3$ and nine with $^{14}NH_4^{15}NO_3$. One group was used for gas flux measurement, and the others for measuring concentration and ^{15}N abundance of NH_4^+, NO_3^- and organic N. After adjusting to the target moisture content, all flasks were covered with cling film (punctured with needle holes to maintain aeration) and incubated in the dark at 25°C for 72 h. Lost water was replaced daily on a weight basis. Gas fluxes were measured at 6, 24, 48, and 72 h after ^{15}N labeling. Each time, the flasks were capped using rubber stoppers fitted with septa and silicone rubber was used to ensure airtightness. Then all the flasks were connected to a multiport vacuum manifold to be flushed with fresh air. The procedure was repeated 3 times (each for 15 s) to ensure the N_2O concentration in the flask headspace was equal to that of fresh air. After incubation for 6 h, 40 mL of gas was collected from the flasks using an airtight plastic syringe and injected into two preevacuated 20 mL glass vials for the analyses of N_2O concentration and isotopic composition, respectively. After sampling, stoppers were removed and flasks were covered with cling film. At each gas sampling, one group of flasks was used to extract NH_4^+ and NO_3^- by adding 150 mL of 2 M KCl solution. Subsequently, soil was washed with distilled water to remove the residual mineral N and then oven dried at 50°C. N remaining in the soil was considered as organic N. N_2O concentrations were measured using GC, and flux was calculated by assuming a linear relationship between concentration and time during the 6 h incubation period. The ^{15}N enrichment of N_2O was analyzed on a mass spectrometer

(MAT 253, Thermo Finnigan, Bremen, Germany). The content and ^{15}N enrichment of NH_4^+, NO_3^-, and organic N were measured using the method described by *Zhang et al.* [2009].

2.3. N_2O Emission Following NO_3^- and/or Glucose Addition

The second incubation experiment involved four treatments: no substrate addition (OA) as a control, sodium nitrate addition (NA), glucose addition (CA), and both sodium nitrate and glucose addition (NCA). All incubations were conducted in triplicate. The amount of sodium nitrate and glucose added was equivalent to 100 mg N kg^{-1} soil and 300 mg C kg^{-1} soil, respectively. Sodium nitrate and glucose solutions were sprinkled evenly to the 250 mL flasks containing 30 g of fresh soil (on an oven-dried basis) after preincubation. Soil moisture content was adjusted to 60% WFPS, and flasks were covered with cling film and incubated in the dark at 25°C. During the incubation, lost water was replaced every other day. Gas fluxes were measured at 6, 12, 24, 48, and 72 h after addition of the above solutions. When sampling, flasks were capped using rubber stoppers after 30 min of aeration. Headspace gas of 20 mL was sampled with a syringe at time zero and 6 h after flask closure. N_2O and CO_2 concentrations were analyzed by GC, and gas fluxes were calculated as described above.

2.4. Calculations

Cumulative N_2O emissions (E_{N_2O}, kg N ha^{-1}) in the field were calculated as follows:

$$E_{N_2O} = \sum (f_i + f_{i+1})/2 \times (t_{i+1} - t_i) \times 24 \times 10^{-5} \tag{1}$$

where f represents the N_2O flux from soil (μg N m^{-2} h^{-1}), i is the ith measurement, $(t_{i+1} - t_i)$ is the time (day) between two adjacent measurements, and 24×10^{-5} was used for unit conversion. Cumulative CO_2 emissions in the field and cumulative gas emissions in the laboratory experiments were calculated using the similar method.

The N_2O emission factor of applied N (EF) was calculated by dividing the difference in annual N_2O emissions in the planted subplots between the fertilized treatments and CK by the total amount of N added (150 kg N ha^{-1}) in the field. The decomposition proportion of added manure C was estimated by dividing the difference in annual CO_2 emissions in the unplanted subplots between the manure and NPK treatments by the amount of organic C added. This calculation is based on the assumption that the addition of manure would not stimulate the decomposition of native SOC, compared with NPK [*Walela et al.*, 2014].

Gross N mineralization has been found linearly related to C mineralization and could be predicted by taking the C:N ratio of the mineralized pool into consideration [*Herrmann and Witter*, 2008]. The amount of mineralized N ($M_{N\text{-}SOM}$, kg N ha^{-1}) from the decomposition of SOM in the CK and NPK treatments was calculated using the following equation [*Flavel and Murphy*, 2006]:

$$M_{N-SOM} = E_{CO_2}/(1-E)/R_{C:N-soil} \tag{2}$$

where E_{CO_2} is the cumulative CO_2 emission (kg C ha^{-1}) in the unplanted subplot of the CK or NPK treatment, E is microbial C use efficiency and assumed to be 0.4 [*Flavel and Murphy*, 2006], and $R_{C:N\text{-}soil}$ is the C:N ratio of soil being mineralized and is assumed to be the initial value. Mineralized N from the decomposition of manure ($M_{N\text{-}OM}$, kg N ha^{-1}) was estimated by

$$M_{N-OM} = (E_{CO_2-OM} - E_{CO_2-NPK})/(1-E)/R_{C:N-OM} \tag{3}$$

where E_{CO_2-OM} and E_{CO_2-NPK} are cumulative CO_2 emissions (kg C ha^{-1}) from the unplanted subplots of the manure and NPK treatment, respectively, and $R_{C:N\text{-}OM}$ is the C:N ratio of manure. The total mineral N pool ($M_{N\text{-}T}$, kg N ha^{-1}) was assumed to be the sum of N applied as urea and mineralized N from the decomposition of SOM or organic manure during the experimental period.

The relative contributions to N_2O production of the different N turnover processes in the ^{15}N tracing experiment were calculated using equation (4) as described by *Rütting et al.* [2010], assuming that N_2O was produced via three processes: autotrophic nitrification (AN), heterotrophic nitrification (HN), and denitrification (DN) from the NH_4^+, organic N, and NO_3^- pools, respectively.

$$a_{N_2O} = f_{AN} \times a_a + f_{HN} \times a_h + f_{DN} \times a_d \tag{4}$$

where a_{N_2O}, a_a, a_h, and a_d are the ^{15}N atom% excess of N_2O, NH_4^+, organic N, and NO_3^-, respectively, and f_{AN}, f_{HN}, and f_{DN} are the fractions (%) of N_2O derived from AN, HN, and DN, respectively. N_2O was assumed to be sourced from the above mentioned three processes, therefore $f_{AN} + f_{HN} + f_{DN} = 1$. Using the measured ^{15}N atom%
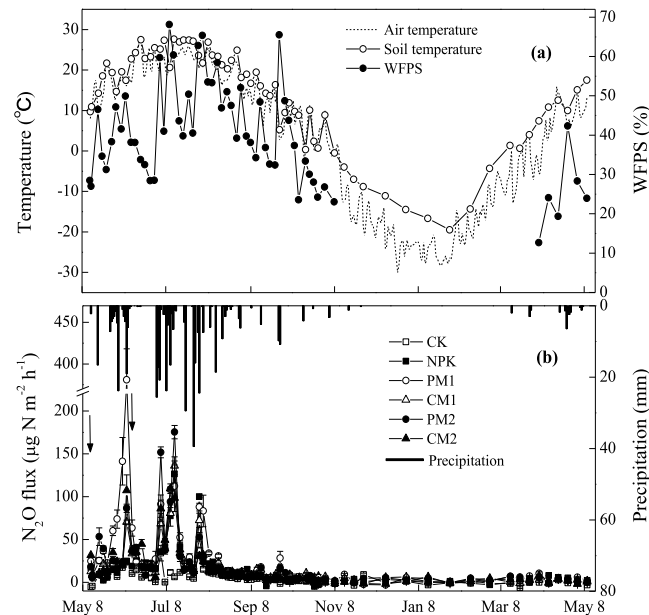
Figure 1. (a) Air temperature, soil water-filled pore space (WFPS), soil temperature at 5 cm depth, (b) precipitation, and N_2O fluxes from soil treated with different fertilizers in the field from 14 May 2011 to 10 May 2012. Soil WFPS and temperature values are the averages of all treatments. Vertical bars denote the standard error of the means of the N_2O fluxes ($n = 3$). Solid arrows indicate the time of fertilizer applications.

excess of NH_4^+, organic N and NO_3^- in the paired $^{15}NH_4NO_3$ and $NH_4^{15}NO_3$ treatments, f_{AN}, f_{HN}, and f_{DN} were calculated with Microsoft Excel 2003. The N_2O flux from each process was calculated by multiplying the total N_2O flux by the corresponding fractions.

The gross rate of oxidation of NH_4^+ (O_{NH_4}) and recalcitrant organic N ($O_{N_{rec}}$) to NO_3^- were calculated using the ^{15}N tracing model [*Müller et al.*, 2007] and considered to be the gross rate of AN and HN, respectively. The gross denitrification rate could not be calculated using this model.

2.5. Statistical Analysis

Before statistical analysis, the normality of data frequency distribution for all the variables was tested using the Kolmogorov-Smirnov test. If the data were not normally distributed, natural logarithm transformation was used, and an integer (7) was added to all field N_2O fluxes to make the data positive and included in the logarithmic transformations [*Davidson et al.*, 2008].

