Biol Fertil Soils (2013) 49:153–163
DOI 10.1007/s00374-012-0711-4

ORIGINAL PAPER

Effects of organic material amendment and water content on NO, N_2O, and N_2 emissions in a nitrate-rich vegetable soil

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Received: 13 January 2012 / Revised: 21 May 2012 / Accepted: 23 May 2012 / Published online: 5 June 2012

Abstract Amending vegetable soils with organic materials is increasingly recommended as an agroecosystems management option to improve soil quality. However, the amounts of NO, N_2O, and N_2 emissions from vegetable soils treated with organic materials and frequent irrigation are not known. In laboratory-based experiments, soil from a NO_3^--rich (340 mg N kg^{-1}) vegetable field was incubated at 30°C for 30 days, with and without 10 % C_2H_2, at 50, 70, or 90 % water-holding capacity (WHC) and was amended at 1.19 gC kg^{-1} (equivalent to 2.5 tC ha^{-1}) as Chinese milk vetch (CMV), ryegrass (RG), or wheat straw (WS); a soil not amended with organic material was used as a control (CK). At 50 % WHC, cumulative N_2 production (398–524 μg N kg^{-1}) was significantly higher than N_2O (84.6–190 μg N kg^{-1}) and NO (196–224 μg N kg^{-1}) production, suggesting the occurrence of denitrification under unsaturated conditions. Organic materials and soil water content significantly influenced NO emissions, but the effect was relatively weak since the cumulative NO production ranged from 124 to 261 μg N kg^{-1}. At 50–90 % WHC, the added organic materials did not affect the accumulated NO_3^- in vegetable soil but enhanced N_2O emissions, and the effect was greater by increasing soil water content. At 90 % WHC, N_2O production reached 13,645–45,224 μg N kg^{-1} from soil and could be ranked as RG>CMV>WS>CK. These results suggest the importance of preventing excess water in soil while simultaneously taking into account the quality of organic materials applied to vegetable soils.

Keywords Vegetable soil · Organic material · Water content · Nitrate · N_2O · NO

Introduction

Agricultural activities have altered the global nitrogen (N) cycle stimulating the production of N, such as nitric oxide (NO), nitrous oxide (N_2O), and N_2 gas. Nitric oxide plays a critical role in the chemistry of the lower troposphere, since with organic radical species regulates both the photochemical formation of O_3 and the production of photochemical smog with effects on acid rain (Hutchinson and Davidson 1993). Nitrous oxide is a greenhouse gas being much more effective in the absorption of infrared radiation than carbon dioxide (CO_2) on a molecular basis (Bouwman 1990).

In China, the area devoted to vegetable crops has risen from 3.33 million ha in 1976 to 24.8 million ha in 2006, accounting for 14.5 % of the total cropland in China (FAO 2011). Vegetable cultivation in China involves high N application rates, multiple croppings, and irrigations. The N application rates to intensive vegetable cultivation fields have been as high as 1782 kg N ha^{-1} year^{-1} in the Shandong province (Ju et al. 2006) and 1,340 kg N ha^{-1} year^{-1} in the Jiangsu province (Xiong et al. 2006), which are three to four times the levels used for nonvegetable crops in fields where two crops are grown per year (Zheng et al. 2004). High amounts of N fertilizer applied to vegetable fields result in soil nitrate (NO_3^-) accumulation, which have stimulated, and will most likely continue to stimulate, high levels of N_2O, and NO emissions (Xiong et al. 2006; Li and Wang 2007; Pang et al. 2009). Both N_2O and NO emissions from

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intensively cultivated vegetable fields are higher than those from other agricultural soils, i.e., upland, orchard, and forest (Meng et al. 2005; Lin et al. 2010).

Nitrous oxide and NO are mainly produced in soils by two microbial processes, nitrification (the oxidation of NH_4^+ to NO_2^-, or NO_3^-) and denitrification (the anaerobic reduction of NO_2^- or NO_3^- to gaseous NO, N_2O, or N_2) (Freney 1997). The magnitude of NO and N_2O emissions is affected by several environmental factors regulating soil N transformations. Among these factors, soil N supply, available organic carbon (C), and soil water content are regarded as the major controllers (Tiedje 1988; Firestone and Davidson 1989; Weier et al. 1993; Huang et al. 2004; Ciarlo et al. 2007; Miller et al. 2008; Jäger et al. 2011). Generally, NO and N_2O emissions increase as N fertilization rates increase due to increasing soil N availability (Xiong et al. 2006; Meng et al. 2005; Pang et al. 2009). Higher soil water content restricts the O_2 diffusion into the soil, thus increasing N_2O emissions through denitrification (Wolf and Russow 2000; Jäger et al. 2011) but reducing NO production and diffusion (Firestone and Davidson 1989; Bollmann and Conrad 1997). Available organic C also affects the relative availability of soil reductants and oxidants and changes the ratio of N_2O/N_2 (Firestone and Davidson 1989). On the other hand, available organic C stimulates heterotrophic microbial activity, which creates anaerobic conditions in soil and subsequently affects the sources of NO, N_2O, and N_2 (Weier et al. 1993; Baggs et al. 2000).

Recently, crop residues have been increasingly applied to vegetable fields as a practical method to provide plant nutrients and to improve soil quality (Wyland et al. 1995). The C to N (C/N) ratio of organic materials is often the best indicator of the availability of C (Huang et al. 2004), although it does not absolutely explain the differences in organic material decomposition rates (Millar and Baggs 2004). Considering the excessive NO_3^- contents in vegetable soil, available organic C from organic material can markedly increase denitrification rates, especially when there is an ample supply of soil water. However, higher denitrification rates do not necessarily result in higher N_2O evolution, since enhanced denitrification may also alter the N_2O/N_2 ratio. It has been observed that this ratio is affected not only by quality and quantity of organic materials but also by the NO_3^- and water content (Weier et al. 1993; Huang et al. 2004; Ciarlo et al. 2007; Miller et al. 2008). However, the combined effects of organic materials and water content on the nitrogenous gases (e.g., NO, N_2O, and N_2) evolution from vegetable soils are still not clear.

In this study, Chinese milk vetch (CMV), ryegrass (RG), and wheat straw (WS), the representative local organic plant materials, were chosen as C amendments. An incubation experiment was conducted (1) to investigate the influence of different organic materials on the emissions of NO, N_2O, and N_2 from a NO_3^- accumulated vegetable soil at 50−90% water holding capacity (WHC) and (2) to evaluate ways of reducing NO and N_2O emissions when applying organic materials to vegetable fields with frequent irrigation.

