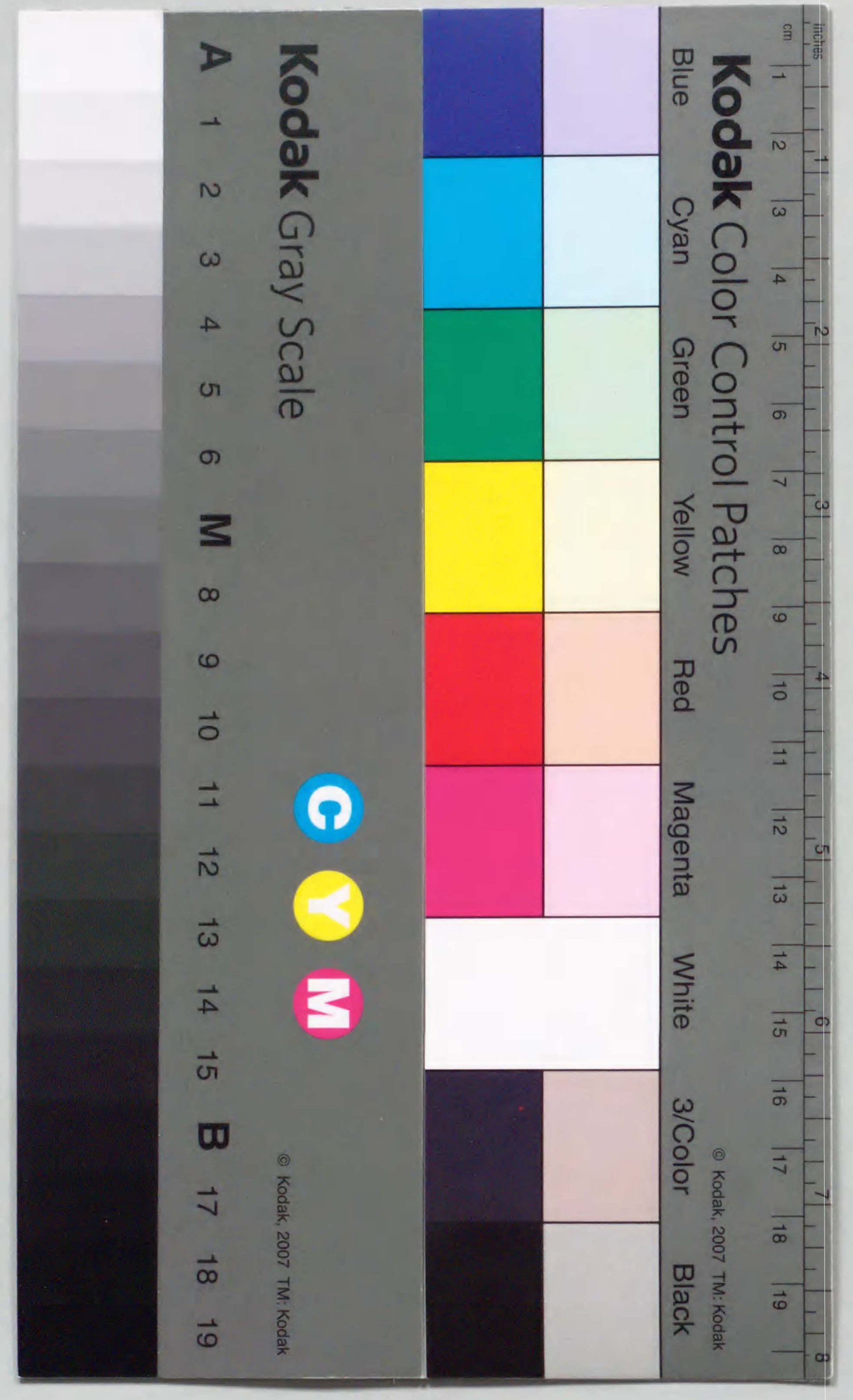

NATURAL ABUNDANCES OF NITROGEN AND OXYGEN STABLE ISOTOPES OF NITROUS OXIDE IN GROUNDWATERS AND OTHER ENVIRONMENTS: AS TRACERS FOR THEIR GENERATION

