

Originals

Potential of Soil and Sediment for the Reduction of Nitrous Oxide

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Abstract

Potential activity of soil and sediment samples along a watershed is determined for the reduction of nitrous oxide (N₂O), which is a doubly important trace gas controlling the energy budget of the troposphere and the ozone level in the stratosphere. ¹⁵N tracer technique is applied for the determination of the potential activity using labelled N₂O. Most of the soil and sediment samples have been proved to have large potential activity for reduction of N₂O. The potential activity is highest for the river and bay subsurface sediment at the coast, where anaerobic micro site predominates. The present results indicate that the nitrate reduction system of soil and sediment, especially in coastal area is an important process in consuming N₂O.

Introduction

Nitrous oxide is one of the most important trace gases which have a potential to destroy ozone layer in the stratosphere¹⁾ and to control the temperature of the Earth by greenhouse effect²⁾. New approaches to the geochemical cycle of nitrous oxide through the natural nitrogen isotope abundance in N₂O³⁻⁶⁾ have provided additional information on the budget of N₂O. One of the great achievements of them is about the contribution of two important processes, denitrification and nitrification, for the cycle of N₂O. Nitrous oxide was considered to be mainly produced by denitrification and nitrification process in terrestrial

and aquatic systems. A considerable attention has been focused on N₂O production during denitrification. However, much less emphasis has been laid on denitrification process as N₂O consuming process. In fact, in an initial few days of incubation of pure culture of denitrifying or soils, significant amount of N₂O accumulates, but its concentration decreases in the latter period of incubation⁷⁾. Therefore, studies on the short period of denitrification reaction tend to give higher N₂O producing activity of the systems by denitrification. Former estimates based on the shorter period of incubation experiments favored over estimates of N₂O production during denitrification process.

Production and/or consumption of N₂O by denitrification process are determined by the subtle balance of the kinetics of nitrate reduction to N₂O, diffusion of N₂O between reaction site and atmosphere, and N₂O reduction⁸⁾. Almost all the studies on the kinetics of denitrification process included either the kinetics of all these three processes or the kinetics of the two preceding processes. The purpose of this study is to estimate the contribution of last process individually in the whole process of nitrate reduction in natural environments.

Very little is known about the N₂O reductase system⁹⁾ as well as N₂O reduction activity in natural environments because of the faintness of the enzyme¹⁰⁾ and of lack in ¹⁵N-tracer studies using ¹⁵N labelled N₂O. So, the number of studies have been limited concerning the N₂O reduction activities of soil and sediments¹¹⁻¹⁴⁾.

In this study, ¹⁵N tracer technique has been firstly applied to measure N₂O reducing activity of natural soils and sediments using ¹⁵N labelled N₂O. The

Key words : Nitrous oxide (N₂O), Nitrogen isotope ratio, ¹⁵N, Denitrification, N₂O reduction, Soil and sediment, Geochemical cycle

potential activity of soil and sediments was measured along the watershed to clarify where the N_2O reduction can proceed actively. A relation between N_2O and nitrate reduction activity has been studied precisely to provide useful information for the global estimate of N_2O reduction on the basis of a number of denitrification data hitherto obtained in various ecosystems.

Materials and Methods

Locations and sampling

Samples of soil and sediment are collected along the watershed of the Otsuchi River, Iwate Pref. Japan for the following reasons ;

- 1) The watershed is in appropriate size for a model ecosystem including forest areas, river valleys, a small town and coastal area. So that the sampling is relatively easy.
- 2) There have been an accumulation of available information obtained by interdisciplinary studies, organized by Otsuchi Marine Research Center, Ocean Research Institute, University of Tokyo.
- 3) The disturbance of the ecosystem by human activities is localized and small.

The sampling locations are shown in Fig. 1. Surface soil sample and river floor sediments at shallow sites have been collected by shovel and a scoop from 0 to 5 cm depth with least disturbance of the sample. Sediments in the bay have been collected by an Ekman Birge sampler from the surface to 15 cm depth and also by a gravity core sampler for the core sample of 36 mm in diameter from the surface to 34 cm depth. All the samples have been through a 2 mm sieve as soon as possible and subjected to the activity measurements.

