

# 农田和森林土壤中氧化亚氮的产生与还原\*

于克伟\*\* 陈冠雄 (中国科学院沈阳应用生态研究所, 沈阳 110015)

Sten Struwe Annelise KjØller (哥本哈根大学基础微生物系, DK-1307, 丹麦)

**【摘要】** 采用土壤淤浆方法对丹麦农田和山毛榉森林土壤反硝化过程中  $N_2O$  的产生与还原进行了研究, 同时考察了硝酸根和铵离子对反硝化作用的影响. 结果表明, 森林土壤反硝化活性大于农田土壤, 但农田土壤中  $N_2O$  还原活性大于森林土壤, 表现在农田和森林土壤中  $N_2O/N_2$  的产生比率分别为 0.11 和 3.65. 硝酸根和铵离子能促进两种土壤中的  $N_2O$  产生, 但可降低农田土壤中的  $N_2O$  还原速率. 与农田土壤相比, 硝酸根可降低森林土壤  $N_2O$  的还原能力, 而铵离子却表现出对  $N_2O$  还原的促进作用. 培养 1 周内尽管农田土壤中硝酸根始终存在, 但几乎所有产生的  $N_2O$  都被还原成  $N_2$ . 然而, 只有当硝酸根几乎耗尽, 森林土壤中  $N_2O$  的还原才开始进行. 由于两种土壤 pH 值的不同造成硝酸根对  $N_2O$  还原抑制作用的差异, 因为酸性条件下硝酸根对  $N_2O$  还原的抑制较强. 因此, 土壤消耗大气  $N_2O$  的适宜条件为厌氧, pH 中性和硝酸根含量低.

**关键词** 反硝化作用 温室气体  $N_2O$  还原  $N_2O/N_2$  比率

**Production and reduction of nitrous oxide in agricultural and forest soils.** YU Kewei, CHEN Guanxiang (*Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110015, China*), Sten Struwe, Annelise KjØller (*Department of General Microbiology, University of Copenhagen, Sølvgade 83H, DK-1307 Copenhagen K, Denmark*). - *Chin. J. Appl. Ecol.*, 2000, 11(3): 385~389.

A soil-water slurry experiment was conducted to study the potentials of  $N_2O$  production and reduction in denitrification of agricultural and beech forest soils in Denmark. The effects of nitrate and ammonium additions on denitrification were also investigated. The forest soil showed a higher denitrification potential than the agricultural soil. However,  $N_2O$  reduction potential of the agricultural soil was higher than the beech forest soil, shown by the ratio of  $N_2O/N_2$  approximately 0.11 and 3.65 in the agricultural and the beech forest soils, respectively. Both nitrate and ammonium additions stimulated the  $N_2O$  production in the two soils, but reduced the  $N_2O$  reduction rates in the agricultural soil slurries. In contrast to the effect on the agricultural soil, nitrate reduced the  $N_2O$  reduction rate in the beech forest soil, while ammonium showed a stimulating effect on the  $N_2O$  reduction activity. After one week incubation, all of the  $N_2O$  produced was reduced to  $N_2$  in the agricultural soil when nitrate was still present. Nitrous oxide reduction in the beech forest soil occurred only when nitrate almost disappeared. The different nitrate inhibitory effect on the  $N_2O$  reduction activity in the two soils was due to the difference in soil pH. Inhibition of nitrate on  $N_2O$  reduction was significant under acidic condition. Consequently, soil could serve as a sink of atmospheric  $N_2O$  under the conditions of anaerobic, pH near neutral and low nitrate content.

**Key words** Denitrification,  $N_2O$  reduction, Greenhouse gases,  $N_2O/N_2$  ratio.

## 1 Introduction

Nitrous oxide ( $N_2O$ ) is not only one of the greenhouse gases, but also one of the gases which react with ozone and may contribute to depletion of the ozone layer in the stratosphere<sup>[10,27]</sup>. Since preindustrial times the amount of  $N_2O$  present in the atmosphere has increased from about 275 ppb(v) to about 310 ppb(v) with a continuously increasing rate about 0.2 to 0.3% yr<sup>-1</sup>. The atmospheric lifetime of  $N_2O$  is about 120 years, much longer than some other important greenhouse gases, such as  $CO_2$  and  $CH_4$ , indicating that the present increase will have a long term effect on the global cli-

mate<sup>[15]</sup>.