Statistically significant differences were tested using the one-way analysis of variance (ANOVA) procedure followed by the least significant difference test at $P < 0.05$. The interaction of the effects of manure type and application rate on N_2O emissions were tested using two-way ANOVA analysis, and correlation analysis was used to probe the relationship between N_2O flux and the other factors. Partial correlation between N_2O flux and soil NO_3^- or mineral N (NH_4^+-N plus NO_3^--N) concentration was conducted, in which the effect of soil temperature was controlled. To determine the key soil factors influencing N_2O flux and the quantitative relationships between them, stepwise multiple linear regression analysis was applied, based on the criteria of $P < 0.05$ to accept and $P > 0.1$ to exclude variables. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software package for Windows (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Environmental Parameters

The daily air temperature (T_{air}) ranged from −30 to 28°C, with an average of 2°C during the field measurement period (Figure 1a). Soil temperature (T_{soil}) at 5 cm depth varied following a similar pattern to air temperature ($T_{soil} = 3.831 + 0.887 \, T_{air}$, $R^2 = 0.96$, and $P < 0.0001$). A total of 544 mm of rainfall occurred between 14 May 2011 and 10 May 2012, of which 91% fell during the maize growing season, and up to 49% fell in July. During the winter (November 2011 to February 2012), the total amount of snowfall was 13 mm, which was less than the 30 year average of 19 mm (1971 to 2000). Soil WFPS varied from 12% to 69%, and was significantly ($P < 0.01$) correlated with the cumulative rainfall during the 3 days prior to measurement (Figure 1). The mean WFPS of all treatments varied from 39.4% to 40.8%, and there were no significant differences between the treatments.

3.2. Soil NH_4^+, NO_3^-, and LOC

Soil NH_4^+ concentration ranged from 1.01 to 8.56 mg N kg^{-1} with an average of 2.76 mg N kg^{-1} in all treatments (Figure 2a). No significant differences in soil NH_4^+ concentration were found among the treatments at each measurement time. Soil NO_3^- concentration was at high level at the beginning of the experiment probably due to the residue of fertilizer N applied in the previous year, and no significant increase was found after the basal fertilizer application compared with CK (Figure 2b). Following application of the supplemental fertilizer,
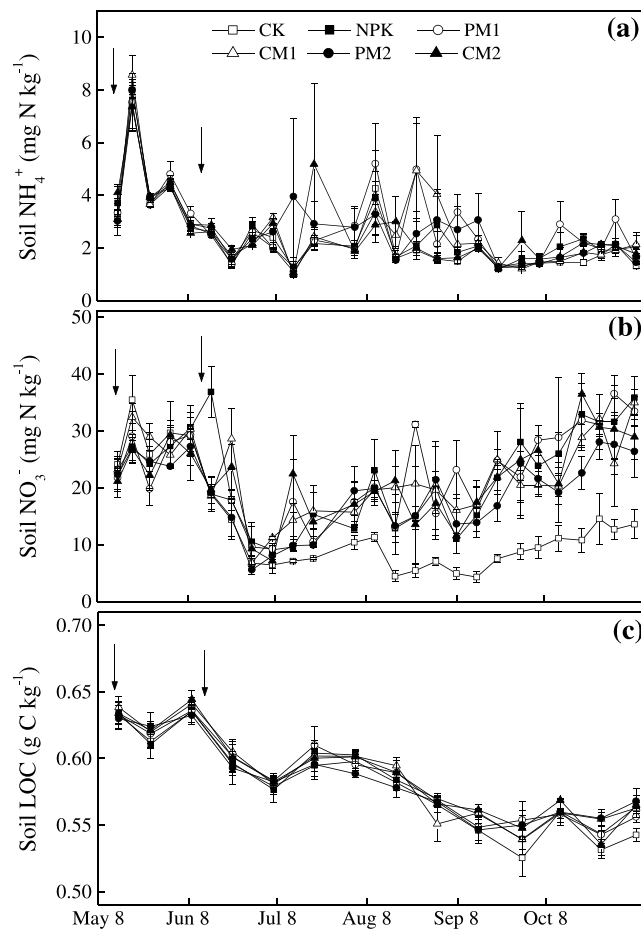
Figure 2. (a) Ammonium (NH_4^+), (b) nitrate (NO_3^-), and (c) labile organic carbon (LOC) concentrations in soil treated with different fertilizers from 15 May 2011 to 18 November 2011. Vertical bars denote the standard error of the means ($n = 3$). Solid arrows indicate the time of fertilizer applications.

however, soil NO_3^- concentration increased obviously compared with CK. After that, soil NO_3^- concentration decreased due to the uptake by crops and gradually increased again in the late summer and autumn. Over the measured period, significantly ($P < 0.05$) higher NO_3^- concentrations in the fertilized soils than in the CK soil were observed from July onward. LOC concentration decreased after fertilization and tended to be stable after harvest, varying from 0.515 to 0.644 g C kg^{-1}; however, no significant difference was found among the treatments at all measurement times (Figure 2c).

3.3. Field N_2O Emissions and Maize Biomass

N_2O fluxes in the CK treatment were consistently low, never greater than 38 μg N m^{-2} h^{-1}, averaging just 3.94 μg N m^{-2} h^{-1} over the entire year (Figure 1b). In the fertilized treatments, N_2O fluxes maintained at high levels for around 3 months after basal fertilization and decreased to background levels by late August. N_2O flux peaks occurred primarily following rainfall rather than immediately after fertilization and were significantly ($P < 0.01$) correlated to the cumulative rainfall during the 3 days prior to N_2O flux measurement in all treatments (Table 2). The highest peak

of N_2O flux was 381 μg N m^{-2} h^{-1} and was observed in the PM1 treatment on 9 June 2011. From June to August, there were 11 flux peaks when N_2O flux was >100 μg N m^{-2} h^{-1} while soil temperature was >17°C and WFPS was >40% (once for CM1, twice for NPK, PM2, and CM2, and 4 times for PM1; Figure 1b). From mid-August 2011 to May 2012, however, N_2O flux was <30 μg N m^{-2} h^{-1} in all treatments. No apparent N_2O flux peaks were observed during the spring thaw in March and April 2012. On a few occasions, especially during winter when soil temperature was below freezing, N_2O fluxes were less than zero, which mainly

Table 2. Correlation Between N_2O Flux and Soil or Environmental Parameters Over the Experimental Period[a]

Treatment	CO₂ Flux	WFPS	Rainfall	T_{5cm}	T_{10cm}	NH_4^+	NO_3^-	Mineral N	LOC	Partial Correlation[b] NO_3^-	Partial Correlation[b] Mineral N
CK	0.64**	0.57**	0.46**	0.53**	0.55**	0.14	0.09	0.10	0.21	0.17	0.16
NPK	0.74**	0.63**	0.55**	0.69**	0.70**	−0.04	−0.51**	−0.49**	0.43	0.01	0.00
PM1	0.76**	0.61**	0.62**	0.63**	0.64**	0.13	−0.28	−0.24	0.57*	0.11	0.12
CM1	0.76**	0.61**	0.60**	0.67**	0.70**	−0.01	−0.35*	−0.33	0.57*	0.14	0.07
PM2	0.78**	0.65**	0.67**	0.62**	0.63**	0.53**	−0.24	−0.10	0.41	0.28	0.37
CM2	0.73**	0.51*	0.58**	0.66**	0.67**	0.14	−0.32	−0.29	0.70**	0.29	0.28

[a]*, $P < 0.05$; **, $P < 0.01$. Rainfall, cumulative rainfall during the 3 days prior to N_2O flux measurement; T_{5cm} and T_{10cm} denote soil temperature at 5 and 10 cm depth, respectively; Mineral N = NH_4^+-N + NO_3^--N.
[b]Partial correlation excluding the masking influence of soil temperature.

Table 3. Cumulative N_2O and CO_2 Emissions, Fertilizer Induced N_2O Emission Factors, and Total Mineral N Over the Experimental Period[a]

Treatment	Cumulative N_2O Emission (kg N ha^{-1})			Emission Factor (%)	Cumulative CO_2 Emission (kg C ha^{-1})	Total Mineral N[c] (kg N ha^{-1})
	Growing Season[b]	Nongrowing Season[b]	Annual			
CK	0.33 ± 0.01d	0.01 ± 0.02b	0.34 ± 0.03d	—	1822 ± 88b	239 ± 12c
NPK	0.77 ± 0.05c	0.08 ± 0.01ab	0.86 ± 0.06c	0.34 ± 0.02d	1919 ± 71ab	402 ± 9a
PM1	1.51 ± 0.14a	0.14 ± 0.04a	1.65 ± 0.11a	0.87 ± 0.06a	2087 ± 82a	397 ± 3a
CM1	0.88 ± 0.05bc	0.14 ± 0.02a	1.02 ± 0.04bc	0.45 ± 0.01bc	2022 ± 82ab	385 ± 3a
PM2	1.02 ± 0.09b	0.15 ± 0.04a	1.17 ± 0.06b	0.55 ± 0.03b	2081 ± 55a	358 ± 3b
CM2	0.86 ± 0.09bc	0.08 ± 0.02ab	0.93 ± 0.07c	0.39 ± 0.03 cd	1987 ± 82ab	341 ± 2b

[a]Values represent means ± stand error ($n = 3$), and different letters following values within the same column indicate significant differences between treatments at $P < 0.05$. The ANOVA table is given in Text S2 (Table S2).
[b]Growing season and nongrowing season denote the period from 14 May 2011 to 15 October 2011, and 16 October 2011 to 10 May 2012, respectively.
[c]Total mineral N = inorganic N added as urea + mineral N released from the decomposition of SOM or added manure.

occurred in the CK treatment. Over the whole year, the mean N_2O flux in the NPK, PM1, CM1, PM2, and CM2 treatments was 9.84, 18.93, 11.71, 13.38, and 10.70 µg N m^{-2} h^{-1}, respectively, which was significantly ($P < 0.01$) higher than that of CK.