Potential activity measurements for reduction of N_2O and nitrate

Potential activity of N_2O reduction has been measured using an incubation vessel as shown in Fig. 2-a. Sample of about 5 g in wet weight is put in the vessel. The atmosphere inside the vessel is replaced with 99.999 % N_2 of which nitrogen isotope composition was measured in advance, by flushing through a syringe needle plunged to the bottom of the vessel and

capped with rubber cap. Reduction of N_2O is started by breaking the capillary tube which contains ^{15}N labelled N_2O of ca. 0.1 cc STP with a stainless steel ball. The preparation of ^{15}N labelled N_2O ($^{15}N^{14}NO$; 51.8 atm % ^{15}N at the labelled position) is described elsewhere¹⁵⁾. The vessel is incubated at 20 °C for a desired period. At the end of the reaction, $HgCl_2$ solution is added and the vessel is stored at -20 °C until analysis. Analytical procedures are as follows ; the vessel is attached to the vacuum system through a syringe needle and the atmosphere inside the vessel is collected via liq. N_2 traps by the help of a Toepfer pump. Thorough purification of N_2 from ^{15}N labelled N_2O is executed by trap work using the vapor pressure difference between N_2 and N_2O at the liq. N_2 temperature. Nitrogen isotope ratio of purified N_2 is determined on a precise ratio determining mass spectrometer VG 602E (VG Isogas, Cheshire, U.K.). Potential activity (A) of the sample for the reduction of N_2O to N_2 is calculated by the following equation.

$$A = fVS^{-1}T^{-1} \\ = \frac{u(uR-2t)}{xu+yt-2tu-u(y-u)R} VS^{-1}T^{-1} \quad (1)$$

where f is the fraction of $^{15}N_2$ in total N_2 , V is the amount of total N_2 , S is the amount of soil or sediment, T is the duration of the incubation, R is $^{29}N_2/^{28}N_2$ ratio of total N_2 , x is the atomic fraction of ^{15}N at $^{*}N$ of $^{*}NNO$, y is the atomic fraction of ^{14}N at $^{*}N$ of $^{*}NNO$, t is the atomic fraction of ^{15}N of bomb N_2 , and u is the atomic fraction of ^{14}N of N_2 .

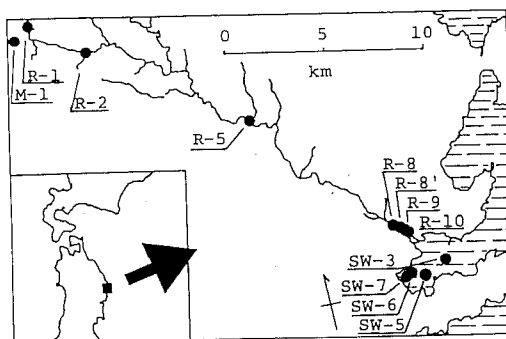


Fig. 1 Sampling locations for soil and sediments at Otsuchi, Iwate Pref. Japan.

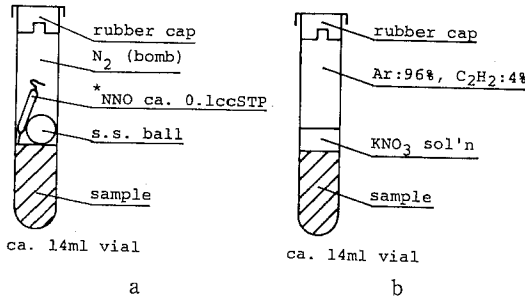


Fig. 2 Arrangements of incubation experiments for the measurement of activities for the reduction of N₂O (a) and nitrate (b).

Potential activity of nitrate reduction is measured using a reaction vessel as shown in Fig. 2-b. Sample of about 5 g in wet weight is put in the vessel. One ml of 0.5 mg-N/l nitrate solution is added to the sample. The atmosphere inside the vessel is replaced with research grade mixed gas of Ar (96 %) and C₂H₂ (4 %) which prevents further reduction of N₂O, in the same manner as described above. The accumulation of N₂O is measured by TCD gas chromatograph (GC-4APT, Shimadzu, Kyoto) and the potential activity of nitrate reduction is estimated¹⁹.