Materials and methods

Site description and sample collection of soil and organic materials

The soil used in this study was collected from a typical vegetable field in Zhongxin village, suburban Yixing (31° 22′ N and 119°57′ E), Jiangsu Province, China. Before vegetable cultivation, the soil (i.e., a typic Hapli-Stagnic Anthrosol) was cropped with rice–wheat cropping systems. The vegetable cultivation lasted 9 years with four to five vegetable crops every year under open-field conditions for 4 years and then alternating open-field conditions from May to November with greenhouse conditions from November to May for 5 years.

Soil samples were collected inside the greenhouse from an area of 6×70 m in May 2010. Five plots (1×1 m) were randomly staked out at 15-m intervals. After harvesting baby bok choy (*Brassica rapa* Pekinensis), two soil cores (5 cm diameter) were sampled from the 0–20 cm layer in each plot and mixed into one composite sample. Fresh soil was sieved (< 2 mm), and stones, living roots, and vegetable residues were removed with tweezers. Soil was immediately stored at 4 °C until the beginning of the incubation experiment. The soil properties were as follows: pH 5.7; total C (TC), 16.4 $g kg^{-1}$; total N (TN), 2.57 $g kg^{-1}$; NH_4^+, 4.25 mg $N kg^{-1}$; NO_3^-, 340 mg $N kg^{-1}$; bulk density, 1.05 $g cm^{-3}$; WHC, 66.0 %; silt, 83.8 %; sand, 11.7 %; and clay, 4.52 %.

The organic materials used in this experiment were CMV (*Astragalus sinicus* L.), RG (*Lolium perenne* L.), and WS (*Triticum aestivum* Linn.), which were collected in Weidu village, suburban Yixing, Jiangsu Province, China. Characteristics of CMV were 449 gC kg^{-1} TC, 29.2 gN kg^{-1} TN, and C/N of 15.4; characteristics of RG were 431 gC kg^{-1} TC, 11.4 gN kg^{-1} TN, and C/N of 37.7; characteristics of WS were 419 gC kg^{-1} TC, 4.67 gN kg^{-1} TN, and C/N of 87.9. Organic materials were chopped into 5-cm segments and oven-dried at 60 °C to a constant weight, then cut and sieved (<2 mm).

Experimental design

The experiment was based on a factorial arrangement of treatments in a completely randomized design with 12 treatments, and each treatment was replicated three times. The factors were three values of soil moisture (50, 70, and 90 % WHC), three types of organic material amendment (CMV, RG, and WS) and one control (CK). Fresh soil (30 g oven-dried) was placed in 250-mL Erlenmeyer flasks and thoroughly mixed with 1.19 gC kg^{-1} dry soil, equivalent to 2.5 tC ha^{-1}; this rate was chosen by considering local agricultural rates. Additional N resulting from CMV, RG, and WS was added into vegetable soil at a rate of 77.4, 31.5, and 13.3 mg Nkg^{-1}, respectively. Distilled water was added as necessary to bring the soil water to 50, 70, or 90 % WHC. All flasks were then capped with plastic film with small holes to allow gas exchange and incubated under laboratory conditions at 30°C for 30 days. During the incubation experiment, the flasks were weighed every 2 days, and water lost by evaporation was replaced to maintain constant soil water. The emission rates of N_2O, NO, and CO_2 were measured on days 1, 3, 5, 7, 10, 14, 18, 22, 27, and 30. After gas sampling, soils from flasks without C_2H_2 were destructively sampled and analyzed for NH_4^+ and NO_3^- contents on days 1, 3, 7, 14, 22, and 30 (three replicates per treatment).

At the above-mentioned sampling times, six flasks of each treatment were sealed with airtight silicone rubber stoppers fitted with butyl rubber septa after the plastic film removal. Silicone sealant was used around the stopper to ensure airtight conditions. The flasks were connected to a multiport vacuum manifold, which allowed 20 flasks to be simultaneously vacuumed and flushed with ambient air (Zhang et al. 2009a, b). The procedure was repeated three times (for 1 min each time). Following equilibration at atmospheric pressure, 28 mL acetylene (C_2H_2, inhibits the reduction of N_2O to N_2) were used to immediately replace an equal amount of headspace air for three flasks of each treatment and pumped several times for an even distribution in the substrata, water, and headspace, whereas the other three flasks of each treatment contained no C_2H_2. According to the method developed by Ciarlo et al. (2007), C_2H_2 was added at the beginning of each measurement rather than during the entire incubation in order to reduce the negative effect of C_2H_2 on soil N transformations, which affect the concentrations of different N form and the emissions of nitrogenous gases (Xu and Inubushi 2005; Zhang et al. 2009a, b). Preliminary tests were performed to determine the most effective method for estimating rates. If C_2H_2 had not been evenly distributed, we would have expected to see increasing rates of N_2O accumulation as the acetylene diffused into the sample. This was not the case since rates were linear throughout the incubation period. Four hours after closing the flask, a 20-mL gas sample was collected through the septum using a 25-mL plastic syringe and stored in pre-evacuated 18.5-mL vials. Gas samples were taken every 20 min during preliminary experiments to determine the appropriate incubation period, and subsequent incubations only required an initial and final gas sampling since there was a steady linear accumulation of N_2O and NO.

Analyses

Soil pH was measured in a 1:2.5 (*w*/*v*) soil/water ratio using a DMP-2 mV/pH detector (Quark Ltd, Nanjing, China). Total organic C of both soil and organic material was determined by wet digestion with H_2SO_4–$K_2Cr_2O_7$ (Bremner 1960). Total N was quantified by semimicro-Kjeldahl digestion using Se, $CuSO_4$, and K_2SO_4 as catalysts (Bremner and Jenkinson 1960). Soil texture was determined by a laser particle characterization analyzer (Beckman Coulter, Brea, CA, USA). Soil NH_4^+ and NO_3^- were extracted with a 2 M KCl solution (1:5*w*/*v*) by shaking soil for 1 h. Extracts were filtered through qualitative filter paper and stored at −20 °C until analysis. Ammonium and NO_3^- concentrations were determined with a continuous flow analyzer (Skalar, Breda, The Netherlands).