There is a very broad range of values in the estimated global sources and sinks of  $N_2O$  from Intergovernmental Panel on Climate Change (IPCC), which indicates that there still are considerable uncertainties in the estimation due to the temporal and spatial variations in  $N_2O$  production and consumption. Denitrification is one of the major sources of  $N_2O$  production and emission influenced by different environmental factors. Denitrifi-

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\*\* 通讯联系人. Corresponding author.

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cation represents the only known biological process for consumption of  $N_2O$  and the rate of this last step in the reduction of  $NO_3^-$  determines the ratio between  $N_2O$  and  $N_2$ <sup>[13]</sup>. There is some evidences in field measurements that soils consume ambient  $N_2O$ <sup>[1,5,6,11,23]</sup>, but there are not enough data to evaluate the global capacity of  $N_2O$  removal by soil and consequently there is no estimated value in the IPCC report on this issue<sup>[14, 15]</sup>. In previous research carried out in this field, the effect of nitrate on the reduction of  $N_2O$  to  $N_2$  was studied<sup>[3, 4, 26]</sup>. In this study, we included both nitrate and ammonium treatments in anaerobic incubation with an agricultural and a beech forest soils to investigate the potentials of  $N_2O$  reduction to  $N_2$  and the effects of nitrate and ammonium.

## 2 Materials and Methods

### 2.1 Sample soils and treatments

Surface soils (0~20cm) from an agricultural field and a beech forest in Denmark were collected in March. The agricultural field is planted with barley and fertilized once a year during sowing of the crop in May at a rate of 80 kg N hm<sup>-2</sup> with  $NO_3^-$ -N :  $NH_4^+$ -N 5.5 : 7.0. The soils were air-dried at room temperature, sieved (2 mm) and stored at 4°C before the use. Basic soil characteristics were analyzed, and the results are shown in table 1.

**Table 1 Characteristics of the agricultural and the beech forest soils**

	Agricultural soil	Beech forest soil
Total C (%)	1.30	1.69
Total N (%)	0.19	0.17
Ammonium ( $\mu\text{g N g}^{-1}$ dry soil)	0.08	1.26
Nitrate ( $\mu\text{g N g}^{-1}$ dry soil)	7.42	6.64
pH	6.60	3.80
Water content (% , w/w)	4.22	9.84

The soil-water slurry experiment was carried out by the following procedure: 15g (fresh weight) air-dried soil was weighted into a 50 ml bottle with 10 ml distilled water. Each of the two soils has six treatments, control, nitrate and ammonium additions treated with and without acetylene.  $KNO_3$ -N or  $NH_4Cl$ -N solution was added to make the final addition of 10  $\mu\text{g N g}^{-1}$  in the slurry. Each bottle was sealed with a rubber stopper and evacuated and then refilled with pure nitrogen to ensure an anaerobic incubation environment. Afterwards, acetylene was injected to 10% of the headspace of the corresponding bottles to inhibit the reduction of  $N_2O$  to  $N_2$ . Each treatment has six replicates. Samples were kept at 15°C after the initial gas sampling. The incubation lasted for 7 days and gas samples were drawn every 24 hours for analysis of  $N_2O$  (in triplicate) and  $CO_2$  (in duplicate) concentrations in the

headspace of the bottles. Immediately after the gas sampling, one bottle of each treatment was used to analyze the nitrate, nitrite and ammonium contents in the slurry.