Both activities are measured once more by using the same sample stored at 4°C two months later. Other characteristics of soil and sediment samples are determined by ordinary methods.

N₂O reduction activity as a function of partial pressure of N₂O

Dependence of the N₂O reduction on the partial pressure of N₂O is investigated for the sample R-10 at 5 different magnitudes of partial pressure between 1.5×10^{-7} and 5.9×10^{-3} atm. For the N₂O partial pressure between 10^{-2} and 10^{-4} , the same vessel as mentioned above is used. For ca. 10^{-5} atm, sample is incubated in ca. 130 ml flask, and for the lower N₂O partial pressures, each sample is put in the flask of about 1000 ml with ca. 0.1 ml of ¹⁵N labelled N₂O. The low partial pressure of N₂O is achieved by diluting the ¹⁵N labelled N₂O with CO₂ and the amount of the mixed gas is precisely measured by McLeod gauge.

Results and Discussion

Time course of N₂O reduction

In order to determine the adequate duration of incubation, the time course of N₂O reduction is checked using the sample R-10. The result is shown in Fig. 3. The reduction rate is rather constant until 24 hours. Because of the high sensitivity of this method, a few to a few tens of minutes incubation is sufficient for the determination of N₂O reduction. N₂O reduction activity is determined by taking the average of the data at 3 and 24 hours incubation for practical reason.

Potential activity for the reduction of N₂O and nitrate

The results of incubation experiments are summarized in Table 1 (Series I) and schematically illustrated in Fig. 4. Both activities are unexpectedly high for particular samples such as the soil (M1) and the river floor sediment at the river head (R1). This fact may be due to high standing stock of micro-organisms together with high nitrate concentration¹⁷. It is an additional reason for high activity for the soil sample M1 that the high water content is favorable for the existence of anaerobic micro site to proceed denitrification including N₂O reduction. Except for these two exceptions, general trend is shown in Fig. 4. That is, both activities are high for the estuarine river floor sediments and the bay sediments. Potential activity of sediments is concluded to be promoted by the increase in the anaerobic site in sediments and also by the increase in nitrate concentration in the lower reaches of the river¹⁷.

Accordingly, estuarine area and bay are considered to be greatly responsible for the consumption of N₂O. This is supported by a large extent of undersaturation of N₂O in the water column of bay where anaerobic conditions are developed in subsurface layer¹⁸⁻²⁰.

In order to obtain more information on the bay sediments other than SW-3 (SW-5, 6 and 7), another set of incubation are carried out as shown in Series II in Table 1. The general trend is conserved and the higher activities are also observed in estuarine and bay sediments. Some characteristics are, however, disturbed; both activities are reduced for the sample M-1 from the mountain area. The soil materials with

Table 1 Experimental results and some characteristics of soil and sediments

Sample	pH		Water (%)	Fe ²⁺ (mg/g)	Kjeldahl-N		Munsell color	Series I *		Series II *		
	H ₂ O	KCl			mgN/g	permil		N ₂ O	NO ₃ ⁻	N ₂ O	NO ₃ ⁻	CO ₂
M- 1	4.84	4.07	53.6	0.12	2.52	9.5	7.5R2/1	13.58	13.65	1.44	4.04	9.27
R - 1	6.54	5.51	47.6	0.07	0.97	0.6	10 Y R3/4	6.22	4.67	15.54	3.41	10.32
R - 2	7.40	6.26	25.6	0.04	0.31	3.5	10 Y R3/3	0.85	2.28	2.29	1.55	4.80
R - 5	7.22	5.86	19.4	0.02	0.19	4.1	2.5Y R4/3	1.33	1.42	1.08	0.45	1.18
R - 8	7.31	6.08	20.1	0.02	n.d.	n.d.	2.5Y R5/3	5.82	6.32	4.64	1.34	2.79
R - 8'	7.11	5.93	21.1	0.36	0.33	5.1	2.5Y R4/2	5.07	6.57	2.35	4.05	9.32
R - 9	7.81	6.38	19.7	0.18	0.29	5.4	10 Y R3/2	5.28	4.16	3.16	4.18	10.19
R-10	7.80	6.80	23.7	0.47	0.43	3.1	2.5G Y2/1	13.53	10.27	70.63	26.37	36.79
SW-7 (7)	7.66	7.50	38.6	1.10	0.56	0.8	2.5Y3/2	n.d.	10.57	1.28	3.47	8.49
SW-6 (10)	7.65	7.55	53.7	2.32	0.86	-0.7	5 Y2/2	n.d.	11.16	6.80	5.83	19.61
SW-5 (20)	7.50	7.54	56.9	1.19	1.06	1.4	2.5Y3/2	n.d.	6.06	8.19	5.89	23.73
SW-3 (32)	7.66	7.49	58.4	1.85	1.19	3.7	5 Y2/2	8.67	11.73	22.16	11.19	28.28
SW-3F-1 (0- 2)			63.8	1.08			5 Y3/2			9.33	9.32	53.24
SW-3F-2 (4- 6)			58.4	2.13			5 Y3/2			13.55	5.03	26.88
SW-3F-3 (6- 8)			59.1	2.30			7.5Y2/2			9.88	6.24	26.73
SW-3F-4 (10-12)			57.0	1.68			7.5Y2/2			3.93	0	13.67
SW-3F-5 (24-26)			57.1	1.39			7.5Y2/1			0.74	0	6.65

