Journal of Environmental Sciences Vol.16, No.3, pp.353—357, 2004

ISSN 1001—0742
CN11—2629/X

Article ID: 1001-0742(2004)03-0353-05 **CLC number**: X511 **Document code**: A

Nitrous oxide emission and reduction in a laboratory-incubated paddy soil response to pretreatment of water regime

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Abstract: A laboratory incubation experiment was conducted to investigate nitrous oxide (N_2O) emission and reduction in a paddy soil (Stagnic Anthrosol) response to the pretreatment of water regime. The paddy soil was maintained under either air-dried (sample D) or submerged (sample F) conditions for 110 d before the soil was adjusted into soil moisture of 20%, 40%, 60%, 80% and 100% water holding capacity (WHC) respectively, and then incubated with or without 10% (v/v) acetylene for 138 h at 25℃. At lower soil water content ($\leq$60% WHC), N_2O emission from the sample F was 2.29 times higher than that from the sample D ($P < 0.01$). While, N_2O emission from the sample F was only 29 and 14 percent of that from the sample D at the soil moisture of 80% and 100% WHC, respectively ($P < 0.01$). The maximal N_2O emissions observed at soil moisture of 80% WHC were about 24 and 186 times higher than the minima obtained at the soil moisture of 20% WHC for the sample F and D, respectively. But at the soil moisture of 80% and 100% WHC, N_2O emission from the sample F with acetylene (F + ACE) was comparable to that of the sample D with acetylene (D + ACE). The results showed that the F sample produced N_2O ability in denitrification was similar to the sample D, however, the sample F was in the better reduction of N_2O to N_2 than the sample D even after the soil moisture was adjusted into the same level of 80% or 100% WHC. Therefore, the pretreatment of water regime influenced the strength and product composition of denitrification and N_2O emission from the paddy soil.

Keywords: denitrification; nitrous oxide reduction; acetylene; product composition; paddy soil

Introduction

Nitrous oxide (N_2O) is a greenhouse gas. Increasing atmospheric N_2O concentration may also be detrimental to the stratospheric ozone layer (Crutzen, 1981). Agricultural soil is a major source of N_2O. Most N_2O originates with soil processes, as an intermediate product from microbial nitrification and denitrification (Granli, 1994). N_2O can be reduced to N_2 by the labile enzyme N_2O reductase in denitrification (Granli, 1994; Klemedtsson, 1988). Depending on conditions, the intermediate product can emit from the soil as N_2O or the mixture of N_2O and N_2. The pronounced temporal and spatial variability of denitrification has confounded attempts to make quantitative prediction of gaseous-N loss from agricultural soils (Parsons, 1991). But it is not well known how changing environmental conditions alter the gaseous-N loss from soils. The water regime is a dominant factor influencing gaseous-N transformations in soil denitrification (Cai, 2001). The effects of soil moisture on N_2O emission have been investigated at certain constant water contents (Bandibas, 1994; Stevens, 1997) or at wetting-drying alternates (Mummey, 1994; Cai, 2001) using fresh soil (Stevens, 1997), air-dried soil (Bandibas, 1994) and fresh but air-dried soil to a certain level of water content (Cai, 2001). It has been documented that N_2O emission depends on the history of the water regime (Granli, 1994; Dendooven, 1996). A flush of N_2O emission was observed in irrigated rice fields after floodwater was drained (Cai, 2001). In practice, it is common that rice fields are flooded for certain period during rice growing season, but under well aeration conditions during non-rice growing period.

Whether the N_2O emission from soil was determined in the field *in situ* or in a laboratory, little was considered about the effect of long-term soil water status prior to measurement. Even in a same plot, some are depressions saturated in water and others are hummocks lacked of water perennially. The N_2O emission varied at different sampling sites. The soils were sampled in the same region at different arid or waterlogging season or year. Even if the tested soil water was regulated at the same level, the N_2O emission has also large varieties. It was the antecedent water status that led to the great difference in N_2O emission. The current or immediate soil moisture was considered in most of the existed studies. The antecedent water status was little consideration. Therefore we choose two extreme antecedent water statuses (wetness and air-dryness) to investigate the N_2O emission, the reduction of N_2O to N_2 and further the composition of denitrification products at the same water content tested.