1992

THE UNITED GRADUATE SCHOOL
TOKYO UNIVERSITY OF AGRICULTURE AND TECHNOLOGY
SCIENCE OF RESOURCES AND ENVIRONMENT

SHINGO UEDA

NATURAL ABUNDANCES OF NITROGEN AND OXYGEN STABLE ISOTOPES OF NITROUS OXIDE IN GROUNDWATERS AND OTHER ENVIRONMENTS: AS TRACERS FOR THEIR GENERATION

A DISSERTATION
SUBMITTED TO THE UNITED GRADUATE SCHOOL
TOKYO UNIVERSITY OF AGRICULTURE AND TECHNOLOGY
IN PARTIAL SATISFACTION OF THE REQUIREMENTS
FOR THE DEGREE DOCTOR OF PHILOSOPHY
IN ENVIRONMENTAL SCIENCE

by

SHINGO UEDA

1992

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Acknowledgments

I wish to devote my heartily thanks to Prof. N. Ogura, TUA&T. His uncoutable suggestions and generous encouragements were invaluable to finish my Ph.D. work. I learned the true role of a scientist through his vital activity in both scientific and social matters.

I wish to devote my acknowledgment to Prof. E. Wada, Kyoto Univ. and the previous Director of Mitsubishi Kasei Institute of Life Sciences. This dissertation would not be possible without much of stimulating discussion with him. He lectured me the fascination and power of the stable isotope study. I appreciate his strategy in the field of biogeochemistry.

I would like to express my gratefulness to Dr. T. Yoshinari, New York State Department of Health and SUNYA. I am very much proud that I could work with the pioneering investigator of nitrous oxide. Nothing compares to the exciting discussion at his laboratory and also by lots of letters after my leaving Albany to date.

T. Asami, Ibaraki Univ., and M. Okazaki, TUA&T critically reviewed this dissertation. N. Yoshida, Toyama Univ. advised me a lot to improve the thesis. K. Minami, National Institute of Agro-Environmental Sciences and M. Tamaki, Environmental Science Institute of Hyogo Pref. kindly sent me literatures I asked.

I thank to staffs of the Laboratory of Social and Environmental Sciences, MKILS, M. Minagawa, H. Mizutani, T. Tsuji, N. Naitoh, S. Egawa, and Y. Kabaya. T. Yoshioka, M. Uzaki, A. Sugimoto gave me helpful suggestion during their working as post doctoral fellows at the laboratory.

B. Fry, The Ecosystem Center, MBL permitted me to use his mass spectrometers to analyze US groundwater samples. C. Hopkinson and S. Weinghlight helped me in technical aspects there. Thanks must be devoted to L.A. Codispoti and G. Friedelich, Monterey Bay Aquarium Research Institute to determine sea water samples.

R.L. Smith, US Geological Survey reviewed my draft for publication in GRL. K. Mashimo and R. Hamada, TUA&T gave me facility for preparing its camera-ready manuscript.

I. Koike, Tokyo Institute of Oceanography kindly lent me a TCD. N. Nakamoto, Shinshyu Univ., and T. Hojo, Nagano-Ken Kankyo Gijyutsu Center, helped me in field at Nagano Pref..

I appreciated great friendship of H. Ogawa and J.-M. Oh in both scientific and private aspects. H. Kato and R. Miyazaki assisted with sampling and chemical analyses of groundwaters. C.-S. Ko and Y. Shiomi helped me in preparing illustrations. It's my pleasure to work with all other colleagues of the Laboratory of Soil and Aquatic Environmental Sciences, TUA&T, especially H. Takada, K. Onozaki, H. Tatsuzawa, S. Onozaki, I. Suzuki, S. Shimizu, J. Tamura, K. Saeki, M. Kobayashi, T. Usui, and S. Takeshima.

Special thanks must be due to people who kindly gave me well water samples and also to my research vehicles, *Lite Ace* & *Acty*. Finally, let me express my heartily thank to my parents who trusted and encouraged me at anytime.