Concentrations of N_2O and CO_2 were determined using a Shimadzu, GC14B gas chromatograph (Tokyo, Japan). The NO concentration was measured using the EC 9841T NOx analyzer (ECOTECH, chemiluminescence detector, Australia). Before determination of NO, the gas sample drawn from a flask was diluted to 1,000 mL using highly purified N_2. The amounts of NO, N_2O, and CO_2 released from the soil were corrected for these dissolved gases in water using Bunsen absorption coefficients (Moraghan and Buresh 1977) and for that in the headspace at the start of incubation.

Statistical analyses and calculations

Headspace NO, N_2O, and CO_2 values in the absence of C_2H_2 addition were considered the NO, N_2O, and CO_2 emission rates, whereas the headspace N_2O value in the presence of C_2H_2 addition was considered the N_2O+N_2 emission rate. The N_2 emission was obtained by subtracting the N_2O emission without acetylene from the N_2O emission with acetylene (Ryden et al. 1979). Cumulative emissions of NO, N_2O, N_2, and CO_2 were calculated by summing the amounts over all the sampling intervals during the incubation period. The amount at each sampling interval was calculated by multiplying the daily rate, estimated as the mean value of the two measurements spanning the interval, by the number of days of this interval. The N_2O/N_2 ratio was the ratio of the cumulative N_2O production to the cumulative N_2 production. The net change of inorganic N was calculated as the total amounts of N_2O+NO+N_2+NO_3^-+NH_4^+ at the end of 30-day incubation minus the initial amounts of NO_3^-+NH_4^+ (Zhu et al. 2012)

Simple linear regression was used to evaluate the relationships between CO_2 evolution and N_2O flux. The difference in cumulative productions of NO, N_2O, N_2, and CO_2, the ratios of cumulative N_2O/N_2, and cumulative NO/N_2O and the net change of inorganic N over the 30-day incubation period was evaluated by one-way ANOVA followed by a Tukey multiple-comparison test. Two-way ANOVA followed by a Tukey multiple-comparison test was performed to test the significance of the soil moisture and organic material content on cumulative productions of NO, N_2O, N_2, and CO_2, the ratios of cumulative N_2O/N_2 and cumulative NO/N_2O. Significance was accepted at a probability level of $P<0.05$. All statistical analyses were completed using SAS 9.1 (SAS Institute, Cary, NC, USA).

Results

Nitrous oxide emissions

Nitrous oxide emission rates (0.65–5277 μg Nkg^{-1} day^{-1}) varied greatly among the soils depending on the amendments of organic material and moisture (Fig. 1). At the beginning of the incubation, organic material amendment considerably increased the N_2O emission in the vegetable soil, which was relatively higher in CMV and RG soils than in the WS soil, especially at higher than lower soil moisture values. The N_2O emissions were consistently higher ($P<0.05$) at 90 % WHC than at 70 and 50 % WHC, irrespective of organic material amendments. The peaks of N_2O emission in both CMV and RG soils were observed on days 1–3 at 50–90 % WHC, and the N_2O emission rate in the WS soil reached a maximum on day 7 at 50 % WHC and on day 2 at 70 and 90 % WHC. Nitrous oxide production from the three organic treated soils mostly occurred within 14 days, and as much as 64.2–80.5, 90.4–98.9, and 70.9–88.8 % of the cumulative N_2O production in organic treated soils were emitted in the first 14 day at 50, 70, and 90 % WHC, respectively, while approximately 43.8–85.2 % of the cumulative N_2O production was observed in the CK treatment at the soil moisture of 50–90 % WHC. After 10 days, the difference of N_2O emissions between organic treated soils and CK soil decreased.

Two-way ANOVA revealed the effect of soil moisture ($P<0.001$) and organic material ($P<0.001$) on the cumulative N_2O production over the 30-day incubation, and the interaction between the two factors was also significant ($P<0.001$, Table 1). At 50 % WHC, the cumulative N_2O production was only 84.6 μg Nkg^{-1} in the CK soil but reached 107–190 μg Nkg^{-1} in the organic-treated soils (Table 2). As the soil water content increased from 70 to 90 % WHC, cumulative N_2O production greatly increased from 84.6 to 13,645 μg Nkg^{-1} in the CK soil and from 481–2543 to 33,296–45,224 μg Nkg^{-1} in the organic-treated soils. There were also significant differences in cumulative N_2O production among the organic-treated soils at the same soil moisture value, with higher values in CMV and RG soils than in the WS soil (Table 2).

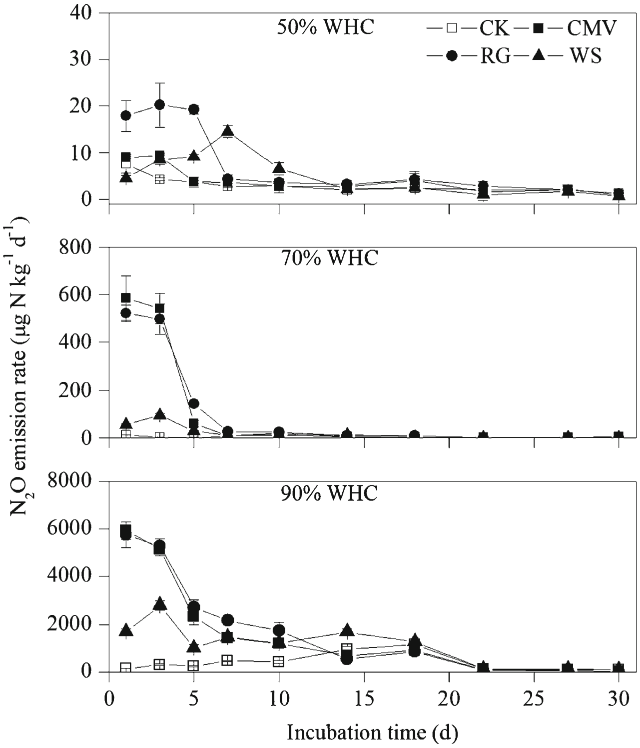

Fig. 1 Nitrous oxide emission rates from the soils amended with different organic materials at 50, 70, and 90 % WHC over a 30-day incubation period. *CK* no input, *CMV* Chinese milk vetch, *RG* ryegrass, *WS* wheat straw