1 Materials and methods

1.1 Pretreatment

A typical paddy soil (Stagnic Anthrosol) used for the experiment was collected from plough layer (about 0—15 cm depth) after wheat harvest from Wuxi, Jiangsu, China (120° 24′E, 31°36′N). The crop rotation of the field was summer rice and winter wheat. The fertilization management for rice

Foundation item: The National Key Basic Research Development Foundation (No. G1999011805) and the Innovative Foundation for Graduate Student of Chinese Academy of Sciences; * Corresponding author

and wheat production in the field and soil properties were described in detail by Cai and Mosier(Cai, 2000).

The fresh paddy soil was air-dried, ground and then passed through a 2 mm sieve. The soil was divided into two portions. One(refers to sample D hereafter) was stored as air-dried for 110 d(the same period as rice-growing season) at room temperature within from 24℃ to 27℃(similar to the seasonally averaged rice soil temperature) and another(refers to sample F hereafter) submerged with distilled water in the same period and then dried quickly using forcing-air flow at the temperature of 25℃ before carrying out the incubation described below. Physico-chemical properties of sample D and F after pretreatment were determined and shown in Table 1.

Table 1 Selected properties of the paddy soil material(top 15 cm layer) after pretreatment

	D[a]	F[a]
pH_{KCl}	4.98	5.80
Organic carbon, g/kg	15.7	15.0
Total N, g/kg	1.39	1.21
KCl-extractable NO_3^--N, mg/kg	11.5	2.05
KCl-extractable NH_4^+-N, mg/kg	8.05	72.6
Water holding capacity, g/kg	518	539
Sand content, g/kg	35	35
Silt content, g/kg	584	584
Clay content, g/kg	381	381

Notes: D[a] and F[a], the soil was kept under air-dried and submerged for 110 d before experiment

1.2 Incubation program

Twenty-five gram of pretreated soil (oven-dry basis) were mixed homogeneously and put into a 150 ml glass jar. The contents of KCl-extractable NH_4^+-N and NO_3^--N of the samples were adjusted to 80 and 20 mg/kg, respectively, at the start of incubation. Nitrogen solution containing the desired amount of NH_4HCO_3-N and KNO_3-N was added by pipetting uniformly over the soil surface. The final soil moisture was 20%, 40%, 60%, 80% and 100% water holding capacity (WHC) after the addition of nitrogen solution, respectively. Immediately after the solution addition, the jar was flushed with compressed standard air (Nanjing Special Gas Factory) for 10 s and then a silicone rubber lids with a septum for gas sampling by syringe was fitted to each jar using NQ-704 silicone adhesive sealant (Nanjing Quri Chemical and Electrical Institute) to form a gas-tight seal. For treatments with acetylene(refers to F + ACE and D + ACE hereafter), 10% of the air(v/v) was removed from the jars and injected with 10%(v/v) acetylene (C_2H_2) following the description of Dendooven *et al*. (Dendooven, 1996). The acetylene was pre-scrubbed through 6 mol/L H_2SO_4 and H_2O to remove acetone and other impurities(Aulakh, 2001). The distribution of C_2H_2 in the jars was promoted by pumping 3 times with a 25 ml syringe (Dendooven, 1996). The headspace air was sampled for N_2O analysis at 3, 6, 12, 18, 30, 42, 66, 90 and 138 h after the addition of nitrogen solution, respectively. Before sampling, the headspace air in the jar was mixed by withdrawing and injecting headspace air three times using a 5 ml gas-tight syringe with a stopcock, and then gas sample of the headspace air of each jar were removed by the syringe. After each sampling, the headspace air was renewed to compensate for consumed oxygen and to avoid N_2O over build-up in the headspace. For renewing headspace gases, jars were opened, re-flushed with the same compressed air for 10 s to remove the produced gases, and then the jars were closed again. For the treatments of F + ACE and D + ACE, C_2H_2 concentration was readjusted by injecting pure C_2H_2 to obtain 10%(v/v) as described above. Water loss through evaporation was negligible in this short period.