As shown in Table 2, N_2O flux was significantly correlated with the CO_2 flux ($P < 0.01$), soil WFPS ($P < 0.05$), and soil temperature at 5 and 10 cm depth ($P < 0.01$) in all treatments. However, a significant relationship between N_2O flux and soil NH_4^+ concentration was observed only in PM2 ($P < 0.01$). In contrast, there was a negative correlation between soil NO_3^- concentration and N_2O flux in the fertilized treatments, and this was most significant for NPK ($P < 0.01$) and CM1 ($P < 0.05$). After excluding the masking influence of soil temperature, however, partial correlation analysis showed a weak positive correlation between soil NO_3^- concentration and N_2O flux instead. A negative correlation and a positive partial correlation after removing the effect of soil temperature were also found between soil mineral N (NH_4^+ plus NO_3^-) concentration and N_2O flux in the fertilized treatments (Table 2). N_2O flux was significantly ($P < 0.05$) correlated with LOC in the PM1, CM1, and CM2 treatments. Using stepwise multiple linear regression analysis, equations linking soil WFPS, 5 cm temperature, or LOC concentration with N_2O flux were able to explain 94–99% of seasonal variation in N_2O flux (Table S1). In the stepwise regression equations, LOC was included whereas soil mineral N was excluded in all treatments and soil WFPS was also included for NPK and PM, and soil temperature for NPK and CM. These results implied that soil moisture, temperature, and particularly LOC supply were more important than the mineral N substrates in regulating the seasonal variation of N_2O emissions.

Annual N_2O emission in the CK treatment was estimated to be 0.34 kg N ha^{-1}, which was equivalent to 0.14% of mineralized N released from the decomposition of SOM (Table 3). N_2O emission significantly ($P < 0.01$)

increased to 0.86–1.65 kg N ha^{-1} in the fertilized treatments, of which 60–79% was sourced from N applied as urea or manure, and 86–92% was emitted during the growing season. N_2O emissions in the manure treatments were higher than in the NPK treatment, and a significant difference was observed between NPK and PM1 or PM2 ($P < 0.01$). Annual N_2O emissions in the pig manure plots were significantly ($P < 0.05$) higher than the chicken manure plots under identical application rate (PM1 > CM1 and PM2 > CM2); however, it decreased with increasing manure application rate, i.e., PM1 > PM2 and CM1 > CM2. Two-way ANOVA analysis showed that there were significant effects of organic manure type ($P < 0.01$), application rate ($P < 0.01$),
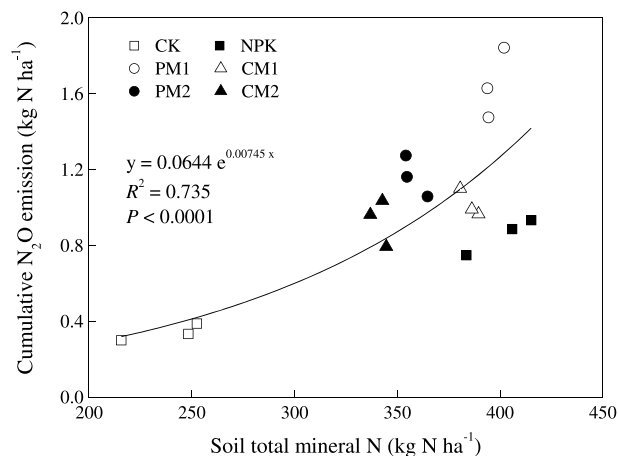
Figure 3. Correlation between cumulative N_2O emissions and soil mineral N derived from urea and the decomposition of organic matter or manure.

In figure:
$y = 0.0644\ e^{0.00745\ x}$
$R^2 = 0.735$
$P < 0.0001$

Table 4. Maize Yield and Aboveground Biomass (kg ha^{-1})[a]

Treatment	Grain	Straw	Total
CK	5031 ± 209a	7482 ± 734a	12513 ± 879a
NPK	5314 ± 81a	7715 ± 264a	13029 ± 184a
PM1	5299 ± 52a	8072 ± 644a	13371 ± 659a
CM1	5342 ± 302a	8571 ± 273a	13913 ± 330a
PM2	5348 ± 315a	7803 ± 703a	13150 ± 790a
CM2	5192 ± 503a	7781 ± 406a	12973 ± 462a

[a]Values represent means ± stand error ($n = 3$), and different letters following values within the same column indicate significant differences between treatments at $P < 0.05$.

and their interaction ($P = 0.03$) on N_2O emissions (Table S3). Annual N_2O emissions were significantly correlated to total mineral N (M_{N-T}) derived from urea and the decomposition of SOM or manure (Figure 3). The N_2O EF increased from NPK (0.34%) to CM2 (0.39%) and CM1 (0.45%), and further to PM2 (0.55%) and PM1 (0.87%). The decomposition rate of added manure organic C was estimated as 52.2% for PM1 and 34.1% for CM1, and this decreased to 25.0% for PM2 and 11.8% for CM2 over the entire year.

There was no statistically significant effect of fertilizer application on maize grain yield and aboveground biomass in our experiment (Table 4), which was probably due to the relatively high background level of soil mineral N in the studied field (Figure 2).

3.4. N_2O Production Processes at Different Soil WFPS Levels

Mean N_2O flux significantly ($P < 0.05$) increased from 69.8 ng N kg^{-1} h^{-1} at 30% WFPS to 89.5 ng N kg^{-1} h^{-1} at 70% WFPS (Table 5). The ^{15}N enrichment of N_2O was lower than that of NH_4^+ and NO_3^- after 24 h incubation in the $^{15}NH_4^+$ treatments, indicating that heterotrophic nitrification of organic N with low ^{15}N abundance contributed to N_2O. In the $^{15}NO_3^-$ treatments, the ^{15}N enrichment of N_2O was always very low, indicating that denitrification contributed little to N_2O production (Figures S1 and S2). On average, nitrification contributed 71.2–79.3% of total N_2O production in the range of 30–70% WFPS. The amount of N_2O produced through autotrophic nitrification increased with increasing soil WFPS, and its contribution to total N_2O increased from 22.3% at 30% WFPS to 45.8% at 70% WFPS. In contrast, the contribution of heterotrophic nitrification decreased from 48.9% at 30% WFPS to 25.3% at 70% WFPS. Interestingly, the contribution of denitrification did not significantly change as soil WFPS increased from 30% to 70%, varying from 20.7% to 28.9%. The gross rate of autotrophic nitrification was 2.27 mg N kg^{-1} d^{-1} at 30% WFPS and increased to 7.97 mg N kg^{-1} d^{-1} at 70% WFPS. Conversely, heterotrophic nitrification rate was highest at 30% WFPS and became too low to be detected at 70% WFPS.

3.5. N_2O Emissions Following Nitrate and/or Glucose Addition

Over the 72 h incubation, cumulative N_2O emission increased from 2.41 µg N kg^{-1} in the control soil (OA) to 2.62 µg N kg^{-1} in the nitrate-added soil (NA), but decreased to 1.80 µg N kg^{-1} in the glucose-added soil (CA). However, these values were not significantly different from each other (Figure 4). In contrast, up to 4.62 µg N kg^{-1} N_2O was emitted from the soil added with nitrate plus glucose (NCA), which was significantly higher than those in the other treatments ($P < 0.05$). The significantly ($P < 0.05$) higher CO_2 emissions were observed from the glucose-added soil (CA and NCA) compared with the control soil (OA).

Table 5. N_2O Flux, the Contribution of Autotrophic (AN) and Heterotrophic Nitrification (HN), and Denitrification (DN) to N_2O Production and Gross Transformation Rate of AN and HN at Different Soil WFPS in the ^{15}N Tracing Study[a]

WFPS (%)	N_2O Flux (ng N kg^{-1} h^{-1})				Relative Contribution (%)			Transformation Rate[b] (mg N kg^{-1} d^{-1})	
	Total	AN	HN	DN	AN	HN	DN	AN	HN
30	69.8b	15.6b	34.2a	20.0ab	22.3bB	48.9aA	28.8aA	2.27	1.06
50	83.1ab	31.6a	34.3a	17.2b	38.0aA	41.3abA	20.7aA	5.93	0.01
70	89.5a	41.0a	22.6b	25.9a	45.8aA	25.3bB	28.9aA	7.97	0.00

[a]Different lowercase letters following values indicate significant differences between different WFPS levels at $P < 0.05$. Different capital letters denote significant differences in contributions between different processes within each WFPS level at $P < 0.05$.
[b]Gross transformation rates of AN and HN were calculated by a ^{15}N tracing model [*Müller et al.*, 2007] regarding oxidation of NH_4^+ and recalcitrant organic N to NO_3^- as AN and HN, respectively.
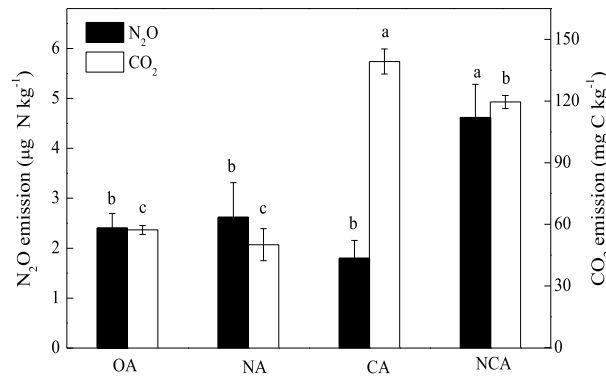
Figure 4. Cumulative N_2O and CO_2 emissions from soils added with nitrate (NA), glucose (CA), both nitrate and glucose (NCA), and without nitrate or glucose (OA) during the 72 h incubation period. Vertical bars denote the standard error of the means ($n = 3$), different letters indicate significant differences between treatments for the same gas ($P < 0.05$), and the ANOVA table is given in Text S2 (Table S4).