Nitrogen gas emissions

Generally, the pattern of N_2 emissions followed that of N_2O emissions during the incubation period (Fig. 2). Organic amendments immediately enhanced N_2 emissions at the onset of the incubation period, with greater effects at higher than lower soil moisture. At 70 and 90 % WHC, maximum N_2 emissions were found on day 1 in CMV and RG soils and on day 3 in WS soil. In contrast, the peak of N_2 emission in the CK soil was delayed at day 5 at 90 % WHC. Thereafter, the N_2 emission decreased, and the difference in N_2 emission among the four treatments disappeared at longer incubation times. At 90 % WHC, the cumulative N_2 production from the four treated soils ranged from 2,330 to 40,130 μg Nkg^{-1}, which was 67.0–98.7 and 7.38–63.7 times higher than that at 50 % WHC and 70 % WHC, respectively. Organic amendment increased the cumulative N_2 production as compared with that from the CK soil, regardless of soil water content (Table 2). Similar to N_2O, the repeated measures ANOVA revealed the significant effect of organic material ($P<0.001$) and soil moisture ($P<0.001$) on the cumulative N_2 production, and there was a significant interaction effect between the two factors ($P<0.001$, Table 1). As the soil water content increased, the ratio of cumulative N_2O to N_2 also increased (Table 2), with higher values (0.59–1.31) at 90 % WHC than at 70 %

Table 1 Statistical analysis of effect of main factors and their interaction on average emissions and the ratios of indicated gases after 30 days of incubation

Factor	*df*	N_2O	NO	CO_2	N_2	N_2O/N_2	NO/N_2O
Soil water content (W)	2	***	***	***	***	***	***
Organic material (O)	3	***	*	***	***	***	***
Soil water content × organic material (W×O)	6	***	*	**	***	***	***

$^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$

ns nonsignificant

(0.36–0.84) and at 50 % WHC (0.24–0.37). At the same soil water content, higher ratios of cumulative N_2O/N_2 were observed in CMV and RG soils than in the WS soil (Table 2).

Nitric oxide emissions

Soil moisture ($P<0.001$) and organic material ($P<0.05$) had a significant effect on the cumulative NO production, and the interaction between the two factors was also significant ($P<0.05$, Table 1). The NO emission rates in the four treatments showed small changes throughout the incubation time (Fig. 3) compared to N_2O emission rates; the NO emission rates ranged from 4.30 to 11.0 μg Nkg^{-1} day^{-1} at 50 % WHC, from 4.88 to 11.1 μg Nkg^{-1} day^{-1} at 70 % WHC and from 2.62 to 9.36 μg Nkg^{-1} day^{-1} at 90 % WHC. Over the 30-day incubation period, the cumulative NO productions were the highest at 70 % WHC (211–261 μg Nkg^{-1}) and the lowest at 90 % WHC (124–158 μg Nkg^{-1}, Table 2). Organic amendments did not affect the cumulative NO productions at 50 % WHC ($P>0.05$, Table 1). At 70 % WHC, the highest cumulative NO emission was observed in the CMV-treated soil (261 μg Nkg^{-1}) and at 90 % WHC in the WS-treated soil (158 μg Nkg^{-1}) (Table 2).

At 50 % WHC, the NO/N_2O ratio ranged from 1.07 to 2.33 and declined as soil water content increased (Table 2). At 70 % WHC, the NO/N_2O ratio varied between 0.09 and 0.44 in organic treated soils, and it was 1.70 in the CK soil. At 90 % WHC, the cumulative N_2O productions were significantly higher than NO production in the four soils ($P<0.001$), resulting in very low NO/N_2O ratios (below 0.01).

Carbon dioxide emissions

The pattern of CO_2 evolution from soil was similar to that of N_2O fluxes (Fig. 4). The CO_2 emission rates at soil moistures of 50–90 % WHC were high at the beginning of incubation and decreased with time in all treated soils except CK; the CK soil was characterized by a low and relative constant CO_2 emission rates. Over 60 % of total CO_2 production during the 30-day incubation period was evolved in the first 14 days after the addition of organic residue to soil. The correlation between CO_2 emission rate (CO_{2ET}, in mg C kg^{-1} day^{-1}) and N_2O emission rate (N_2O_{ET}, in μg N kg^{-1} day^{-1}) during the incubation period was expressed by the following relationships: $CO_{2ET}=0.06\ N_2O_{ET}+2.34$ ($R^2=0.33$, $P<0.05$) at 50 % WHC, $CO_{2ET}=2.91\ N_2O_{ET}-92.7$ ($R^2=0.69$, $P<0.001$) at 70 % WHC and $CO_{2ET}=47.6\ N_2O_{ET}-1263$ ($R^2=0.83$, $P<0.001$) at 90 % WHC.

Table 2 Cumulative emissions and ratios of gases in soils amended with different organic materials and at different soil water contents over 30 days of incubation

Soil water content	Organic material treatment	N_2O	NO	N_2	N_2O/N_2	NO/N_2O	CO_2	Net change of inorganic N
		μg Nkg^{-1}	μg Nkg^{-1}	μg Nkg^{-1}			mg Ckg^{-1}	mg Nkg^{-1}
50%WHC	CK	84.6±7.94c	196±7.50a	348±5.67b	0.24±0.02b	2.33±0.19a	644±24.0d	43.1b
	CMV	134±24.4ab	224±19.7a	398±41.2b	0.34±0.04ab	1.67±0.19b	1,372±21.3b	71.7a
	RG	190±38.4a	200±7.12a	524±31.4a	0.37±0.07a	1.07±0.19c	1,453±48.9a	37.6bc
	WS	107±12.1b	201±25.9a	343±13.9b	0.31±0.05ab	1.89±0.24b	1,110±31.1c	28.6c
75%WHC	CK	132±3.85c	224±34.5ab	366±50.6d	0.36±0.04b	1.70±0.24a	925±30.6b	58.6b
	CMV	2,472±267a	261±9.39a	2,991±291b	0.84±0.17a	0.11±0.01c	1,684±49.4a	96.9a
	RG	2,543±80.7a	225±15.0ab	5,434±272a	0.47±0.04b	0.09±0.01c	1,762±57.3a	42.0c
	WS	481±13.7b	211±9.09b	1,265±86.8c	0.38±0.02b	0.44±0.03b	1,618±50.0a	32.6c
90%WHC	CK	13,645±926c	124±12.1b	23,330±954c	0.59±0.06c	0.009±0.001a	986±92.8b	67.3a
	CMV	41,739±1,789a	132±16.0b	32,168±3,280b	1.31±0.18a	0.003±0.000bc	1,720±54.9a	−99.8c
	RG	45,224±1,617a	133±9.40b	40,130±1,840a	1.13±0.09a	0.003±0.000c	1,706±26.8a	−79.2c
	WS	33,296±403b	158±17.8a	39,270±3,705a	0.85±0.08b	0.005±0.001b	1,603±59.6a	17.1b