At the end of 138 h incubation, the soil samples were immediately extracted by shaking with 2 mol/L KCl solution (1:2 soil-solution ratio) for 1 h on a 250 r/min mechanical shaker at 25℃ followed by filtration (Aulakh, 2001). Filtrates were determined immediately or stored at 4℃ prior to analyzing the concentrations of NO_3^--N and NH_4^+-N by a flow injection analyzer(Skalar, The Netherlands) within one week.

1.3 Nitrous oxide measurement

N_2O was analyzed using a gas chromatograph(Shimadzu GC-14B) equipped with a ^{63}Ni electron capture detector (ECD) operated at 300℃ and a stainless-steel column packed with 80/100 mesh Porapak-Q at 65℃. The injecting port temperature was 100℃. The carrier gas was 5% CH_4 in argon(Ar) gas with a flow rate of 40 ml/min.

N_2O standard gas made in Nanjing Special Gas Factory were used for calibration. The standard gases were calibrated once again by the standard gases supplied by the National Institute for Agro-Environmental Sciences, Tsukuba, Japan. Peak areas were integrated on a Shimadzu Chromatopac C-R8A integrator. Concentration of the N_2O was quantified by comparing the peak area with those of calibration mixture. The cumulative N_2O emission from the paddy soil during the whole incubation was the sum of the net N_2O emission at every measurement. All treatments had three replicates.

1.4 Data analysis

For testing the significance of the pretreatment of water regime on N_2O emission from the paddy soil, one-way ANOVA was employed in this study. At each level of water content, the cumulative N_2O emission from the sample D and sample F was subjected to Student's *t*-test analysis at $P < 0.05$ and $P < 0.01$.

2 Results

2.1 Effects of pretreatment on soil properties

As shown in Table 1, pretreatment significantly altered soil properties, which regulate nitrogen transformation and N_2O emission. KCl extractable NH_4^+-N was built up to 72.6 mg/kg in the sample F after 110 d submergence. The sum of extractable NH_4^+-N and NO_3^--N was also much higher than that in the sample D, which was stored at air-dried moisture. Because submergence created anaerobic conditions, NO_3^--N in the sample F was much lower than that in the sample D.

For comparing N_2O emission at the same levels of NH_4^+-N and NO_3^--N, their contents in both samples were adjusted into the same levels of 80 and 20 mg/kg at the start of incubation. Pre-submergence decreased organic carbon and total N contents, while, raised soil pH (in KCl solution) 0.82 units and water holding capacity slightly, compared to the sample D. In this experiment, there was a slight difference of in water holding capacity between sample D and F.

2.2 Nitrous oxide emission rate

There was a peak in the temporal pattern of N_2O emission rate during the incubation (Fig. 1). The pretreatment affected the time when the peak emission appeared. Compared to the maximal rate of N_2O emission from the sample D, the peak rate of N_2O emission from the sample F lagged at soil moisture $\leq 60\%$ WHC. The N_2O emission rate from the sample D achieved its maximum at around 3- or 6-h incubation, while the peak rate of N_2O emission from the sample F appeared at around 12- or 18-h incubation. At soil moisture of 80% and 100% WHC, the peak N_2O emission rate of the sample D and sample F appeared synchronously, presented at 12- to 18-h incubation. After peaked emission, N_2O emission rate decreased gradually to near zero with the incubation time for both samples (Fig. 1).

2.3 Nitrous oxide emission from the sample F and D

Soil moisture significantly influenced the cumulative N_2O emission from the paddy soil ($P < 0.01$, Fig. 2). The response pattern of N_2O emission to soil moisture was similar in the sample F and sample D, increasing with the increase in soil moisture and reached the maximum at 80% WHC for both samples. However, the cumulative N_2O emission from the sample F was 2.25, 2.34 and 2.28 times of that from the sample D at 20%, 40% and 60% WHC, respectively, and the difference was significant ($P < 0.01$). Conversely, the cumulative N_2O emission from the sample F was only 29% and 14% of that from the sample D at the soil moisture of 80% and 100% WHC, respectively, and the difference was also significant ($P < 0.01$).

2.4 Nitrous oxide emission from the sample F + ACE and D + ACE

Below the soil moisture of 60% WHC, the cumulative N_2O emissions from the samples with acetylene was similar to that from the samples without acetylene ($P > 0.05$, Fig. 3), but dramatically increased at soil moisture of 80% and 100% WHC ($P < 0.001$, Fig. 3). Especially, the ratios of the cumulative N_2O emission from the sample F + ACE to that from the sample D + ACE were 0.95 at 80% and 1.02 at 100% WHC. The corresponding ratios were 1.71 at 20% WHC, 1.80 at 40% WHC and 2.27 at 60% WHC, respectively.