3.6. Modeling N_2O Emissions From Global-Cultivated Black Soil

We compiled available field measurements of N_2O emissions and ancillary data in the cultivated black soils applied with inorganic fertilizer N in the world (Table S5). Here the results of experiments under application of fertilizer together with nitrification inhibitors were not included. The database consisted of 252 observations from 45 study sites in Argentina, Canada, China, Uruguay, and the United States.

Pairwise correlation analysis showed that N_2O emissions were not significantly correlated to air temperature, soil pH, and the measurement period but correlated to SOC concentration ($P = 0.001$), clay content ($P = 0.004$), WFPS ($P = 0.002$), and particularly precipitation and fertilizer N application rate ($P < 0.0001$) (Table S6). Linear regression analysis was used to fit N_2O emissions to these relevant factors, and model fitness was assessed by power analysis and the residual distribution pattern [*Zou et al.*, 2007]. A regression equation including precipitation (P_r, m) and fertilizer N application rate (N_r, kg N ha^{-1}) was derived as follows: N_2O (kg N ha^{-1}) = 1.533 ($\pm$1.384; 95% confidence interval) P_r + 0.0238 ($\pm$0.0092; 95% confidence interval) P_r N_r ($R^2 = 0.57$, $F = 166$, $P < 0.0001$, and $n = 249$). This equation could more efficiently account for the variability of N_2O emissions than the equation of N_2O = 0.541 ($\pm$0.702; 95% confidence interval) +0.0138 ($\pm$0.0049; 95% confidence interval) N_r ($R^2 = 0.11$, $F = 31$, $P < 0.0001$, and $n = 249$), which was established similar to the Intergovernmental Panel on Climate Change (IPCC) methodology [*Intergovernmental Panel on Climate Change*, 2006]. The second equation implies that the EF for global black soils is 1.38% $\pm$ 0.49%, which is slightly, but not statistically significantly, higher than the IPCC default value of 1.00% for all agricultural soils. The first equation also includes precipitation as a factor, thus permitting the calculation of an EF for black soils at each level of precipitation: EF = $0.0238 \times P_r$, where P_r is the mean annual precipitation (m).
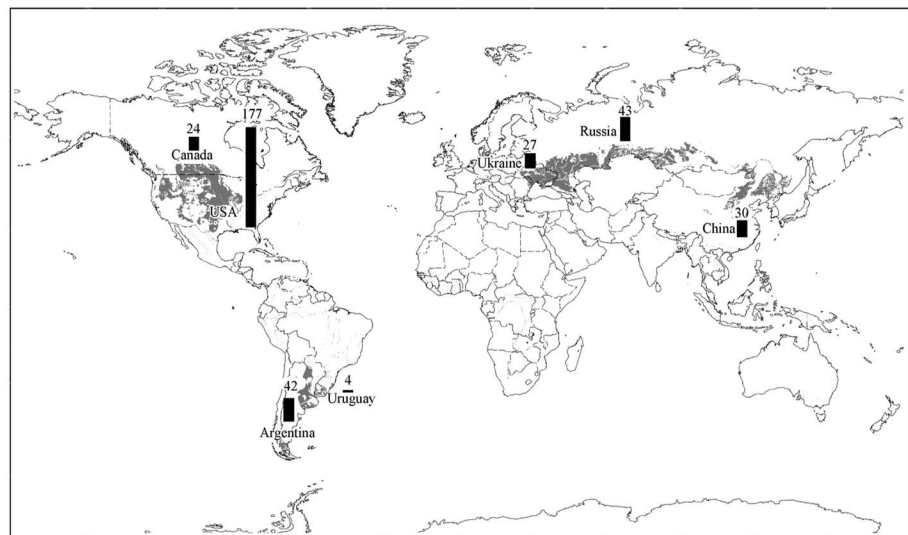

Figure 5. Estimated N_2O emissions (Gg N yr^{-1}) from cultivated black soil under inorganic fertilizer application in major countries of the world.

Although this regression model explains a larger fraction of the variation globally, it still overestimates the EF for the relatively dry climate of the present study site. Based on this regression equation, we estimated N_2O emissions from cultivated black soils applied with inorganic fertilizer N in the major countries across the world (Text S6). The mean N_2O emission rate varied from 0.94 kg N ha^{-1} yr^{-1} in Russia to 2.80 kg N ha^{-1} yr^{-1} in Uruguay (Table S7). Total annual N_2O emission from black soils was estimated to be 42, 24, 30, 43, 27, 4, and 177 Gg N for Argentina, Canada, China, Russia, Ukraine, Uruguay, and United States, respectively (Figure 5).

4. Discussion

4.1. N_2O Production Processes in the Cultivated Black Soil in China

In this study, inorganic fertilizer was found to significantly promote N_2O emission ($P < 0.01$). However, the EF was only 0.34%, which was lower than the current IPCC default value of 1.00%, a reported national average (0.57%) for uplands in China [*Xing*, 1998], the reported ranges of regional means (0.65% to 1.57%) for uplands of China [*Zhou et al.*, 2014], and the mean value (1.06%) for global maize-cultivated cropland estimated by *Linquist et al.* [2012]. From the compiled data set, the EF in China was also found to be lower than in both North and South America, suggesting that inorganic fertilizer N applied in black soil of China was less efficiently converted into N_2O. We found that N_2O flux in the field experiment was significantly correlated to rainfall (Table 2), and N_2O emissions primarily depended on precipitation according to the empirical model for the global-cultivated black soil applied with inorganic fertilizer N. These results suggested that the relatively low N_2O emission from black soil in Northeast China was putatively attributed to low rainfall in part.

Soil WFPS has been demonstrated to play an important role in regulating N_2O emissions [*Dobbie and Smith*, 2003]. Indeed, high soil WFPS favors the formation of anaerobic condition and stimulates denitrification, which produces more N_2O than nitrification under low WFPS [*Wang et al.*, 2011]. *Ma et al.* [2010] proposed 45% WFPS as the threshold value above which anaerobic denitrification predominated. Using a combination of ^{15}N labeling and acetylene inhibition techniques, *Bateman and Baggs* [2005] found that all emitted N_2O at 70% WFPS was derived from denitrification in a silt loam soil. In our field experiment, N_2O flux peaks always occurred following rainfall events (Figure 1b). However, the peak flux was <130 μg N m^{-2} h^{-1} even at 69% WFPS following continuous rainfall of up to 50 mm in NPK. This value was significantly lower than the results reported by *Ma et al.* [2010] and *Garland et al.* [2011], and these authors suggested that large N_2O emissions following rainfall were predominantly produced from denitrification at increased WFPS.

Using ^{15}N tracer technique, we found that nitrification contributed 71.2–79.3% of total N_2O at 30–70% WFPS in the test soil (Table 5). Several other studies also suggested that nitrification was the primary source of N_2O at <75% WFPS [*Khalil and Baggs*, 2005; *Ding et al.*, 2007a; *Wang et al.*, 2011]. As the substrate for denitrification, NO_3^- has been reported to be positively correlated with N_2O flux [*Sanchez-Martin et al.*, 2010; *Pelster et al.*, 2012]. However, in the field we found that there was no significant correlation between N_2O flux and soil NO_3^- (Table 2), and soil NO_3^- concentration was always higher than 5 mg N kg^{-1}, the threshold value for N_2O production through denitrification [*Conen et al.*, 2000]. In the laboratory incubation, nitrate addition alone did not significantly induce N_2O emission, but when nitrate and glucose were added together, N_2O emission was significantly increased by 92% at 60% WFPS (Figure 4). Mineral N appeared not to be the limiting factor for N_2O production by denitrification, but organic C availability seemed to be crucial for this process. Previous studies demonstrated that denitrification was not only controlled by soil moisture and nitrate but also by organic C supply, and increasing organic C availability could reduce the moisture threshold for denitrification [*Rochette et al.*, 2000; *van Groenigen et al.*, 2004; *Chantigny et al.*, 2013]. Although black soil in Northeast China has the highest SOC concentration in the nationwide cropland [*Xie et al.*, 2007], mean CO_2 flux from the decomposition of native SOC was only 46 mg C m^{-2} h^{-1} in this study, which was remarkably lower than the values in an Inceptisol in North China [*Ding et al.*, 2010] and a Utisol in South China [*Lou et al.*, 2004]. LOC, an indicator of biologically active organic C, was 0.67 g kg^{-1} of our soil, which was lower than that reported by *Melero et al.* [2011] and *Wang et al.* [2013], although SOC concentrations in these studies were much lower. These results indicated that the low LOC supply in the test soil suppressed denitrification and thus N_2O emissions. It should be noted that the incubation using sieved (<2 mm) soil, compared with intact soil, might to some extent underestimate the contribution of denitrification due to breakup of part of anaerobic microsites.