Personal Data

The author was born in downtown Yokohama on July 6, 1962 as an only child of his parents. He moved to Omiya with his family in 1972. He was getting a mischievous kid by being familiar with variety of wildlife there. He received a bachelor's degree in environmental science from Tokyo University of Agriculture and Technology in 1987. During a master course student, he studied nitrogen metabolism in the hypertrophic Lake Teganuma in particular for sedimental denitrification. At the later half of the master course, he was interested in the accumulation of nitrous oxide in groundwaters. After the author entered the United Graduate School of TUA&T, he began stable isotope study to think about origin of the gas. Eight months from November in 1989, the author got a chance to work as a participant in the project of nitrogen and oxygen stable isotopic characterization of marine N_2O, which was financially supported by NSF grant to Drs. T. Yoshinari and L.A. Codispoti. He got much of scientific and cultural stimuli there, but little progress in English. In effect, he likes to laze time away at sunny spots best.

Publications

<u>Journals:</u>

Ueda, S. and N. Ogura, Determination of denitrification activity in bottom sediment of Lake Teganuma and its role in selfpurification of the lake, *Jpn. J. Limnol.*, *50*, 15-24, 1989 (in Japanese with English abstract).

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Ueda, S and N. Ogura, Transaction of the 1987 annual meeting of the *Japanese Society of Limnology*.

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Yoshinari T., S. Ueda, L. Codispoti, and G. Friedlich, Nitrogen and oxygen isotope composition of N_2O in the water column of Monterey Bay, *EOS 71*, 1413, 1990 (Transactions of the *American Geophysical Union*).

Abstract of the Dissertation

In this dissertation, origins of nitrous oxide (N_2O) in different environments with emphasis of a groundwater were discussed from the view point of natural abundances of stable isotopes. The study intended to develop the application of nitrogen and oxygen isotopic characterization of environmental N_2O to realizing the biogeochemical N_2O cycle.

New methods to analyze both nitrogen and oxygen isotopic compositions in N_2O were developed. N_2O was converted to N_2 and CO_2 by using copper or graphitic carbon as a reductant. $^{15}N/^{14}N$ in N_2 and $^{18}O/^{16}O$ in CO_2 were measured mass spectrometry, then the abundances of stable isotopes were expressed as variations from standard in terms of δ-values in per mil notation. Atmospheric N_2 and Standard Mean Ocean Water were used as the standard. Analytical precision of $\delta^{15}N$ and $\delta^{18}O$ were ± 0.2 and ± 0.5 ‰, respectively.

N_2O concentrations in 61 groundwaters in Kanto Plain, Nagano Prefecture and New York State were 0.02 to 7 μM, corresponding 1.3 to 540 times of the atmospheric equilibria at *in situ* water temperatures. These groundwaters were oxygenated (20-300 μM) and contaminated with NO_3^- (530 μM in average). Low concentrations of dissolved organic carbon (40 μM in average) and depletions of NO_2^- and NH_4^+ suggest an inhibition of nitrate respiration in the aquifers. $\Delta N_2O/NO_3^-$ molar ratios (0.02-0.9 %) in the groundwaters, where "Δ" means measured concentration minus atmospheric equilibrium at *in situ* water temperature, agreed with the reported N_2O yield in aerobic nitrification (< 1 %). $\delta^{15}N$ of N_2O and NO_3^- in the groundwaters were -32 to +3 ‰ and -1 to +8 ‰, respectively. $\delta^{18}O$ of the groundwater N_2O ranged from +31 to +42 ‰, with exceptions of the lowest (+22 ‰) and the highest (+50 ‰) values. Since $\delta^{18}O$ of +3 to +10 ‰ was reported for NO_3^- in oxic groundwaters, the N_2O could be characterized by its ^{15}N-depletion and ^{18}O-enrichment with respect to the coexistent NO_3^-. In the light of evidence that *Nitrosomonas europaea* produces ^{15}N-depleted N_2O to a substrate NH_4^+ and an end product NO_2^-, the nitrogen isotopic findings suggest nitrification as a major source for the gorundwater N_2O, because the $\delta^{15}N$ of NO_3^- in an oxic groundwater is equal to that of the source material. If the groundwater N_2O with lower $\delta^{15}N$ than NO_3^- were the product of an initial stage of denitrification, when the $NO_3^- \rightarrow N_2O$ step overwhelms the $N_2O \rightarrow N_2$ in rate, such N_2O should be depleted in ^{18}O as compared with the NO_3^-. Thus, the N_2O with higher $\delta^{18}O$ than NO_3^- could not be explained by assuming denitrification as a mechanism of N_2O production. Therefore, the two-isotopic data identified nitrification to be a dominant mechanism producing the N_2O accumulating in oxic groundwaters. As nitrate contaminated groundwaters globally spread, the results depict groundwater to be evaluated as a biogeochemical pathway of N_2O.