2.5 Mole fraction of nitrous oxide

The mole fraction of N_2O, which was calculated as the N_2O emission in the absence of C_2H_2 divided by that in the presence of C_2H_2 (Aulakh, 2001), ranged from 0.86 to 0.96

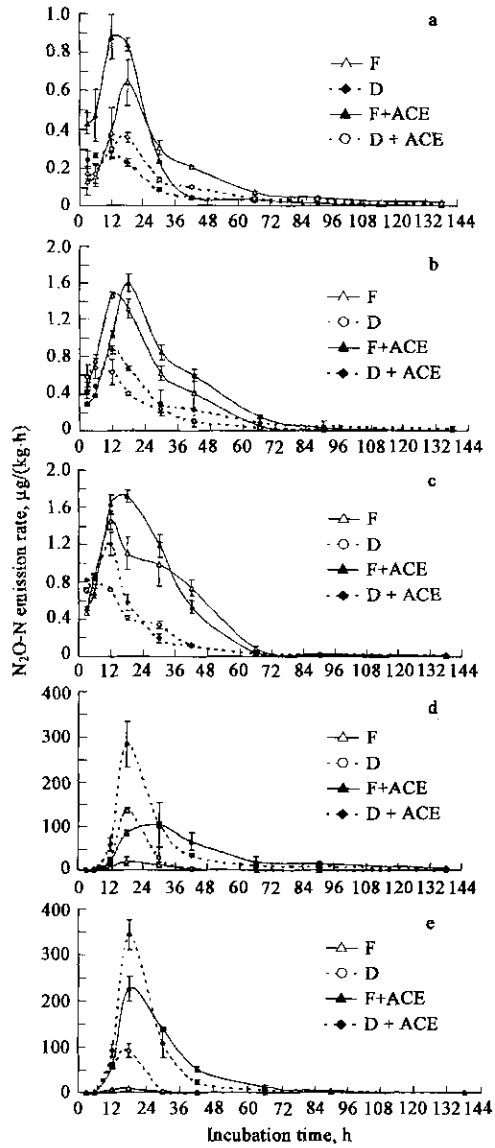

Fig. 1 The N_2O emission rate from the paddy soil through the 138 h incubation at 25℃ at soil moisture of 20% (a), 40% (b), 60% (c), 80% (d), and 100% (e) water holding capacity (WHC), respectively. The vertical bars refer to one standard deviation ($n = 3$). F means that the soil sample was air-dried after the submergence for 110 d. D means that the soil sample was air-dried and stored for 110 d. ACE means that the acetylene was added in the headspace at a rate of 10% v/v

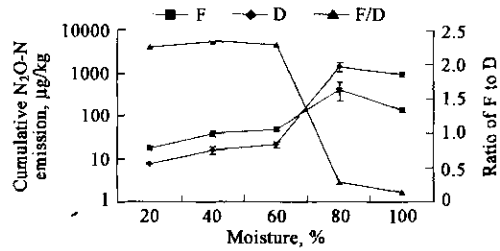

Fig. 2 The 138 h cumulative N_2O emission from the paddy soil incubation at 25℃ at different soil water holding capacity (WHC). The vertical bars refer to one standard deviation ($n = 3$). For abbreviations, see Fig. 1

at soil moisture of 20% to 60%, dropped to 0.10 at soil moisture of 80% WHC, and further dropped to 0.03 at 100% WHC for the sample F (Fig. 4). A similar trend was

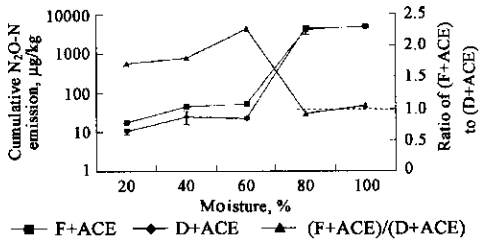

Fig. 3 The 138 h cumulative N_2O emission from the paddy soil with acetylene incubation at 25℃ at different soil water holding capacity (WHC). The vertical bars refer to one standard deviation ($n = 3$). For abbreviations, see Fig. 1

observed in the sample D but the change with soil moisture was much modest. The mole fraction of N_2O in the sample D was 0.73 at 20% WHC, 0.66 at 40% WHC, 0.95 at 60% WHC, 0.32 at 80% WHC, and 0.21 at 100% WHC (Fig. 4).