Generally, heterotrophic nitrification is regarded as an important pathway in forest and grassland soil [*Wrage et al.*, 2001; *Stange et al.*, 2013]. Interestingly, we found that heterotrophic nitrification contributed 25.3–48.9% of total N_2O production at 30–70% WFPS in this cropland soil. Similarly, *Cai et al.* [2010] reported that 38% of N_2O was derived from heterotrophic nitrification at 70% water-holding capacity in a cultivated black soil using acetylene inhibition method. The gross rate of heterotrophic nitrification in our soil was 0.00–1.06 mg N kg^{-1} d^{-1}, which was similar to the rates measured in soil from subtropical woodland in South China (0.13–0.85 mg N kg^{-1} d^{-1}) [*Zhang et al.*, 2013], acid pasture in Australia (0.22–0.86 mg N kg^{-1} d^{-1}) [*Islam et al.*, 2007], and temperate grassland in north Ireland (0.02–2.58 mg N kg^{-1} d^{-1}) [*Müller et al.*, 2011]. We would expect the relatively high SOC concentration (27.5 g kg^{-1}) and low pH (5.9) in the soil of the present study to favor heterotrophic nitrification. Fungi have been found to be more acid tolerant than bacteria and more dominant in soil with high recalcitrant SOM concentration [*Strickland and Rousk*, 2010], and are considered to be the most efficient microorganisms performing heterotrophic nitrification [*Pederson et al.*, 1999]. *McLain and Martens* [2006] demonstrated that fungi played a predominant role in N_2O production via heterotrophic nitrification in a semiarid soil. Therefore, we speculated that the N_2O produced through heterotrophic nitrification in this study might be related to fungi. As an aerobic process, heterotrophic nitrification could produce more N_2O under conditions of higher oxygen availability [*Anderson et al.*, 1993; *Stange et al.*, 2013]. Thus, the higher N_2O flux and contribution of heterotrophic nitrification at lower WFPS (Table 5) could be attributed to more aerobic condition. To our knowledge, this is the first study that reports the important role of heterotrophic nitrification in N_2O production in cultivated soil at different WFPS using the ^{15}N tracing method. Given that ammonium is also the substrate for heterotrophic nitrification [*Zhang et al.*, 2014] and the microbial mechanisms are unclear, further research is needed.

In contrast to heterotrophic nitrification, the contribution of autotrophic nitrification to N_2O production increased from 22.3% to 45.8% when soil WFPS was increased from 30% to 70% (Table 5). *Bateman and Baggs* [2005] also found that the proportion of N_2O production from autotrophic nitrification increased as soil WFPS increased from 20% to 60%. Autotrophic nitrification is performed primarily by ammonia oxidizing bacteria (AOB) and ammonia oxidizing archaea under aerobic conditions [*Hayatsu et al.*, 2008]. In this study, increasing soil moisture was putatively better for substrates diffusion and simultaneously did not yet restrict O_2 diffusion within the range of 30–70% WFPS, thus favored more N_2O production through autotrophic nitrification at higher WFPS [*Parton et al.*, 1996]. Furthermore, AOB can carry out nitrifier denitrification at higher-soil moisture content where short-term O_2 limitation occurs and aeration condition is suboptimal for denitrification [*Wrage et al.*, 2001]. N_2O is produced as an intermediate in the reduction of NO_2^- to N_2 in nitrifier denitrification, which may become a major contributor to N_2O emission under the subanaerobic condition [*Kool et al.*, 2011; *Zhu et al.*, 2013]. Accordingly, nitrifier denitrification could be an important process for N_2O production at higher soil WFPS, and further studies are required to quantify the extent of this contribution to N_2O emissions.

4.2. Effects of Manure on N_2O Emissions

The influence of organic materials on soil N_2O emissions remains controversial in the literature, with authors disagreeing as to whether it leads to stimulation, inhibition, or has no effect [*Aguilera et al.*, 2013]. In this study, although the soil mineral N pool (M_{N-T}) derived from applied inorganic fertilizer and decomposition of SOM or manure was highest in the NPK treatment, N_2O emission was higher in manure-treated soil, significantly so for PM (Table 3). As discussed above, N_2O production in this soil is primarily derived from nitrification, as denitrification is limited by the availability of labile organic C under the field moisture condition. In the field, N_2O flux was significantly correlated with CO_2 flux and also with LOC in the PM1, CM1, and CM2 treatments but not with soil NO_3^- or mineral N. This suggests that increasing available organic C rather than mineral N would stimulate N_2O emissions. Our incubation results showed that the input of organic C sources such as glucose enhanced denitrification potential and N_2O production, even at relatively low soil WFPS [*Garcia-Ruiz and Baggs*, 2007], presumably due to the formation of anaerobic condition [*Hayakawa et al.*, 2009; *Pelster et al.*, 2012] and increase of the activity and abundance of denitrifiers [*Miller et al.*, 2012]. Consequently, larger N_2O emission in the manure treatments than in the NPK treatment was attributed to the enhancement of denitrification potential [*Rochette et al.*, 2000; *Hayakawa et al.*, 2009].

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The composition of the organic materials was suggested to be the primary determinant affecting N_2O emissions [*Millar and Baggs*, 2004; *Garcia-Ruiz and Baggs*, 2007]. *Cayuela et al.* [2010] suggested that N form and content of the applied organic materials were responsible for their effects on soil N_2O emission. However, in this study, we found that N_2O emissions in soil treated with PM were significantly higher than those of CM treated soil at both application rates, despite the fact that the inorganic N concentration of CM was sixfold higher than that of PM (Table 1). This indicates that difference of N concentration in manure was not the controlling factor. DOC and NDF concentrations were higher in PM, while ADL concentration was higher in CM (Table 1), indicating that the organic C in PM was more decomposable [*Serramiá et al.*, 2012]. The in situ organic C decomposition rate of PM was significantly higher than CM (52.2% for PM1 versus 34.1% for CM1 and 25.0% for PM2 versus 11.3% for CM2). Thus, we concluded that application of PM resulted in a more anaerobic soil environment due to the higher labile organic C supply and resulted in more N_2O emissions from denitrification [*Cayuela et al.*, 2010; *Aguilera et al.*, 2013].

Cai et al. [2013] found that compost addition alone increased N_2O emissions more than the addition of half compost N plus half inorganic fertilizer N, presumably due to the high input of easily decomposable organic C. Contrary to our original expectation, increasing the manure application rate from 37.5 to 75 kg N ha^{-1} by reducing the amount of applied urea N lowered N_2O emissions (Table 3). Since fertilizer was applied in bands at the ridges, manure was deeper in the soil and formed a larger volume in PM2 and CM2 than in PM1 and CM1 treatments. This may have resulted in the lower diffusion rate of O_2 from air to soil, lower O_2 availability in soil and poor manure-soil contact [*Henriksen and Breland*, 2002], which may have impeded the colonization and growth of microorganisms, especially fungi, the primary decomposers of recalcitrant organic materials, and decreased the activity of cellulases, hemicellulase, and polyphenol oxidase [*Henriksen and Breland*, 2002; *Zibilske and Bradford*, 2007]. Therefore, no significant difference in CO_2 emission was found between PM1 and PM2, and between CM1 and CM2 (Table 3), leading to no apparent difference of mineralized N from manure decomposition between PM1 and PM2, and between CM1 and CM2. Cumulative N_2O emissions increased exponentially with M_{N-T} (Figure 3), and the substitution of 50% urea N by manure N in the PM2 or CM2 treatments significantly reduced M_{N-T} compared to PM1 or CM1 ($P < 0.01$; Table 3). The lower N_2O emissions in the PM2 and CM2 treatments were therefore likely due to the decreased mineral N supply for nitrifiers and denitrifiers. However, due to uncertainties existed in the estimation of M_{N-T}, further study is needed to confirm our finding using the ^{15}N tracer technique.

4.3. N_2O Emissions From the Cultivated Black Soil

Based on the empirical model, we evaluated the contribution of cultivated black soil to N_2O emissions from arable soils on the national and global scales (Figure 5). In China, N_2O emission from cultivated black soil was 30 Gg N yr^{-1}. This equaled to 9.7% of 308 Gg N_2O-N yr^{-1} emitted from the cropland treated with inorganic fertilizer of China [*Zhou et al.*, 2014]. United States had the largest area of cultivated black soil in the world, and annual N_2O emission amounted to 177 Gg N, accounting for 24.0–35.5% of the total emission (0.50–0.74 Tg N) from national croplands [*Li et al.*, 1996]. In Canada, N_2O emission rate was 1.13 kg N ha^{-1} yr^{-1} and total annual emission was 24 Gg N estimated in this study (Table S7). These values were close to the results calculated by *Smith et al.* [2004] using the denitrification-decomposition model that N_2O flux varied from 0.75 to 1.69 kg N ha^{-1} yr^{-1}, and total emission from all agricultural black soils of Canada was 27 Gg N_2O-N. The EF was 1.33% for black soil in Russia calculated from our model, which was similar to the value of 1.26% reported by *Romanovskaya* [2008]. As the dominant arable soil type, black soil emitted 43 Gg N_2O-N yr^{-1}, contributing 38.6% of total N_2O emission (111 Gg N yr^{-1}) from all agricultural soils in Russia [*Romanovskaya*, 2008]. Cultivated black soil in the seven countries covered 17.4% of global cropland area (1229 Mha), and N_2O emission from black soil under inorganic fertilizer application was 347 Gg N yr^{-1}, which accounted for 10.4% of the global annual emission of 3345 Gg N from fertilized cropland [*Stehfest and Bouwman*, 2006].