At the Monterey Bay, California, depth profiles of N_2O, NO_3^- and O_2 suggested nitrification as a source for N_2O (0.01-0.05 μM) through the water column. There were two of $\delta^{15}N$-$\delta^{18}O$ maxima just below a photic layer and at O_2 minimum in an aphotic zone. The shallow maximum ($\delta^{15}N$ of +8 ‰, $\delta^{18}O$ of +42 ‰) was a result of nitrification of heavier isotopically enriched NH_4^+ and, which were owing to an active regeneration of nutrients at the expense of O_2 within the photic zone. The deep maximum ($\delta^{15}N$ of +9 ‰, $\delta^{18}O$ of +47 ‰) was due probably to N_2O production via nitrifier denitrification under low O_2 stress, in which NO_2^- is used as a terminal electron acceptor in place of O_2. In an oxic surface layer of the Fayettiville Green Lake, New York, ^{15}N depletion in N_2O (-3 to -6 ‰) was demonstrated as it accumulated with depth (0.02-0.07 μM). In the Lake Teganuma, Chiba, N_2O (0.08 μM) with a negative $\delta^{15}N$ (-1 ‰) was observed in the upper basin water where N_2O and NO_3^- were produced at consumption of riverine NH_4^+. At the lower basin, where an active denitrification in sediment had been revealed, N_2O concentration decreased (0.02 μM) and high $\delta^{15}N$ (+15 ‰) was obtained. In the River Minami-Asakawa Tokyo, significant increase of N_2O concentration (0.25 μM) with concurrent depletion of $\delta^{15}N$ (-38 ‰) was observed in the lower reaches of the stream, where active nitrification of an externally loaded NH_4^+ had been confirmed by the nitrogen mass balance. According to the above $\delta^{15}N$ determination, aquatic N_2O can be explained by productions of ^{15}N-depleted N_2O via nitrification and ^{15}N-enriched N_2O via denitrification.

Two-isotope map of N_2O from different environment was compiled. The tropospheric N_2O ($\delta^{15}N$ of +7 ‰, $\delta^{18}O$ of +36 ‰) is similar in $\delta^{15}N$ to the oceanic N_2O ($\delta^{15}N$ of + to +9 ‰), whereas close in $\delta^{18}O$ to the groundwater N_2O ($\delta^{18}O$ of +31 to +42 ‰). Combustion origin N_2O ($\delta^{15}N$ of -23 to +2 ‰, $\delta^{18}O$ of +15 +17 ‰) was lighter in both $\delta^{15}N$ and $\delta^{18}O$ than the ambient atmospheric one. Because the isotopic composition of the tropospheric N_2O is controlled by relative source strength of the above sources isotopically distinguished and possibly by an isotope effect in N_2O decomposition in the stratosphere, such two-isotopic characterization of N_2O is believed to help in constructing a reliable budget of N_2O as well as in identifying reason for the tropospheric accumulation.

CHAPTER I

GENERAL INTRODUCTION AND LITERATURE REVIEW

I-1. INTRODUCTION

Many places in the world have suffered pollutions of airs, waters and soils after the industrial revolution in the late 18th century. They are mainly attributed to waste materials originated form varieties of mankind practices including mining and manufacturing industries, agricultural practices, and domestic affairs as well. These rather regional deteriorations of natural environments can be resolved or improved to some extent by human intelligence tagged with modern technologies. And today, we are facing world-wide rapid environmental changes we never experienced. Such phenomena as forest decline, acid rain, climate change, destruction of ozone shield are called global environmental matters. All of these are not independent but significantly affected by each other. The global environmental matters are international, intercontinental and inter-cultural issues. We do must manage our planet before the Earth will be inhabitable world.