Different letters within a column and water content indicate significant differences between the means ($P<0.05$). *CK* no-input control, *CMV* Chinese milk vetch, *RG* ryegrass, *WS* wheat straw

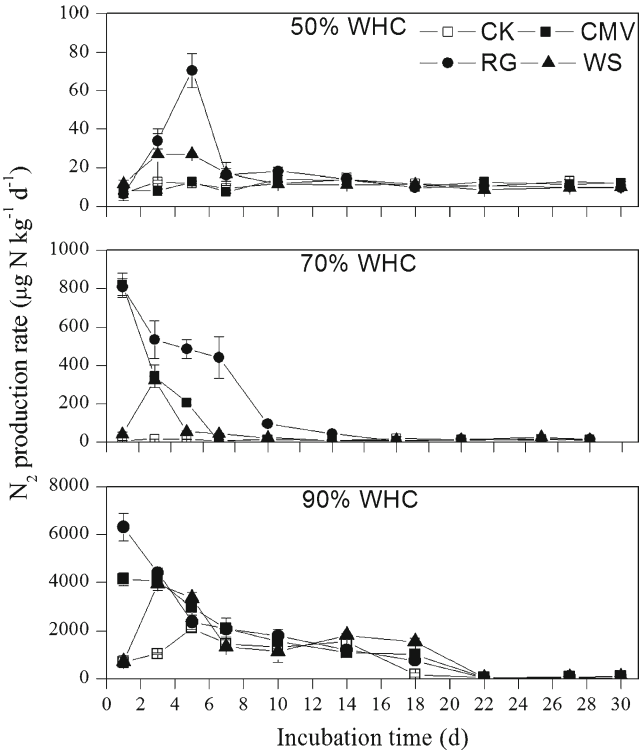

Fig. 2 Nitrogen gas emission rates in soils incorporated with different organic materials at 50, 70, or 90 % WHC over a 30-day incubation period. *CK* no input, *CMV* Chinese milk vetch, *RG* ryegrass, *WS* wheat straw

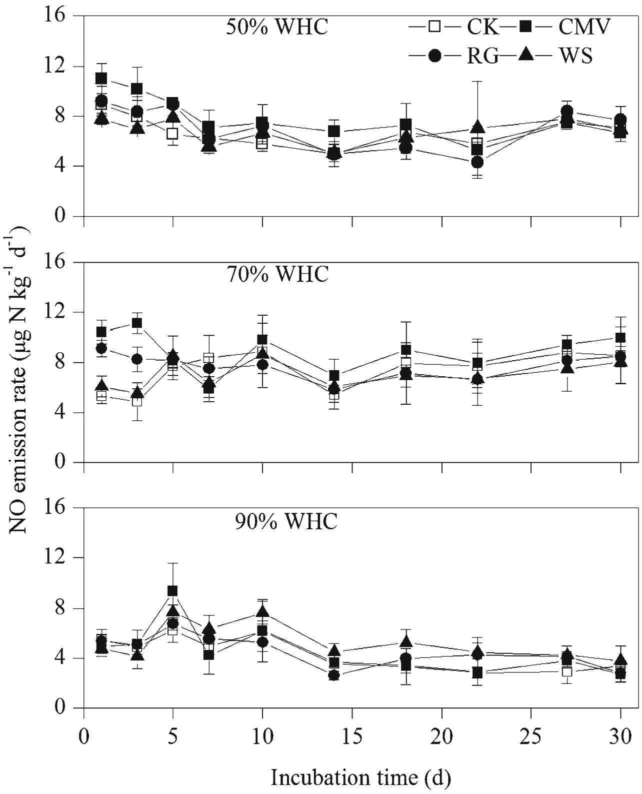

Fig. 3 Nitric oxide emission rates in soils incorporated with different organic materials at 50, 70, or 90 % WHC over a 30-day incubation period. *CK* no input, *CMV* Chinese milk vetch, *RG* ryegrass, *WS* wheat straw

The cumulative CO_2 production was significantly affected by soil moisture ($P<0.001$) and organic amendment ($P<0.001$), and there was a significant interaction effect between the two factors ($P<0.01$, Table 1). Cumulative CO_2 productions for each treatment at 70 and 90 % WHC were comparable and were significantly higher than those at 50 % WHC ($P<0.05$). The addition of organic material significantly increased the cumulative CO_2 production (Table 1). At 50 % WHC, there was a considerable difference among the four treatments ($P<0.05$), with a maximum CO_2 production of 1,453 mg Ckg^{-1} in the RG soil. At 70 and 90 % WHC, however, no significant differences in the cumulative CO_2 production were found between the three organic-treated soils, which were significantly higher ($P>0.05$) than those in the CK soil. The cumulative CO_2 production in the organic-treated soils accounted for 39.1–68.0 %, 58.2–70.3 %, and 51.8–61.6 % of the added organic material C at 50, 70, and 90 % WHC, respectively.

Mineral N dynamics

At 50 and 70 % WHC, organic amendments immediately decreased the NO_3^- concentration, which then slowly increased after 3–7 days. At the end of the incubation period, the NO_3^- concentration in the CMV soil was higher than that in the CK soil (Fig. 5). At 90 % WHC, NO_3^- concentrations in the organic-treated soils considerably decreased throughout the incubation period, declining more rapidly in CMV and RG soils (from 290 to 170 mg Nkg^{-1}) than in the WS soil (from 329 to 287 mg Nkg^{-1}); meanwhile, it increased from 336 to 371 mg Nkg^{-1} in the CK soil. In contrast to NO_3^- concentrations, soil NH_4^+ concentrations in all treatments were below 10 mg Nkg^{-1} (Fig. 5). Soil moisture did not affect soil NH_4^+ concentrations ($P>0.05$), which remained relatively constant in WS and CK soils, peaked after 3 days in the CMV soil and after 7–14 days in the RG soil. At the end of the incubation, there was no significant difference in NH_4^+ concentrations among all treatments.