Taking precipitation into account greatly improved our regression model compared with that including only the variable of fertilizer N application rate; however, our estimates might have high uncertainties. First, field measurement data on N_2O emissions were mainly sourced from Canada and United States, and no data in the literature were available from Russia and Ukraine, which might affect the reliability of the model. Second, the national average of fertilizer N application rate and mean annual precipitation in the region of black soil in each country were used due to the absence of detailed site-specific data [*Zheng et al.*, 2004],

which in turn increased the uncertainty of estimated N_2O emissions. Third, irrigation besides precipitation was also a major factor influencing soil moisture and then N_2O production in irrigated croplands [*Cai et al.*, 2013]. Last, N_2O emission was greatly affected by the type of crop and fertilizer and tillage practices [*Bouwman et al.*, 2002; *van Kessel et al.*, 2013]. In the future, the above factors should be included in the model to obtain more credible estimate as more field measurement data are available.

5. Conclusions

Using field experiments, laboratory incubations, and global analysis, we investigated N_2O emission from cultivated black soil and its dependence on climatic factors, soil conditions, and fertilization. The EF of inorganic fertilizer and annual N_2O emission from the cultivated black soil in Northeast China was generally lower than the national level of Chinese cropland, and that from the cultivated black soil in North and South America based on the compiled global data. The ^{15}N tracing experiment showed that N_2O emission under the field moisture regime was mainly derived from nitrification. Denitrification was limited by low WFPS and low LOC. Thus, combined application of inorganic fertilizer and manure significantly increased N_2O emissions in the field. This stimulation effect depended on the type and application rate of manure, and should be taken into consideration when evaluating C sequestration strategy of organic fertilizer. Heterotrophic nitrification was identified as an important source of N_2O production, which broadened our knowledge of this process in cropland ecosystems. Based on the empirical model relating N_2O emissions to precipitation and fertilizer N rate, N_2O emission from global-cultivated black soil under inorganic fertilizer N application was estimated as 347 Gg N yr^{-1}. This was the first estimation of N_2O emission from global black soil; however, large uncertainty existed in this estimate and further research is needed.

References

Adviento-Borbe, M. A. A., M. L. Haddix, D. L. Binder, D. T. Walters, and A. Dobermann (2007), Soil greenhouse gas fluxes and global warming potential in four high-yielding maize systems, *Global Change Biol.*, *13*(9), 1972–1988, doi:10.1111/j.1365-2486.2007.01421.x.

Aguilera, E., L. Lassaletta, A. Sanz-Cobena, J. Garnier, and A. Vallejo (2013), The potential of organic fertilizers and water management to reduce N_2O emissions in Mediterranean climate cropping systems, A review, *Agri. Ecosyst. Environ.*, *164*, 32–52, doi:10.1016/j.agee.2012.09.006.

Akiyama, H., and H. Tsuruta (2003), Effect of organic matter application on N_2O, NO, and NO_2 fluxes from an Andisol field, *Global Biogeochem. Cycles*, *17*(4), 1100, doi:10.1029/2002GB002016.

Alluvione, F., C. Bertora, L. Zavattaro, and C. Grignani (2010), Nitrous oxide and carbon dioxide emissions following green manure and compost fertilization in corn, *Soil Sci. Soc. Am. J.*, *74*(2), 384–395, doi:10.2136/sssaj2009.0092.

Anderson, I. C., M. Poth, J. Homstead, and D. Burdige (1993), A comparison of NO and N_2O production by the autotrophic nitrifier *Nitrosomonas europaea* and the heterotrophic nitrifier *Alcaligenes faecalis*, *Appl. Environ. Microbiol.*, *59*(11), 3525–3533.

Barton, L., R. Kiese, D. Gatter, K. Butterbach-Bahl, R. Buck, C. Hinz, and D. V. Murphy (2008), Nitrous oxide emissions from a cropped soil in a semi-arid climate, *Global Change Biol.*, *14*(1), 177–192, doi:10.1111/j.1365-2486.2007.01474.x.

Bateman, E. J., and E. M. Baggs (2005), Contributions of nitrification and denitrification to N_2O emissions from soils at different water-filled pore space, *Biol. Fertil. Soils*, *41*(6), 379–388, doi:10.1007/s00374-005-0858-3.

Bouwman, A. F., L. J. M. Boumans, and N. H. Batjes (2002), Emissions of N_2O and NO from fertilized fields: Summary of available measurement data, *Global Biogeochem. Cycles*, *16*(4), 1058, doi:10.1029/2001GB001811.

Bouwman, A. F., E. Stehfest, and C. van Kessel (2010), Nitrous oxide emissions from the nitrogen cycle in arable agriculture: Estimation and mitigation, in *Nitrous Oxide and Climate Change*, edited by K. A. Smith, pp. 85–106, Earthscan Ltd., London, U. K.

Cai, Y. J., W. X. Ding, X. L. Zhang, H. Y. Yu, and L. F. Wang (2010), Contribution of heterotrophic nitrification to nitrous oxide production in a long-term N-fertilized arable black soil, *Commun. Soil Sci. Plant Anal.*, *41*(19), 2264–2278, doi:10.1080/00103624.2010.507833.

Cai, Y. J., W. X. Ding, and J. F. Luo (2013), Nitrous oxide emissions from Chinese maize-wheat rotation systems: A 3-year field measurement, *Atmos. Environ.*, *65*, 112–122, doi:10.1016/j.atmosenv.2012.10.038.

Cayuela, M. L., G. L. Velthof, C. Mondini, T. Sinicco, and J. W. van Groenigen (2010), Nitrous oxide and carbon dioxide emissions during initial decomposition of animal by-products applied as fertilisers to soils, *Geoderma*, *157*(3–4), 235–242, doi:10.1016/j.geoderma.2010.04.026.

Chantigny, M. H., D. E. Pelster, M. H. Perron, P. Rochette, D. A. Angers, L. É. Parent, D. Massé, and N. Ziadi (2013), Nitrous oxide emissions from clayey soils amended with paper sludges and biosolids of separated pig slurry, *J. Environ. Qual.*, *42*(1), 30–39, doi:10.2134/jeq2012.0196.

Chen, H. H., X. C. Li, F. Hu, and W. Shi (2013), Soil nitrous oxide emissions following crop residue addition: A meta-analysis, *Global Change Biol.*, *19*(10), 2956–2964, doi:10.1111/gcb.12274.

Conen, F., K. E. Dobbie, and K. A. Smith (2000), Predicting N_2O emissions from agricultural land through related soil parameters, *Global Change Biol.*, *6*(4), 417–426, doi:10.1046/j.1365-2486.2000.00319.x.

Davidson, E. A. (1991), Fluxes of nitrous oxide and nitric oxide from terrestrial ecosystems, in *Microbial Production and Consumption of Greenhouse Gases: Methane, Nitrogen Oxides and Halomethanes*, edited by J. E. Rogers and W. B. Whitman, pp. 219–236, *Am. Soc. Microbiol.*, Washington, D. C.

Davidson, E. A. (2009), The contribution of manure and fertilizer nitrogen to atmospheric nitrous oxide since 1860, *Nat. Geosci.*, *2*(9), 659–662, doi:10.1038/NGEO608.

Davidson, E. A., D. C. Nepstad, F. Y. Ishida, and P. M. Brando (2008), Effects of an experimental drought and recovery on soil emissions of carbon dioxide, methane, nitrous oxide, and nitric oxide in a moist tropical forest, *Global Change Biol.*, *14*(11), 2582–2590, doi:10.1111/j.1365-2486.2008.01694.x.

Ding, W. X., Y. Cai, Z. C. Cai, K. Yagi, and X. H. Zheng (2007a), Nitrous oxide emissions from an intensively cultivated maize-wheat rotation soil in the North China Plain, *Sci. Total Environ.*, *373*(2–3), 501–511, doi:10.1016/j.scitotenv.2006.12.026.

Ding, W. X., L. Meng, Y. F. Yin, Z. C. Cai, and X. H. Zheng (2007b), CO_2 emission in an intensively cultivated loam as affected by long-term application of organic manure and nitrogen fertilizer, *Soil Biol. Biochem.*, 39(2), 669–679, doi:10.1016/j.soilbio.2006.09.024.

Ding, W. X., H. Y. Yu, Z. C. Cai, F. X. Han, and Z. H. Xu (2010), Responses of soil respiration to N fertilization in a loamy soil under maize cultivation, *Geoderma*, 155(3–4), 381–389, doi:10.1016/j.geoderma.2009.12.023.

Dobbie, K. E., and K. A. Smith (2003), Nitrous oxide emission factors for agricultural soils in Great Britain: The impact of soil water-filled pore space and other controlling variables, *Global Change Biol.*, 9(2), 204–218, doi:10.1046/j.1365-2486.2003.00563.x.