It is widely known that the concentrations of some atmospheric trace gases have changed steadily, mostly increased, over the last two decades, which is believed to disturb the atmosphere's stability. They are principally the tropospheric elevation of carbon dioxide, methane, nitrous oxide and chlorofluorocarbons, and the depletion of ozone in the stratosphere. These trace gases accumulated in the troposphere trap more planetary infrared radiation, then return the heat to the Earth. That will result in temperature increase of the Earth's surface, which is so called "global warming". The present green house effect of the tropospheric trace gases other than carbon dioxide rivals or even exceeds that of the carbon dioxide alone. The stratospheric ozone depletion causes more perturbation of UV light from the space to the planet's face, which is hazardous to life.

Nitrous oxide must be paid careful attention because of its participation in both the global warming and the stratospheric ozone-chemistry. Despite of intensive studies, it must be said that a reliable estimate of the global budget of nitrous oxide has yet to be established. The failure is due mainly to difficulties in estimating the source strength because of variety and complexity in generation of the gas. Since nitrous oxide is produced larger from biological process than abiotic ones, both temporal and spatial fluctuations of circumstantial conditions strongly affect the emission of nitrous oxide to the atmosphere. Thus, full understanding the breath of the Gia is vital to protect life on the Earth from the expected damage.

It is believed that stable isotope finger print is a suitable clue to realize origin and behavior of nitrous oxide in natural environments. This dissertation mainly deals with nitrous oxide dissolved in groundwater, "a forgotten compartment in the biogeochemical cycle". The nitrous oxide is isotopically characterized, then its source mechanism is identified. By performance of case studies in some different environments, rather extensive discussion on development of stable isotope study on nitrous oxide is achieved.

In this chapter, a literature review will be performed to clarify the contribution of this thesis in the field of biogeochemistry of nitrous oxide. In the first half, I will describe the previous knowledge on biogeochemical cycle of nitrous oxide with emphasis on its microbial aspects. The discussion includes an atmospheric chemistry of nitrous oxide and its effects on the progressing climate change. I will secondly assert an importance of stable isotope signatures in constructing the global budget of nitrous oxide after an introduction of general aspects of stable isotope study. Then, the goal and contents of the dissertation will appear.

I-2. BIOGEOCHEMICAL CYCLE OF NITROUS OXIDE

Atmospheric Concentration

Nitrous oxide (N_2O) is the most abundant nitrogen oxides in the present atmosphere. A thermodynamic equilibrium calculation based on the composition of major constituents in the present atmosphere (N_2 = 0.78 atm., O_2 = 0.21 atm., H_2O = 0.01 atm., and $CO_2 = 3.3 \times 10^{-4}$ atm.) gives a mixing ratio of N_2O in the surface atmosphere of 2×10^{-19} [Chameides and Davis, 1982], whereas the worldwide measurements of tropospheric N_2O concentration has converged a mixing ratio of 3×10^{-7} (Table I-1). The overwhelmingness of measured concentration (3×10^{-7}) to the calculated (2×10^{-19}) means that almost all N_2O was produced in the biosphere [Levine and Augustsson, 1985].

There is no doubt that the mole fraction of N_2O in the troposphere is now increasing by annual rate of 0.2-0.4 $\% \cdot y^{-1}$ (Table I-1). Analysis of vertical profiles of N_2O concentration in bubble air occluded in the Antarctic ice core revealed that the elevation had begun after the Industrial Revolution [Pearman *et al.*, 1986]. Weiss [1981] reported higher mixing ratio of tropospheric N_2O in the northern hemisphere than the southern hemisphere by 0.77 ppbv. A slightly larger difference of 1.97 ppbv was observed by Butler *et al.* [1989]. Such interhemispheric difference in N_2O concentration is probably reflect the fact that human activity is localized in northern hemisphere. These observations reflect the fact that the elevation of tropospheric N_2O concentration closely relates to expansion of human activities.

Atmospheric Chemistry

N_2O is chemically inert in the troposphere against both an ultraviolet radiation (UV) and a hydroxyl radical (OH). Since N_2O forms no ionic chemical species, unlike other nitrogen oxides, the gas is not easily washed out by rainfall. There is no identified sink in the troposphere, then almost all N_2O emitted to the troposphere will ultimately enter the stratosphere.