### 2.2 Sample analysis and calculation

Each soil slurry was extracted with 25ml 0.1M KCl. After shaking for 2 hrs, soil solution was filtered to remove soil particles. An aquatic flow injection system was used to analyze  $NH_4^+$ ,  $NO_2^-$  and  $NO_3^-$  contents.  $N_2O$  concentration was measured on a Hewlett-Packard GC 5890 with ECD. The oven, injector and detector temperatures were 40°C, 120°C and 325°C, respectively, and the carrier gas flow rate was 20 ml  $N_2 \text{ min}^{-1}$ . A Hewlett-Packard GC 5890 with TCD was used for  $CO_2$  measurement with oven, injector and detector temperatures being 90°C, 120°C and 85°C, respectively, and carrier gas flow rate being 20 ml  $H_2 \text{ min}^{-1}$ . A pH meter 28 (Radiometer Copenhagen) was used to measure pH value in the slurry.

Denitrification potential was determined by the rate of  $N_2O$  accumulation in  $C_2H_2$ -inhibited treatment. Some of the  $N_2O$  produced can be reduced to  $N_2$  when there is no inhibition of  $N_2O$  reduction by  $C_2H_2$ . Therefore  $N_2O$  reduction potential was determined by subtracting the amount of  $N_2O$  produced in  $C_2H_2$ -inhibited treatment from the  $N_2O$  accumulated without  $C_2H_2$ . The amount of  $N_2O$  dissolved in water phase of the slurry was considered in the calculation<sup>[21]</sup>. All results were calculated based on dry weight of the soil.

## 3 Results and Discussion

### 3.1 Soil general metabolism

Soil anaerobic respiration is expressed by  $CO_2$  production in the experiment. The forest soil showed much higher  $CO_2$  production activity than the agricultural soil, and the ratio between  $CO_2$  and  $N_2O$  evolution in the forest soil was much higher than in the agricultural soil (Table 2 and 3), indicating a larger portion of denitrifiers in the microbial community of the agricultural soil. There was no significant difference in  $CO_2$  production between different treatments of each soil, indicating that the general microbial metabolism did not differ, and therefore the acetylene inhibition technique was ac-

**Table 2  $CO_2$  productions ( $\mu\text{g C g soil}^{-1} \text{ d}^{-1}$ , n = 8) under different treatments**

	Agricultural soil		Beech forest soil	
	With $C_2H_2$	Without $C_2H_2$	With $C_2H_2$	Without $C_2H_2$
Control	3.34	3.29	22.85	22.98
$NH_4^+$	3.67	3.51	21.23	23.83
$NO_3^-$	3.35	3.47	20.98	21.78

Data are calculated by the linear regression of  $CO_2$  production during the incubation. Values in column are not significantly different at 0.05 level (T-test, Microsoft Excel 5.0).

ceptable and the effects of different additions on  $N_2O$  production and reduction were comparable.

### 3.2 $N_2O$ production and reduction potentials

Research to identify sources of  $N_2O$  in soils has indicated that most, if not all, of the  $N_2O$  evolved from soils is produced by biological processes and that little, if any, is produced by chemical processes such as chemodenitrification<sup>[6]</sup>. In most cases, chemodenitrification may be responsible for NO but not  $N_2O$  production<sup>[1]</sup>. Biological denitrification is considered as the only source of  $N_2O$  production in this anaerobic incubation experiment.  $N_2O$  production and reduction potentials were calculated based on the first 24 hrs measurements. Nitrous oxide production potential was estimated by the rate of  $N_2O$  accumulation with acetylene blockage, and  $N_2O$  reduction potential by the rate of  $N_2O$  reduction to  $N_2$  (the difference between  $N_2O$  accumulations with and without acetylene).

Stronger denitrification activity was found in the forest soil illustrated by higher  $N_2O$  production potential (Table 3) and faster decrease in nitrate content (Fig. 1 (c) and 2 (c)). Actually the acidic environment of the forest soil is not favorable for denitrification, because

denitrification activity is generally stronger at neutral conditions, and the initial nitrate content in the forest soil slurry was lower than the agricultural soil slurry due to the difference in original nitrate and water contents of the sample soils. Larger denitrifier population in the forest soil could be responsible for the stronger denitrification activity, and higher organic matter content in the forest soil might partially account for the result.