Flavel, T. C., and D. V. Murphy (2006), Carbon and nitrogen mineralization rates after application of organic amendments to soil, *J. Environ. Qual.*, 35(1), 183–193, doi:10.2134/jeq2005.0022.

Garcia-Ruiz, R., and E. M. Baggs (2007), N_2O emission from soil following combined application of fertiliser-N and ground weed residues, *Plant Soil*, 299(1–2), 263–274, doi:10.1007/s11104-007-9382-6.

Garland, G. M., E. Suddick, M. Burger, W. R. Horwath, and J. Six (2011), Direct N_2O emissions following transition from conventional till to no-till in a cover cropped Mediterranean vineyard (*Vitis vinifera*), *Agri. Ecosyst. Environ.*, 141(1–2), 234–239, doi:10.1016/j.agee.2011.02.017.

Hayakawa, A., H. Akiyama, S. Sudo, and K. Yagi (2009), N_2O and NO emissions from an Andisol field as influenced by pelleted poultry manure, *Soil Biol. Biochem.*, 41(3), 521–529, doi:10.1016/j.soilbio.2008.12.011.

Hayatsu, M., K. Tago, and M. Saito (2008), Various players in the nitrogen cycle: Diversity and functions of the microorganisms involved in nitrification and denitrification, *Soil Sci. Plant Nutr.*, 54(1), 33–45, doi:10.1111/j.1747-0765.2007.00195.x.

Henriksen, T. M., and T. A. Breland (2002), Carbon mineralization, fungal and bacterial growth, and enzyme activities as affected by contact between crop residues and soil, *Biol. Fertil. Soils*, 35(1), 41–48, doi:10.1007/s00374-001-0438-0.

Herrmann, A. M., and E. Witter (2008), Predictors of gross N mineralization and immobilization during decomposition of stabilized organic matter in agricultural soil, *Eur. J. Soil Sci.*, 59(4), 653–664, doi:10.1111/j.1365-2389.2008.01023.x.

Intergovernmental Panel on Climate Change (2006), N_2O emissions from managed soils and CO_2 emissions from lime and urea application, in *IPCC Guidelines for National Greenhouse Gas Inventories 4*, edited by H. S. Eggleston et al., Hayama, Japan.

Islam, A., D. Chen, and R. E. White (2007), Heterotrophic and autotrophic nitrification in two acid pasture soils, *Soil Biol. Biochem.*, 39(4), 972–975, doi:10.1016/j.soilbio.2006.11.003.

Jiang, G. Y., et al. (2014), Soil organic carbon sequestration in upland soils of northern China under variable fertilizer management and climate change scenarios, *Global Biogeochem. Cycles*, 28, 319–333, doi:10.1002/2013GB004746.

Khalil, M. I., and E. M. Baggs (2005), CH_4 oxidation and N_2O emissions at varied soil water-filled pore spaces and headspace CH_4 concentrations, *Soil Biol. Biochem.*, 37(10), 1785–1794, doi:10.1016/j.soilbio.2005.02.012.

Kool, D. M., J. Dolfing, N. Wrage, and J. W. van Groenigen (2011), Nitrifier denitrification as a distinct and significant source of nitrous oxide from soil, *Soil Biol. Biochem.*, 43(1), 174–178, doi:10.1016/j.soilbio.2010.09.030.

Lal, R. (2004), Soil carbon sequestration impacts on global climate change and food security, *Science*, 304(5677), 1623–1627, doi:10.1126/science.1097396.

Li, C. S., V. Narayanan, and R. C. Harriss (1996), Model estimates of nitrous oxide emissions from agricultural lands in the United States, *Global Biogeochem. Cycles*, 10(2), 297–306, doi:10.1029/96GB00470.

Li, C. S., S. Frolking, and K. Butterbach-Bahl (2005), Carbon sequestration in arable soils is likely to increase nitrous oxide emissions, offsetting reductions in climate radiative forcing, *Clim. Change*, 72(3), 321–338, doi:10.1007/s10584-005-6791-5.

Linquist, B., K. J. van Groenigen, M. A. Adviento-Borbe, C. Pittelkow, and C. van Kessel (2012), An agronomic assessment of greenhouse gas emissions from major cereal crops, *Global Change Biol.*, 18(1), 194–209, doi:10.1111/j.1365-2486.2011.02502.x.

Liu, X. B., C. L. Burras, Y. S. Kravchenko, A. Duran, T. Huffman, H. Morras, G. Studdert, X. Y. Zhang, R. M. Cruse, and X. H. Yuan (2012), Overview of Mollisols in the world: Distribution, land use and management, *Can. J. Soil Sci.*, 92(3), 383–402, doi:10.4141/CJSS2010-058.

López-Fernández, S., J. A. Díez, P. Hernáiz, A. Arce, L. García-Torres, and A. Vallejo (2007), Effects of fertiliser type and the presence or absence of plants on nitrous oxide emissions from irrigated soils, *Nutr. Cycl. Agroecosyst.*, 78(3), 279–289, doi:10.1007/s10705-007-9091-9.

Lou, Y. S., Z. P. Li, T. L. Zhang, and Y. C. Liang (2004), CO_2 emissions from subtropical arable soils of China, *Soil Biol. Biochem.*, 36(11), 1835–1842, doi:10.1016/j.soilbio.2004.05.006.

Ma, B. L., T. Y. Wu, N. Tremblay, W. Deen, M. J. Morrison, N. B. McLaughlin, E. G. Gregorich, and G. Stewart (2010), Nitrous oxide fluxes from corn fields: On-farm assessment of the amount and timing of nitrogen fertilizer, *Global Change Biol.*, 16(1), 156–170, doi:10.1111/j.1365-2486.2009.01932.x.

McLain, J. E. T., and D. A. Martens (2006), N_2O production by heterotrophic N transformations in a semiarid soil, *Appl. Soil Ecol.*, 32(2), 253–263, doi:10.1016/j.apsoil.2005.06.005.

Meijide, A., L. García-Torres, A. Arce, and A. Vallejo (2009), Nitrogen oxide emissions affected by organic fertilization in a non-irrigated Mediterranean barley field, *Agri. Ecosyst. Environ.*, 132(1–2), 106–115, doi:10.1016/j.agee.2009.03.005.

Melero, S., M. Panettieri, E. Madejón, H. G. Macpherson, F. Moreno, and J. M. Murillo (2011), Implementation of chiselling and mouldboard ploughing in soil after 8 years of no-till management in SW, Spain: Effect on soil quality, *Soil Till. Res.*, 112(2), 107–113, doi:10.1016/j.still.2010.12.001.

Millar, N., and E. M. Baggs (2004), Chemical composition, or quality, of agroforestry residues influences N_2O emissions after their addition to soil, *Soil Biol. Biochem.*, 36(6), 935–943, doi:10.1016/j.soilbio.2004.02.008.

Miller, M. N., C. E. Dandie, B. J. Zebarth, D. L. Burton, C. Goyer, and J. T. Trevors (2012), Influence of carbon amendments on soil denitrifier abundance in soil microcosms, *Geoderma*, 170, 48–55, doi:10.1016/j.geoderma.2011.11.022.

Mirsky, S. B., L. E. Lanyon, and B. A. Needelman (2008), Evaluating soil management using particulate and chemically labile soil organic matter fractions, *Soil Sci. Soc. Am. J.*, 72(1), 180–185, doi:10.2136/sssaj2005.0279.

Müller, C., T. Rütting, J. Kattge, R. J. Laughlin, and R. J. Stevens (2007), Estimation of parameters in complex ^{15}N tracing models by Monte Carlo sampling, *Soil Biol. Biochem.*, 39(3), 715–726, doi:10.1016/j.soilbio.2006.09.021.

Müller, C., R. J. Laughlin, P. Christie, and C. J. Watson (2011), Effects of repeated fertilizer and cattle slurry applications over 38 years on N dynamics in a temperate grassland soil, *Soil Biol. Biochem.*, 43(6), 1362–1371, doi:10.1016/j.soilbio.2011.03.014.

Parton, W. J., A. R. Mosier, D. S. Ojima, D. W. Valentine, D. S. Schimel, K. Weier, and A. E. Kulmala (1996), Generalized model for N_2 and N_2O production from nitrification and denitrification, *Global Biogeochem. Cycles*, 10(3), 401–412, doi:10.1029/96GB01455.

Pederson, H., K. A. Dunkin, and M. K. Firestone (1999), The relative importance of autotrophic and heterotrophic nitrification in a conifer forest soil as measured by ^{15}N tracer and pool dilution techniques, *Biogeochemistry*, 44(2), 135–150, doi:10.1023/A:1006059930960.

Pelster, D. E., M. H. Chantigny, P. Rochette, D. A. Angers, C. Rieux, and A. Vanasse (2012), Nitrous oxide emissions respond differently to mineral and organic nitrogen sources in contrasting soil types, *J. Environ. Qual.*, 41(2), 427–435, doi:10.2134/jeq2011.0261.

Qiu, J. J., C. S. Li, L. G. Wang, H. J. Tang, H. Li, and E. van Ranst (2009), Modeling impacts of carbon sequestration on net greenhouse gas emissions from agricultural soils in China, *Global Biogeochem. Cycles*, 23, GB1007, doi:10.1029/2008GB003180.