* : Results of the incubation series I and II are expressed in terms of reduction and production potential activities for N₂O, NO₃⁻ and CO₂, respectively in the unit of $\mu\text{l STPg}^{-1}\text{day}^{-1}$.

Figures in the parenthesis indicate the water depth for the samples SW-3-7 in metre, and the core depth for the samples SW-3F-1-5 in cm. n.d. : not determined.

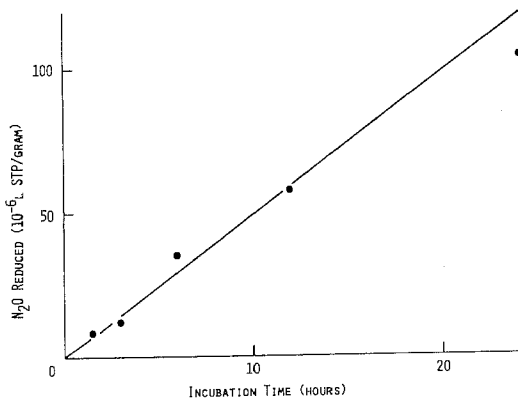


Fig. 3 Time course of N₂O reduction for the sediment sample R-10.

high porosity seemed to be suppressed of its activities under anaerobic conditions. Generally in Series II, potential activity for the reduction of N₂O increased almost twice as much as that of nitrate.

Detailed study is conducted on the vertical profile for the bay sediment core sample SW-3-F, which is collected by a gravity corer. As shown in Table 1,

N₂O reduction activity is the highest at the subsurface layer and decreased with depth but does not vanish even at the depth of 24-26 cm. On the other hand, nitrate reducing activity is limited to the depth of 0 to 8 cm and vanish for the deeper samples, which can still reduce N₂O and produce CO₂.

The assimilation of N₂O by the samples is not observed, which is checked by the fact that the nitrogen isotope ratio of organic nitrogen (Kjeldahl-N) is not altered after incubation.

As expected from Fig. 4, the N₂O reducing activity is closely correlated with that of nitrate reduction. Both activities for the reduction of N₂O and nitrate correspond to each other as shown in Fig. 5 (Note that the units for both activities are the same). This indicates that both potential activities are almost the same for all the samples.