Most of the  $N_2O$  produced was reduced to  $N_2$  in the agricultural soil, leading to only 10% of the end products of denitrification being  $N_2O$  ( $N_2O/N_2$  0.11), but in the forest soil about 78% of the end products was  $N_2O$  ( $N_2O/N_2$  3.65) (Table 3). The results were obtained from soils with different physical and chemical characteristics. The pH values were 6.64 and 3.80 in the agricultural and the forest soil, respectively (Table 1). The difference in  $N_2O$  emission corresponded with other observations that high  $N_2O$  emission occurred at low pH conditions<sup>[3, 12]</sup>. In soil slurry experiments Struwe and Kjølner<sup>[25]</sup> found that the ratio of  $N_2O/N_2$  was inversely related to pH, and increased from 0.5 ~ 2.8 with decreasing pH from 7.2 ~ 4.3. Blackmer and Bremner<sup>[3]</sup> found a strong inhibitory effect of nitrate on

**Table 3 Effects of nitrate and ammonium on  $N_2O$  production and reduction potentials ( $\mu g N_2O-N g soil^{-1} d^{-1}$ )**

	Agricultural soil			Beech forest soil		
	Production	Reduction	$N_2O/N_2$	Production	Reduction	$N_2O/N_2$
Control	1.63 ± 0.15 <sup>a</sup>	1.46 ± 0.14 <sup>a</sup>	0.11 ± 0.01 <sup>a</sup>	1.90 ± 0.07 <sup>a</sup>	0.41 ± 0.02 <sup>a</sup>	3.65 ± 0.02 <sup>a</sup>
$NH_4^+$	2.04 ± 0.01 <sup>b</sup>	1.30 ± 0.06 <sup>a</sup>	0.57 ± 0.07 <sup>b</sup>	1.99 ± 0.12 <sup>a</sup>	0.48 ± 0.07 <sup>a</sup>	3.15 ± 0.47 <sup>a</sup>
$NO_3^-$	1.75 ± 0.06 <sup>a</sup>	1.44 ± 0.01 <sup>a</sup>	0.22 ± 0.02 <sup>c</sup>	1.99 ± 0.13 <sup>a</sup>	0.17 ± 0.02 <sup>b</sup>	10.86 ± 0.97 <sup>b</sup>

Values are expressed as means ± standard deviations of three replications. Values with the same character in column are not significantly different at 0.05 level (T-test, Microsoft Excel 5.0).

the  $N_2O$  reduction under acidic conditions.

Both nitrate and ammonium additions slightly enhanced  $N_2O$  productions in the agricultural soil, while  $N_2O$  reduction potentials decreased (Table 3). The inhibitory effect of nitrate on  $N_2O$  reduction was an expected result. It was also found that ammonium had a stronger stimulating effect on  $N_2O$  production and stronger inhibitory effect on  $N_2O$  reduction than nitrate. There was about 36%  $N_2O$  ( $N_2O/N_2$  0.57) in the gaseous end products when ammonium was added compared with only 10%  $N_2O$  ( $N_2O/N_2$  0.11) in control. If this finding is also true under natural conditions, it may have great implication for agricultural practice, since large areas of cultivated soil are fertilized with urea. The capacity of agricultural soil to consume  $N_2O$  may

be limited by the  $NH_4^+$  released from urea hydrolysis. Blackmer and Bremner<sup>[2]</sup> emphasized that a small reduction of the  $N_2O$  consumption activity may lead to a large increase in  $N_2O$  emission. It could be expected that a small change in the  $N_2O/N_2$  ratio would greatly influence the  $N_2O$  emission from cultivated soil and also the source inventory of  $N_2O$ .

The results obtained from forest soil were different. The ammonium addition did not show an inhibitory effect on  $N_2O$  reduction, but showed a slightly stimulating effect, which resulted in a lower  $N_2O/N_2$  ratio than control. Both ammonium and nitrate additions enhanced the  $N_2O$  productions in the forest soil, but not as much as in the agricultural soil. The inhibitory effect of nitrate on  $N_2O$  reduction was found, corresponding to the

result in the agricultural soil (Table 3).