Robertson, J. B., and P. J. van Soest (1981), The detergent system of analysis and its application to human foods, in *The Analysis of Dietary Fibre in Food*, edited by W. P. Y. James and O. Theander, pp. 123–158, Marcel Dekker, New York.

Rochette, P., E. van Bochove, D. Prévost, D. A. Angers, D. Côté, and N. Bertrand (2000), Soil carbon and nitrogen dynamics following application of pig slurry for the 19th consecutive year: II. Nitrous oxide fluxes and mineral nitrogen, *Soil Sci. Soc. Am. J.*, 64(4), 1396–1403, doi:10.2136/sssaj2000.6441396x.

Romanovskaya, A. A. (2008), Methane and nitrous oxide emissions in the agricultural sector of Russia, *Russ. Meteorol. Hydrol.*, 33(2), 117–124, doi:10.3103/S106837390802009X.

Rütting, T., T. J. Clough, C. Müller, M. Lieffering, and P. C. D. Newton (2010), Ten years of elevated atmospheric carbon dioxide alters soil nitrogen transformations in a sheep-grazed pasture, *Global Change Biol.*, 16(9), 2530–2542, doi:10.1111/j.1365-2486.2009.02089.x.

Sanchez-Martin, L., A. Sanz-Cobena, A. Meijide, M. Quemada, and A. Vallejo (2010), The importance of the fallow period for N_2O and CH_4 fluxes and nitrate leaching in a Mediterranean irrigated agroecosystem, *Eur. J. Soil Sci.*, 61(5), 710–720, doi:10.1111/j.1365-2389.2010.01278.x.

Serramiá, N., A. Roig, and M. A. Sánchez-Monedero (2012), Soil mineralization of two-phase olive mill wastes: Effect of the lignocellulosic composition on soil C dynamics, *J. Environ. Monit.*, 14(2), 499–509, doi:10.1039/c1em10490j.

Smith, W. N., B. Grant, R. L. Desjardins, R. Lemke, and C. Li (2004), Estimates of the interannual variations of N_2O emissions from agricultural soils in Canada, *Nutr. Cycl. Agroecosyst.*, 68(1), 37–45, doi:10.1023/B:FRES.0000012230.40684.c2.

Stange, C. F., O. Spott, H. Arriaga, S. Ménendez, J. M. Estavillo, and P. Merino (2013), Use of the inverse abundance approach to identify the sources of NO and N_2O release from Spanish forest soils under oxic and hypoxic conditions, *Soil Biol. Biochem.*, 57, 451–458, doi:10.1016/j.soilbio.2012.10.006.

Stehfest, E., and L. Bouwman (2006), N_2O and NO emission from agricultural fields and soils under natural vegetation: Summarizing available measurement data and modeling of global annual emissions, *Nutr. Cycl. Agroecosyst.*, 74(3), 207–228, doi:10.1007/s10705-006-9000-7.

Strickland, M. S., and J. Rousk (2010), Considering fungal:Bacterial dominance in soils—Methods, controls, and ecosystem implications, *Soil Biol. Biochem.*, 42(9), 1385–1395, doi:10.1016/j.soilbio.2010.05.007.

United Nations Environment Programme (UNEP) (2013), Drawing down N_2O to protect climate and the ozone layer. A UNEP synthesis report, United Nations Environment Programme (UNEP), Nairobi, Kenya. [Available at http://www.unep.org/pdf/UNEPN2Oreport.pdf.]

Vallejo, A., U. M. Skiba, L. García-Torres, A. Arce, S. López-Fernández, and L. Sánchez-Martín (2006), Nitrogen oxides emission from soils bearing a potato crop as influenced by fertilization with treated pig slurries and composts, *Soil Biol. Biochem.*, 38(9), 2782–2793, doi:10.1016/j.soilbio.2006.04.040.

van Groenigen, J. W., G. J. Kasper, G. L. Velthof, A. van den Pol-van Dasselaar, and P. J. Kuikman (2004), Nitrous oxide emissions from silage maize fields under different mineral nitrogen fertilizer and slurry applications, *Plant Soil*, 263(1–2), 101–111, doi:10.1023/B:PLSO.0000047729.43185.46.

van Kessel, C., R. Venterea, J. Six, M. A. Adviento-Borbe, B. Linquist, and K. J. van Groenigen (2013), Climate, duration, and N placement determine N_2O emissions in reduced tillage systems: A meta-analysis, *Global Change Biol.*, 19(1), 33–44, doi:10.1111/j.1365-2486.2012.02779.x.

van Soest, P. J., J. B. Robertson, and B. A. Lewis (1991), Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition, *J. Dairy Sci.*, 74(10), 3583–3597, doi:10.3168/jds.S0022-0302(91)78551-2.

Vasconcelos, S. S., et al. (2004), Moisture and substrate availability constrain soil trace gas fluxes in an eastern Amazonian regrowth forest, *Global Biogeochem. Cycles*, 18, GB2009, doi:10.1029/2003GB002210.

Velthof, G. L., P. J. Kuikman, and O. Oenema (2003), Nitrous oxide emission from animal manures applied to soil under controlled conditions, *Biol. Fertil. Soils*, 37(4), 221–230, doi:10.1007/s00374-003-0589-2.

Walela, C., H. Daniel, B. Wilson, P. Lockwood, A. Cowie, and S. Harden (2014), The initial lignin:nitrogen ratio of litter from above and below ground sources strongly and negatively influenced decay rates of slowly decomposing litter carbon pools, *Soil Biol. Biochem.*, 77, 268–275, doi:10.1016/j.soilbio.2014.06.013.

Wang, J. Y., Z. Z. Chen, Y. C. Ma, L. Y. Sun, Z. Q. Xiong, Q. W. Huang, and Q. R. Sheng (2013), Methane and nitrous oxide emissions as affected by organic-inorganic mixed fertilizer from a rice paddy in southeast China, *J. Soils Sediments*, 13(8), 1408–1417, doi:10.1007/s11368-013-0731-1.

Wang, W. J., R. C. Dalal, S. H. Reeves, K. Butterbach-Bahl, and R. Kiese (2011), Greenhouse gas fluxes from an Australian subtropical cropland under long-term contrasting management regimes, *Global Change Biol.*, 17(10), 3089–3101, doi:10.1111/j.1365-2486.2011.02458.x.

Wrage, N., G. L. Velthof, M. L. van Beusichem, and O. Oenema (2001), Role of nitrifier denitrification in the production of nitrous oxide, *Soil Biol. Biochem.*, 33(12–13), 1723–1732, doi:10.1016/S0038-0717(01)00096-7.

Xie, Z. B., J. G. Zhu, G. Liu, G. Cadisch, T. Hasegawa, C. M. Chen, H. F. Sun, H. Y. Tang, and Q. Zeng (2007), Soil organic carbon stocks in China and changes from 1980s to 2000s, *Global Change Biol.*, 13(9), 1989–2007, doi:10.1111/j.1365-2486.2007.01409.x.

Xing, G. X. (1998), N_2O emission from cropland in China, *Nutr. Cycl. Agroecosyst.*, 52(2–3), 249–254, doi:10.1023/A:1009776008840.

Zhang, J. B., Z. C. Cai, Y. Cheng, and T. B. Zhu (2009), Denitrification and total nitrogen gas production from forest soils of Eastern China, *Soil Biol. Biochem.*, 41(12), 2551–2557, doi:10.1016/j.soilbio.2009.09.016.

Zhang, J. B., T. B. Zhu, T. Z. Meng, Y. C. Zhang, J. J. Yang, W. Y. Yang, C. Müller, and Z. C. Cai (2013), Agricultural land use affects nitrate production and conservation in humid subtropical soils in China, *Soil Biol. Biochem.*, 62, 107–114, doi:10.1016/j.soilbio.2013.03.006.

Zhang, J. B., W. J. Sun, W. H. Zhong, and Z. C. Cai (2014), The substrate is an important factor in controlling the significance of heterotrophic nitrification in acidic forest soils, *Soil Biol. Biochem.*, 76, 143–148, doi:10.1016/j.soilbio.2014.05.001.

Zheng, X. H., S. H. Han, Y. Huang, Y. S. Wang, and M. X. Wang (2004), Re-quantifying the emission factors based on field measurements and estimating the direct N_2O emission from Chinese croplands, *Global Biogeochem. Cycles*, 18, GB2018, doi:10.1029/2003GB002167.

Zhou, F., et al. (2014), A new high-resolution N_2O emission inventory for China in 2008, *Environ. Sci. Technol.*, 48(15), 8538–8547, doi:10.1021/es5018027.

Zhu, X., M. Burger, T. A. Doane, and W. R. Horwath (2013), Ammonia oxidation pathways and nitrifier denitrification are significant sources of N_2O and NO under low oxygen availability, *Proc. Natl. Acad. Sci. U.S.A.*, 110(16), 6328–6333, doi:10.1073/pnas.1219993110.

Zibilske, L. M., and J. M. Bradford (2007), Oxygen effects on carbon, polyphenols, and nitrogen mineralization potential in soil, *Soil Sci. Soc. Am. J.*, 71(1), 133–139, doi:10.2136/sssaj2006.0167.

Zou, J. W., Y. Huang, X. H. Zheng, and Y. S. Wang (2007), Quantifying direct N_2O emissions in paddy fields during rice growing season in mainland China: Dependence on water regime, *Atmos. Environ.*, 41(37), 8030–8042, doi:10.1016/j.atmosenv.2007.06.049.