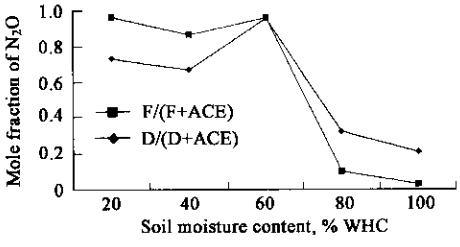

Fig. 4 The mole fractions of N_2O in the 138 h incubation at 25℃ at different soil water holding capacity (WHC). For abbreviations, see Fig. 1

2.6 KCl-extractable NO_3^--N and NH_4^+-N

At the start of incubation, the NO_3^--N and NH_4^+-N for each level of soil moisture content were adjusted into the same levels of 20 and 80 mg/kg, respectively. After 138 h incubation, the NO_3^--N content of the sample F and D increased significantly at low soil moisture ($\leq 60\%$ WHC) ($P < 0.05$, Table 2), and decreased very significantly at the soil moisture of 80% and 100% WHC ($P < 0.01$). But for the samples with acetylene, the NO_3^--N content was similar to the original value of 20 mg/kg at the low soil moisture ($\leq$ 60% WHC). This result showed that the nitrification was inhibited by the 10% C_2H_2 on one hand, the denitrification was little below the soil moisture of 60% WHC on the other hand.

Table 2 KCl extractable NO_3^--N and NH_4^+-N content at the end of the incubation of the paddy soil

N species	Treatments	Moisture (% water holding capacity)				
		20	40	60	80	100
NO_3^--N, mg/kg	F	27.2	30.6	31.6	3.88	1.74
	D	25.8	31.4	31.2	1.71	1.73
	F + ACE	20.5	22.2	21.6	2.89	1.86
	D + ACE	20.6	20.7	21.9	2.88	1.86
NH_4^+-N, mg/kg	F	85.4	88.6	78.5	86.1	89.2
	D	80.9	81.3	77.7	81.3	87.7
	F + ACE	72.9	80.9	81.0	80.3	88.9
	D + ACE	71.4	80.2	82.3	80.9	86.6

Notes: For abbreviation F and D see Table 1; + ACE means that acetylene was added to the headspace at a rate of 10% v/v

Compared to the NO_3^--N content, the NH_4^+-N content was little change before and after incubation for all the samples. Paired t-test showed that NH_4^+-N content in the sample F was significantly higher than that in the sample D at the end of incubation ($P < 0.05$, Table 2). The result indicated that the sample F mineralized stronger than the sample D on one hand, the sample D nitrified higher than the sample F on the other hand.

3 Discussion

The results observed in this experiment showed that the antecedent water regimes changed the N_2O emission from the paddy soil. Because the nitrifying and denitrifying bacteria appear to be well adapted to surviving extreme drought and to becoming active after wetting dry soil (Davidson, 1992), and air-dry storage of soil increased the availability of readily degradable organic carbon (Patten, 1980), N_2O emission from the air-dried soil (sample D) achieved its maximal rate in a shorter time than that from the submerged soil (sample F) at soil moisture content $\leq 60\%$ WHC (Fig. 1). The sample F emitted more greater N_2O than that of the sample D at lower tested soil moisture content $\leq 60\%$ WHC, but at higher soil moisture tested (80% and 100% WHC), the opposite results were observed (Fig. 2). The trace amount of! N_2O emission at soil moisture content $\leq 60\%$ WHC was very significantly lower than that at the 80% and 100% WHC. However, the general relationship between soil moisture content and N_2O emission, i.e. the N_2O emission increase with the increment soil moisture in a certain water content range (Granli, 1994), does not change.