### 3.3 Dynamic accumulation of $N_2O$ during the incubation

The dynamic patterns of  $N_2O$  accumulation and change of nitrate content during the incubation were illustrated in Fig. 1 and 2. We didn't find significant amount of nitrite accumulation and ammonium content kept constant during the anaerobic incubation. The results from the agricultural soil without acetylene showed that virtually all of the  $N_2O$  produced in denitrification was reduced to  $N_2$  in one week, and this pattern was not significantly affected even when nitrate was added. Due to the stronger inhibitory effect of ammonium on  $N_2O$  reduction, largest  $N_2O$  accumulation was found in the ammonium added treatment (Fig. 1 (a)). Nitrate strongly inhibited  $N_2O$  reduction, and this inhibition was stronger under acidic conditions of the forest soil<sup>[24]</sup>. Only when nitrate was almost undetectable in the forest soil slurry and denitrification nearly stopped after incubation for 3 days, the consumption of  $N_2O$  in the headspace occurred (Fig. 2). The result indicates that the capacity of the forest soil to consume  $N_2O$  greatly depended on nitrate content. It has been proposed that the development of nitrous oxide reductase requires more time<sup>[19]</sup>. The duration of this lag increases with  $NO_3^-$  concentration<sup>[9, 17]</sup>.

The agricultural soil showed higher  $N_2O$  reduction potential than the forest soil, and the capacity depended on several environmental parameters. The microorganisms responsible for  $N_2O$  consumption are widely present in different ecosystems. Kromka *et al.*<sup>[18]</sup> observed that 80~90% of the denitrifiers were able to reduce  $N_2O$  in their study. Okereke<sup>[22]</sup> reported that 59 out of 71 denitrifiers isolated from eight different countries utilized  $N_2O$  as terminal electron acceptor. A suitable reduced environment can be found in flooded areas or deeper layers of soil. Observations on consumption of atmospheric  $N_2O$  have been reported from field measurements of cultivated soils<sup>[5, 28]</sup>, grasslands<sup>[7, 11, 23]</sup> and tropical soils<sup>[16, 20]</sup>. Ryden<sup>[23]</sup> reported that an unfertilized control grassland plot in UK served as a sink for atmospheric  $N_2O$  during the period from August to November when nitrate content in the soil was low and soil water content exceeded 20%, which corresponded

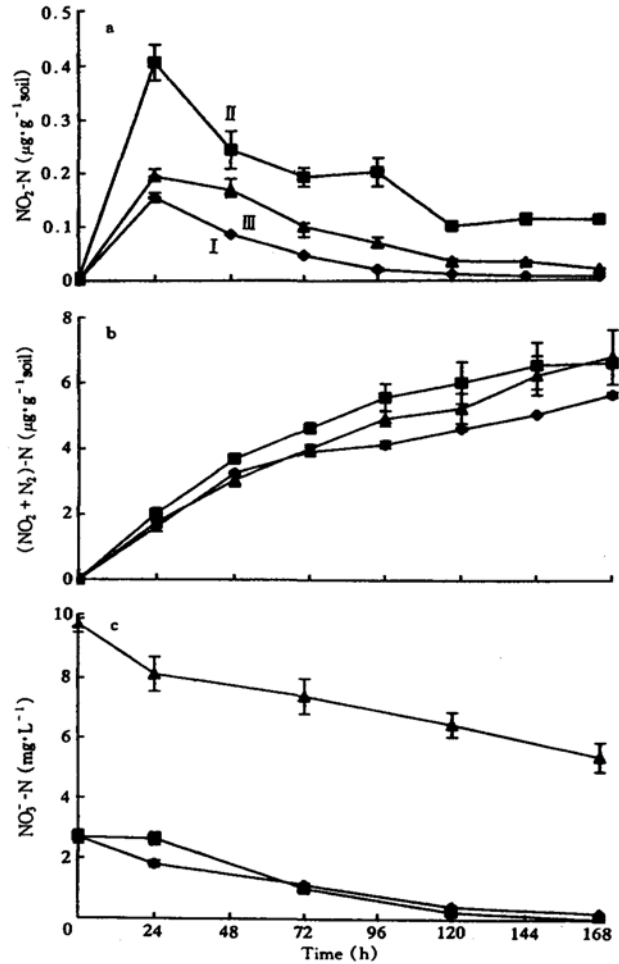


Fig. 1 Accumulation of nitrous oxide and change of nitrate content in the agricultural soil slurry.