To estimate the global amplitude of N₂O reduction, it is one of the best ways to utilize abundant estimates of denitrification in both local and global, on the basis of the relationship between two activities. Therefore, it must be the next step in the present study to

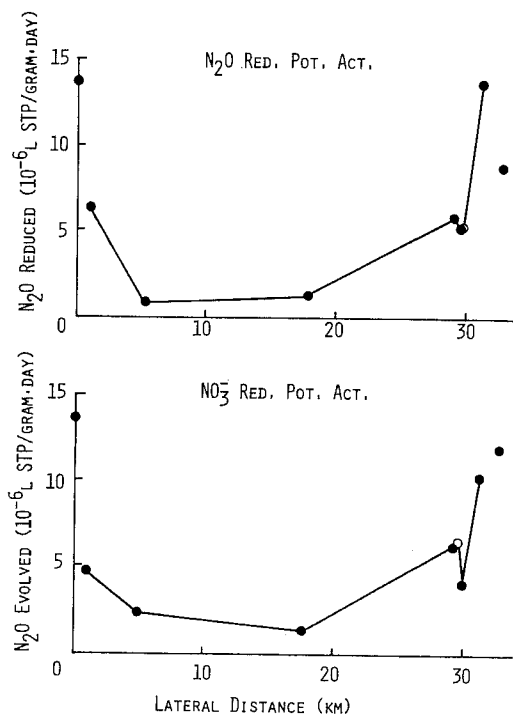


Fig. 4 Potential activities of soil and sediment for the reduction of N_2O (top) and nitrate (bottom).

determine the relationship of substrate concentration dependence on both activities. Fig. 6 depicts the dependence of the N_2O reduction activity for the sample R-10 on the partial pressure of N_2O . The dependence could be approximated by Michaelis-Menten type saturation curve. The substrate concentration that gives the reaction velocity half of that at the maximum (half saturation constant : K_s) is 1.88×10^{-4} atm. This K_s value corresponds to the dissolved N_2O concentration of 5.68×10^{-6} mol/l calculated from the solubility data²¹⁾, assuming that N_2O is in dissolution equilibrium with pore water of the sediments. The K_s value of N_2O is almost the same as that of nitrate for estuarine sediments, which is about 5×10^{-6} mol/l²²⁾. Accordingly, N_2O reduction activity at lower N_2O concentration is also related to nitrate reduction activity at lower nitrate concentration.

Several more steps are required to estimate the global amplitude of N_2O reduction ; such as diffusion studies of N_2O of soil and sediment between atmosphere and/or pore water micro site where reaction

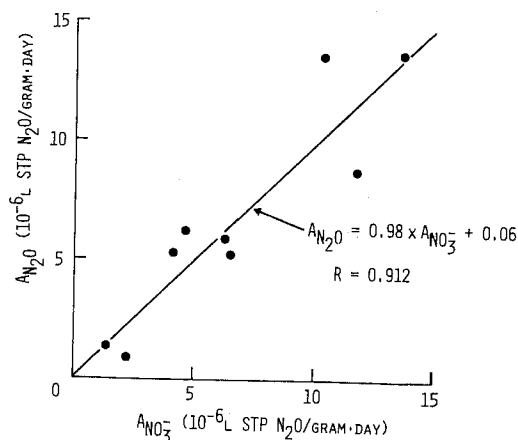


Fig. 5 Relationship between reduction potential activity of soil and sediments for N_2O and for nitrate.

takes place and detailed analysis of sequential kinetics. However, it may be concluded that denitrification system plays an important role in regulating the atmospheric concentration of N_2O through suppressing the enhanced level of N_2O in soil and sediment.

Summary

Soil and sediment samples are collected along a watershed from mountain area to a bay via coastal area. The potential activity of soil and sediment for N_2O reduction is found to be significantly high. The N_2O reduction activity is highest for the sediment of the river mouth, where anaerobic micro site prevails. The vertical profile of N_2O reduction activity is studied for a bay sediment core to reveal that the subsurface is responsible for suppressing the N_2O level of the pore water in the sediment column. There is a good correlation between potential activity of N_2O and that of nitrate, which indicates the possibility to estimate the global amplitude of N_2O reduction through abundant nitrate reduction estimates.

Nitrogen and oxygen isotope studies are another important approaches to the global cycle of N_2O . Combination of isotope studies with flux studies is expected to provide comprehensive information for the global budget of N_2O .