It has been generally accepted that nitrification is a dominant process generating N_2O at low soil moisture and N_2O is mainly contributed by denitrification process at high soil moisture (Mummey, 1994; Aulakh, 2001). Inferring from the increases in KCl extractable NO_3^--N contents after the incubation (Table 2), nitrification process did occur at soil moisture content $\leq 60\%$ WHC. It has also been commonly accepted that acetylene at concentration of 10% (v/v) is sufficiently effective to inhibit nitrification and the conversion of N_2O to N_2 in denitrification process (Klemedtsson, 1988; Mummey, 1994; Stevens, 1997). No significant increases in KCl extractable NO_3^--N contents after the incubation in the treatments with acetylene addition at soil moisture $\leq 60\%$ WHC (Table 2) further confirmed that added acetylene inhibited nitrification process. Therefore, the produced N_2O in the treatments with acetylene addition was reasonably from denitrification process and could be used as an indicator of denitrification strength. The results of N_2O emissions from the treatments with acetylene addition indicated that denitrification occurred at soil moisture of 20% WHC and became intensive with soil moisture increasing (Fig. 3). This meant that even at soil moisture of 20% WHC, the N_2O would be generated from both nitrification and denitrification processes. However, higher N_2O emissions from the sample F + ACE than from the sample D + ACE at the soil moisture $\leq 60\%$ WHC (Fig. 3) suggested

that more anaerobic microsites where denitrification process could take place would be formed when the submerged soil was rapidly air-dried to designed soil moisture (Granli, 1994). But we have not the suitable method to determine the *Eh* value of the microsite in the samples in this experiment. The relative larger contribution of denitrification to N_2O production in the sample F would be one of the main reasons why the N_2O emissions were higher from the sample F than from the sample D at low soil moisture ($\leq$60% WHC).

At soil moistures of 80% and 100% WHC, denitrification was a dominant process generating N_2O, confirmed by the dramatic decrease in NO_3^--N contents from 20 to below 3.88 mg/kg after the incubation (Table 2). Because of the addition of 10% C_2H_2, the denitrification is the process to produce N_2O and the nitrification to produce N_2O was inhibited by the addition (Klemedtsson, 1988). The ratio of N_2O emission from the sample F + ACE to that from the sample D + ACE was around 1.0 at 80% and 100% WHC (Fig.3). This result indicated that the degree of nitrate transformed to N_2O in the microbial denitrification was equivalent between the sample F and D at the soil moisture of 80% and 100% WHC. But the N_2O emission from the sample F was lower than that from the sample D (Fig. 2). The result suggested that the sample F had more stronger reduction of N_2O to N_2 in microbial denitrification, in other words, the sample F was more reduced than the sample D in oxidation-reduction status at the soil moisture of 80% and 100% WHC. This can be further confirmed by the mole fraction of N_2O. It has been well known that the mole fraction of N_2O in the denitrification products decreases with soil redox potential (Masscheleyn, 1993). The mole fraction of N_2O in denitrification products was lower in the sample F (0.10) than in the sample D(0.31) at soil moisture of 80% WHC and decreased to 0.03 for the sample F and 0.21 for the sample D at soil moisture of 100% WHC, respectively.

Soil pH is also an important factor influencing N_2O emission (Bandibas, 1994; Stevens, 1998). The pretreatment significantly differentiated soil pH (Table 1). Pre-submergence raised soil pH, which was consistent with the previous observation (Saraswathi, 1991). The discrepancy in soil pH after the pretreatment would also affect N_2O emissions. But the effect of soil pH on N_2O emission was not clear in this experiment and needed further research.

4 Conclusions

The antecedent water regime alters N_2O emission in the magnitude from the paddy soil at each level of soil moisture. Pre-submergence significantly increased the ability of the reduction of N_2O to N_2 in denitrification at higher soil moisture tested. So the pre-submerged soil decreased the N_2O emission compared to the pre-air-dry storage soil at higher soil moisture tested. But the response pattern of N_2O emission to soil moisture is not changed, i.e. N_2O emission increased with the increment of soil moisture content. Therefore, the effect of antecedent water regime should be considered if N_2O emissions from soils are quantitatively studied.

Acknowledgements: The authors thank Prof. Zhaoliang Zhu's valuable suggestions on the research.

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(Received for review March 28, 2003. Accepted May 10, 2003)