Organic amendment and soil moisture significantly affected the net changes of inorganic N (Table 2). The net changes of inorganic N for each treatment at 50 and 70 % WHC were comparable ($P<0.05$). The net changes of inorganic N ranged from 28.6 to 71.7 mg Nkg^{-1} at 50 % WHC and from 32.6 to 96.9 mg Nkg^{-1} at 70 % WHC, with the highest values in the CMV soil than other three treated soils. As the soil moisture increased to 90 % WHC, net changes of inorganic N in the CK soil reached 67.3 mg Nkg^{-1}, while they considerably decreased in organic-treated soils, with

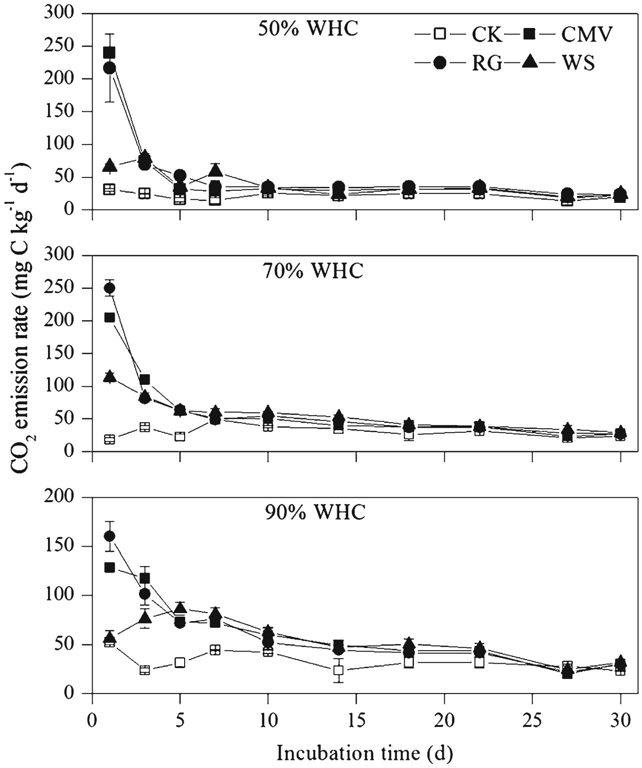

Fig. 4 Carbon dioxide emission rates in soils incorporated with different organic materials at 50, 70 or 90 % WHC over a 30-day incubation period. *CK* no input, *CMV* Chinese milk vetch, *RG* ryegrass *WS* **wheat straw**

lower values in CMV (−99.8 mg Nkg^{-1}) and RG (−79.2 mg Nkg^{-1}) soils than in the WS (17.1 mg Nkg^{-1}) soil (Table 2).

Discussion

Acetylene inhibition technique

The C_2H_2 inhibition technique is a widely used method to measure N_2 production in soil based on the inhibition of the N_2O reductase with high concentrations of C_2H_2 (about 10 %). Generally, the N_2 production is calculated by the difference between the N_2O production with and without C_2H_2, while NO production is frequently neglected and not determined during measurement of denitrification (Weier et al. 1993; Cheng et al. 2004a; Ciarlo et al. 2007). The presence of C_2H_2 at high concentrations not only inhibits the reduction of N_2O to N_2 but also affects the evolution of NO to N_2O in soil. Hence, N_2 production should be considered as the differences in productions of NO and N_2O with and without C_2H_2. Preliminary tests in this study, however, showed a significant relationship between NO production and the added amount of C_2H_2 (P=0.02) (unpublished data), suggesting that C_2H_2 can strongly interfere with chemiluminescent analysis of NO determined using the EC 9841T NO_x analyzer. Additionally, Bollmann and Conrad (1997) found that NO could be converted to NO_2 and NO_3^- in the presence of C_2H_2 and O_2 and subsequently resulted in decreased N_2O production. Due to these problems, the N_2 production can only be calculated using the method by Ryden et al. (1979), which probably underestimates the N_2 production. With the increase in soil denitrification rate, the underestimation of the N_2 production may be exaggerated and subsequently affect the accurate estimate of N losses. In addition, negative effects of C_2H_2 on soil N transformation have been reported by Xu and Inubushi (2005) and Zhang et al. (2009a, b). At the end of 30-day incubation, the net changes of inorganic N in four treated soils ranged from 28.6 to 96.9 mg Nkg^{-1} at 50−70 % WHC, suggesting the occurrence of net mineralization of organic N under aerobic conditions. Our earlier study showed that heterotrophic nitrification was the main process producing NO_3^- in the vegetable soil (Zhu et al. 2011). Probably, high amounts of labile organic N in both soil and organic material induced the increase in NO_3^- concentration over 30-day incubation. As soil moisture increased to 90 % WHC, however, net changes of inorganic N in CMV and RG soils were negative, and the disappeared N was approximately 80 −100 mg Nkg^{-1}. At 90 % WHC, the presence of easily decomposed organic materials, and the accumulated NO_3^- in the vegetable soil increased the denitrification rate, and thus, the underestimation of the N_2 production measured by C_2H_2 inhibition technique may be important and can explain the "missing" N. To accurately evaluate realistic denitrification rates and N_2 emissions in NO_3^--rich soil, other methods (e.g., ^{15}N tracers, direct N_2 quantification) should be used in the future studies.

Production processes of N_2O and NO in soils

The NO/N_2O ratio, N_2 production, and changes in soil NO_3^- concentrations can be used for investigating the N processes responsible for the NO and N_2O production. At 50 % WHC, the NO/N_2O ratios of all treatments ranged from 1.07 to 2.33 (Table 2), suggesting that probably nitrification was the dominant process of NO and N_2O production; indeed, this occurs when the NO/N_2O ratio is higher than 1 (Cheng et al. 2004b; Hayakawa et al. 2009). In addition, soil NO_3^- concentrations of the four treatments increased from 318 to 344 mg Nkg^{-1} on day 1 to 370 to 413 mg Nkg^{-1} on day 30, thus confirming the occurrence of nitrification in soil at 50 % WHC. Nitrogen gas production can be used to estimate the occurrence of denitrification, which is the only biological process converting NO and N_2O into N_2. At 50 % WHC, the N_2 production (398–524 μg Nkg^{-1}) of the four treatments was significantly higher than N_2O (84.6–190 μg Nkg^{-1}) and NO (196–224 μg Nkg^{-1}) production, suggesting the occurrence of denitrification under