Vertical bars represent the standard deviations of means and can not be seen when they are smaller than the symbols of points,  $n = 3$  for (a) and (b),  $n = 2$  for (c). Points in (c) represent means of nitrate contents in the slurries with and without acetylene inhibition, based on the assumption that there is no effect of acetylene on denitrification in slurries except the inhibition of  $N_2O$  reduction to  $N_2$ . I. Control, II.  $NH_4^+$ , III.  $NO_3^-$ . The same below.

to the results of this experiment. Similar results were found in a bare field (cultivated soil with no vegetation), which showed a continuous consumption of ambient  $N_2O$  from September to November, and this consumption was close to the amount of  $N_2O$  emitted in the remaining months of the year<sup>[28]</sup>.

Reduction of  $N_2O$  in soil is probably only a minor sink, but may still play an important role on a global scale. The elimination of  $N_2O$  in the stratosphere is so slow that even a small sink could contribute significantly to reduction of the atmospheric residence time of  $N_2O$ <sup>[8, 14]</sup>. Further studies are needed to evaluate the importance of this sink on a global scale. The potential of soil as a sink for atmospheric  $N_2O$  deserves atten-

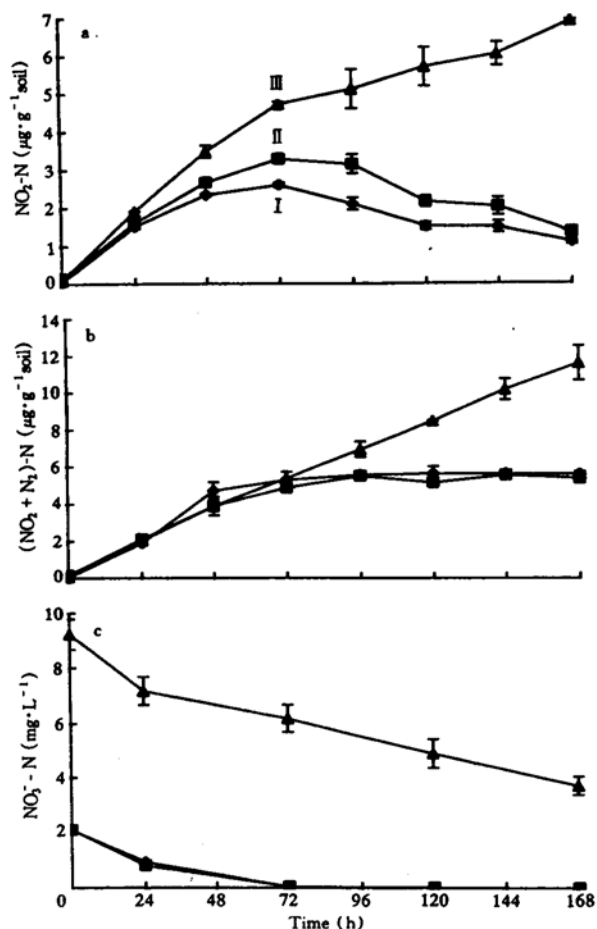


Fig.2 Accumulation of nitrous oxide and change of nitrate content in the forest soil slurry.

Explanations are the same as in Fig. 1, but the scales of y-axis in (a) and (b) are different from Fig. 1.

tion in future attempts to estimate the atmospheric  $\text{N}_2\text{O}$  budget.

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**Author introduction** Kewei Yu, born in 1964, associate research professor, major research in biogenic greenhouse gases and nutrient cycling in terrestrial ecosystem. He has more than 30 publications in Chinese and English. He is currently studying at Louisiana State University, USA for Ph. D, E-mail: kewei.yu@mailcity.com