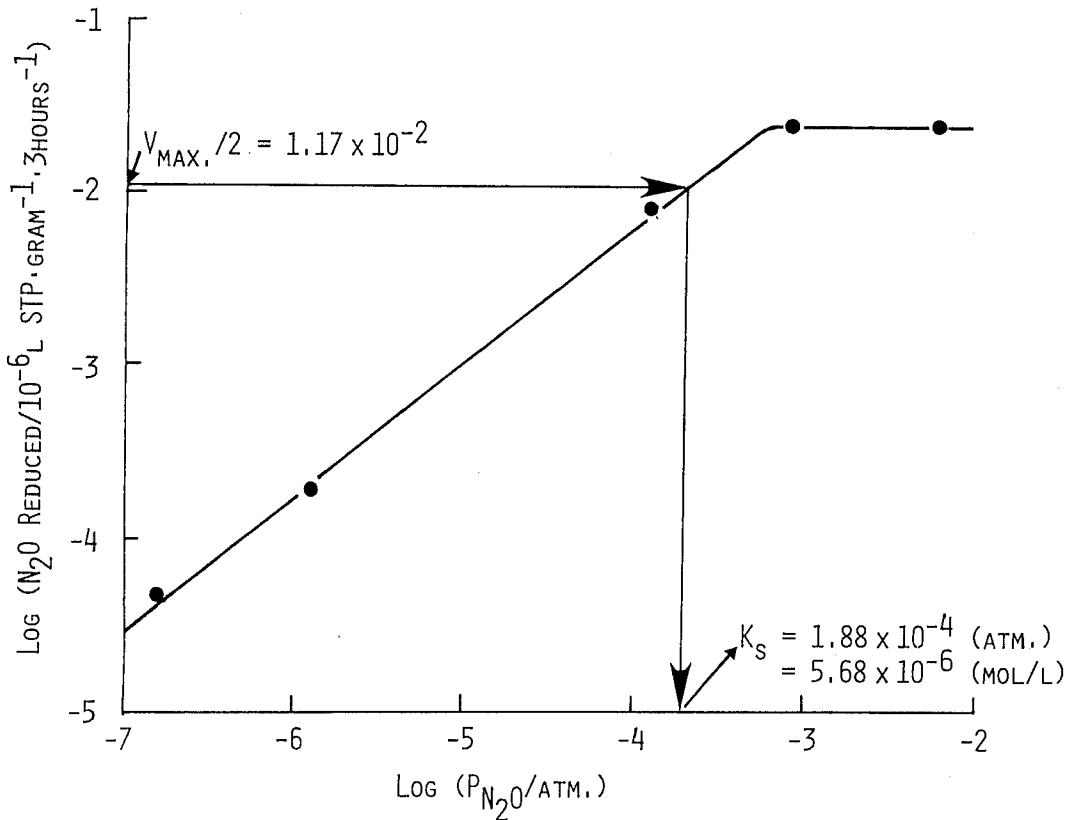


Fig. 6 Dependence of reduction rate on partial pressure of N_2O for sediment sample R-10.

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土壌・堆積物の一酸化二窒素還元活性

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要 約

岩手県大槌川水系に沿って、森林土壌から湾内堆積物にいたる土壌・堆積物試料を採取し、¹⁵Nで標識をつけた一酸化二窒素(N₂O)を用いて、地球温暖化とオゾン層の消長に重要な役割を果たすN₂Oの還元活性を測定した。いずれの土壌・堆積物も高い還元活性を示し、もっとも高い活性を示したのは嫌気的な部位の卓越する河口の堆積物であった。湾内の堆積物コア試料についてN₂O還元活性の鉛直分布を調べたところ、亜表層の堆積物が、堆積物中の間隙水のN₂O濃度の上昇を抑制していることが明らかにされた。N₂Oの還元活性と硝酸の還元活性の間には非常によい相関があり、これを利用することですでに数多く研究されている硝酸還元活性の見積もりから地球規模の還元によるN₂O消滅量を推定することが可能になると思われる。一方、N₂OとN₂Oに関わる化合物について窒素と酸素の安定同位体比を指標とする研究はN₂Oの地球化学的循環を理解するための有効なアプローチである。安定同位体による研究とフラックスの測定により得られた知見の重ね合わせを通して、N₂Oの地球化学的循環の全体像が明らかにされると考えられる。

キーワード：一酸化二窒素(N₂O)、窒素安定同位体比、¹⁵N、脱窒反応、N₂O還元、土壌・堆積物、地球化学的循環