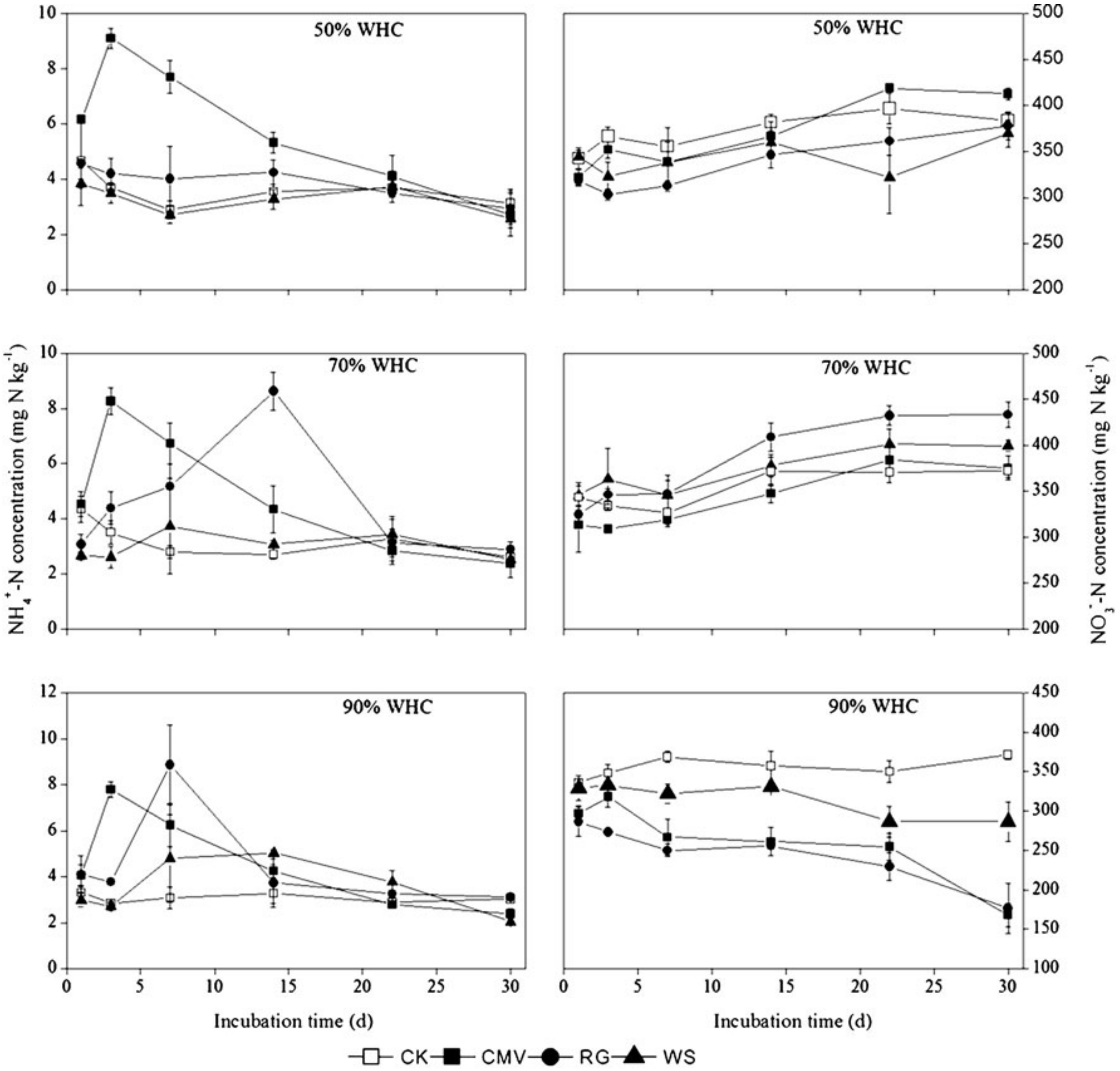

Fig. 5 Change of soil NH_4^+ and NO_3^- concentrations in soils incorporated with different organic materials at 50, 70, or 90 % WHC over a 30-day incubation period. *CK* no input, *CMV* Chinese milk vetch, *RG* ryegrass, *WS* wheat straw

unsaturated conditions. Using a ^{15}N tracing technique, Zhu et al. (2011) found that as much as 23–58 % of the N_2O evolved from adjacent four vegetable soils (cultivated from 0.5 to 10 years) was produced by denitrification at 50 % WHC. Therefore, the denitrification is important in producing N_2O and should be considered when evaluating N losses in vegetable soils under aerobic conditions.

At 70 and 90 % WHC, all soils, except the CK soil at 70 % WHC, produced greater N_2O than NO emissions, with NO/N_2O ratios of 0.09–0.44 at 70 % WHC and lower than 0.01 at 90 % WHC (Table 2). Probably, the soil became anoxic with organic amendments. At 90 % WHC, soil NO_3^- concentrations in the organic-treated soils decreased throughout (Fig. 5), while N_2 production was higher than that at 70 and 50 % WHC (Table 2). Probably denitrification was the dominant process for N_2O production at higher than lower soil water contents, confirming previous reports (Khalil et al. 2002; Liu et al. 2007; Ciarlo et al. 2007). However, the fact that soil NO_3^- contents increased in all treated soils at 70 % WHC and in the CK soil at 90 % WHC at the end of incubation period suggests that not only denitrification but also nitrification contributed to N_2O production. Other reports have also showed that both nitrification and denitrification can simultaneously occur, and the contributions of the two processes to NO and N_2O emissions mainly depends on soil water content and the amendment of soil with organic material (Wolf and Russow 2000; Cai et al. 2001; Zhu et al. 2011).

Influence of soil water content on NO and N_2O emissions in the vegetable soil

Soil water content affected both N_2O and N_2 emissions (Fig. 1 and Table 1). According to the theoretical model developed by Davidson (1991) and Potter et al. (1996), the maximum N_2O release rate was observed at 70−80% water-filled porosity (WFPS). However, we have found the highest cumulative N_2O production during the 30-day incubation at 90 % WHC (equivalent to 100 % WFPS) (Table 2).

Although caution is required in comparing studies because of different soils, methods, and environmental parameters, it is possible that the discrepancy between our study and previous reports was primarily due to differences in soil mineral N content. The NO_3^- concentration (340 mg N kg^{-1}) of our soil was significant higher than the NH_4^+ concentration (4.25 mg N kg^{-1}). Probably, at 50 % WHC, N_2O release from soil was limited by the NH_4^+ content, which remained below 10 mg N kg^{-1} during the 30-day incubation period (Fig. 5). As soil water content increased, denitrification became the dominant process for N_2O emission, due to the development of anaerobic microsites favoring reducing conditions. The high NO_3^- concentration (340 mg N kg^{-1}) of the vegetable soil favored the rate of denitrification by increasing soil water content. However, the cumulative N_2O/N_2 ratio was the highest at 90 % WHC, and this seems to contradict the bibliography reporting low N_2O release rates, with most of the N_2O being reduced to N_2 at soil moisture levels above field capacity (Davidson 1991; Weier et al. 1993; Potter et al. 1996; Ciarlo et al. 2007). Probably, the high cumulative N_2O/N_2 ratio depended on the high NO_3^- concentration of our soil, which was always above 300 mg N kg^{-1} at 50 % WHC and 70 % WHC during the entire incubation period; at 90 % WHC, NO_3^- concentrations decreased, but they were higher than 150 mg N kg^{-1} (Fig. 5). Such high NO_3^- concentrations have been shown to inhibit N_2O reductase activity during denitrification (Blackmer and Bremner 1978), and N_2O can accumulate when the NO_3^- concentration (>10 mg N kg^{-1}) is greater than the reducing demand of the denitrifiers (Swerts et al. 1996). Therefore, N_2O emissions can increase when the vegetable field is irrigated with large amounts of water due to the accumulation of NO_3^- content.

The NO emissions were less sensitive to soil water content than N_2O emissions (Figs. 1 and 3). The cumulative NO production was highest at 70 % WHC (211–261 μg N kg^{-1}), confirming previous results from field and laboratory studies (Potter et al. 1996; Garrido et al. 2002; Zhou et al. 2010). High soil water contents restrict the diffusion of NO, and the NO produced from soil is partially consumed before it can escape from the soil (Firestone and Davidson 1989). Although the cumulative NO emissions during the 30-day incubation only accounted for <1 % of the applied organic material N in this study and was significantly less than the cumulative N_2O emissions at higher water contents (Table 2), it is important to monitor NO production because it is a free obligate intermediate and a precursor of N_2O for most denitrifiers (Ye et al. 1994).

Influence of organic materials on NO and N_2O emissions in the vegetable soil

Organic materials incorporated into vegetable soil considerably enhanced N_2O emissions and cumulative N_2O/N_2 ratios (Table 2), confirming that organic materials stimulate N_2O emissions, especially at higher soil water contents (Weier et al. 1993; Baggs et al. 2000; Huang et al. 2004; Miller et al. 2008; Jäger et al. 2011). The relatively high N_2O emission rates may be due to the stimulation of mineralization at 50 % WHC and thus NH_4^+ availability for nitrification or the availability of organic C to the denitrifier at high soil water content (e.g., 70–90 % WHC). The temporal pattern of N_2O evolution from organic amended soils showed a flush in the early incubation period followed by a decline over time (Fig. 1), since as much as 64.2–98.9 % of total N_2O production occurred in the first 14 days. Likewise, Millar and Baggs (2004) found that over 57 % of N_2O was emitted in the first 7 days, and N_2O emissions were reduced to background levels 22 days after the incorporation of organic residues into soil. The rapid N_2O pulses followed by the decline in organic amended soils paralleled the CO_2 pattern; indeed CO_2 and N_2O evolution were correlated, with the strongest correlation at 90 % WHC. Cheng et al. (2004a) also found a significant positive correlation between CO_2 and N_2O productions from 11 agricultural soils incubated for 2 days at 25°C and 80 % WFPS. These results suggest that soil respiration (i.e., CO_2 emission rate) can be used as an indicator for estimating N_2O production. Considering that most of the NO_3^- remained in the vegetable soil even at the highest soil water content (>150 mg N kg^{-1}, Fig. 5) and that CO_2 and N_2O emissions rapidly decreased after 14 days, it is the readily available soil C from organic materials, rather than NO_3^-, the key limiting factor for regulating N_2O emissions in the vegetable field.

Previous researchers have found that the C/N ratio of organic materials can affect NO and N_2O emissions (Kiese and Butterbach-Bahl 2002; Akiyama and Tsuruta 2003; Huang et al. 2004). Akiyama and Tsuruta (2003) proposed a negative linear correlation between the logarithm of cumulative NO emissions from organic amended soils after 30 days and the C/N ratio of the added organic material. Khalil et al. (2002) reported that N_2O production can increase by decreasing the C/N ratio of the organic matter. In our study, however, organic amendments influenced NO and N_2O differently (Table 2). During the 30-day incubation, NO productions from the three organic-treated soils were within the same order of magnitude, but N_2O productions significantly differed (Table 2), with higher evolution from CMV and RG soils than from the WS soil, especially at the highest soil water content. According the CO_2 production, the amount of available C was the lowest for the WS material, which had the highest C/N ratio (87.9) and had greater amounts of structural woody materials, such as lignin, condensed tannins, and terpenes, and lower available N than CMV and RG materials. Therefore, CMV and RG would provide more available organic C than WS with creation of more anaerobic microsites, favoring the denitrification of NO_3^- and N_2O and N_2 production. Huang et al. (2004) investigated the influence of different plant residuals with a wide range of C/N ratios (from 8 to 118) on N_2O emissions and found a negative linear correlation between N_2O production after plant residual amendment and the C/N ratio. In our study, RG with a higher C/N ratio than CMV resulted in higher N_2O and N_2 emissions regardless of soil water content when it was added to soil (Table 2). Our results indicate that the C/N ratio of organic material alone may be insufficient to predict N_2O emissions, and also other properties of the organic amendment such as TN, TC, lignin, or the protein binding capacity of extractable polyphenols can be important (Millar and Baggs 2004).

Conclusion

In this study, organic amendments apparently enhanced the emissions of N_2O and N_2 and the N_2O/N_2 ratio, and stimulatory effects increased as soil water content increased, most likely due to the high NO_3^- concentration in the vegetable soil. However, the effect of organic amendments on NO emission was relatively small, and the largest NO emission was observed at 70 % WHC. Thus, in NO_3^--rich vegetable soils with frequent irrigations, the beneficial effect of organic matter amendment on soil fertility may be compensated by additional N_2O emissions. However, our results are based on a laboratory incubation under controlled conditions and without plant growth and leaching, which makes easy to identify significant effects of organic amendment and soil moisture changes in mineral N and emissions of NO, N_2O, and N_2. It is still a challenge to determine whether these effects are significant under field conditions where variable environmental factors and soil properties can occur.

Acknowledgments This work is founded by Projects of National Natural Science Foundation of China (40830531, 40921061, and 41101209), China Postdoctoral Science Foundation (2012M511779), Natural Science Foundation of Jiangsu Province (BK2010611 and BK20082282), and the Priority Academic Program Development of Jiangsu Higher Education Institutions.

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