In the stratosphere, N_2O is known to dessociate mainly by photolysis after a reaction (1) and partly though reactions with .pn6

TABLE I-1 Tropospheric Concentration of N_2O and its Annual Increase

Period	mean Conc. (ppbv)	Annu. Inc. (pppv)	(%)	n	Location	[3]Anal.	[4]Ref.
1938	400	–	–	[2]NA		IR	1
1949	470	–	–	NA	Texas	MS	2
1953	350	–	–	NA	Arizona, England	IR	3
1961	300	–	–	NA	N.Atlantic	TCD	4
1961-1974	1) 299	0.5	0.15	NA	Pacific	TCD	5
1967-1969	246-292	–	–	NA	Germany	TCD	6
1970-1971	247-280	–	–	NA	N.Atlantic	TCD	7
1970-1971	285	–	–	NA	Germany	TCD	7
1972	328	–	–	20	Atlantic, Caribbean	HID	8
1974	296	–	–	NA	California	USD	9
1975	340	–	–	21	Arctic	ECD	10
1975-1980	1) 330	1.2	0.4	100	S.Pole, Oregon	ECD	11
1975-1985	307	1.0	0.3	NA	S.Pole, Oregon	ECD	12
1976	332	–	–	212	Pacific	ECD	13
1976	329	–	–	207	N.America, Pacific	ECD	14
1976	330	–	–	148	Alaska, New Zealand	ECD	10
1976	328	–	–	9000	Washington	ECD	10
1976	312	–	–	59	California	ECD	15
1976	329	–	–	17	Oregon	ECD	16
1976	306	–	–	27	N.Hemisphere	ECD	17
1976	314	–	–	52	S.Hemisphere	ECD	17
1976	280	–	–	NA	England	ECD	18
1976-1977	326	–	–	75	Colorado	ECD	19
1976-1977	330	–	–	47	Michigan	ECD	20
1976-1978	338	–	–	NA	Australia	ECD	21
1976-1979	1) 316	1.8	0.6	14		ECD	22
1976-1980	1) 299	0.5	0.2	418	N.Hemisphere	USD	23
1977	287	–	–	12	E.T.N.Pacific	ECD	24
1977	294	–	–	14	Massachusetts	ECD	25
1977	327	–	–	14	Japan	TCD	26
1977	316	–	–	NA	Pacific	USD	27
1977	305	–	–	NA	Iowa	USD	28
1977-1978	300	–		12	Great Lakes	ECD	29
1978-1979	310	–	–	90	Japan	ECD	30
1977-1980	1) 299	0.8	0.3	600		ECD	31
1978-1981	1) 325	1.5	0.4	140		ECD	32
1979-1981	1) 303	0.9	0.3	500		ECD	33
1979-1982	1) 302	0.8	0.3	9000	Oregon, Tasmania	ECD	34
1980	298	–	–	26	Colorado	IR	35
1980	332	–	–	NA	E.T.Pacific	ECD	36

TABLE I-1 continued

Period	mean Conc. (ppbv)	Annu. Inc. (pppv)	(%)	n	Location	[3]Anal.	[4]Ref.
1980	319	–	–	31	Kobe	ECD	37
1980-1983	315	–	–	48	Japan, N.Pacific	VM	38
1982	325	–	–	60	Japan	ECD	39
1982	311	–	–	4300	Kobe	ECD	40
1982-1985	301	0.7	0.24	190	Antarctica	ECD	41
1986	311	–	–	15	T.Atlantic	USD	42
1988-1988	305	0.9	0.3	2000	W.Pacific, E.Indian	ECD	43

[1] Value extrapolated to Jan. 1975 by by Kahlil and Rasmussen [1983].

[2] NA: not available

[3] Analytical methods: IR-infrared absorbance; MS-mass spectrometry; TCD-thermal conductivity detector gas chromatography; HID-helium ionization detector gas chromatography; USD-ultrasonic detector gas chromatography; VM-volumetry.

[4] This table is mainly after the reviews by Khalil & Rasmussen [1983] and Tamaki [1983]. Literature cited are as follows: 1. Adel [1938]; 2. Slobod [1950]; 3. Goody & Walshaw [1953]; 4. Craig [1963]; 5. Craig *et al.* [1976]; 6. Schultz *et al.* [1970]; 7. Hahn [1974]; 8. Yoshinari [1976]; 9. Weiss & Craig [1976]; 10. Pierotti *et al.* [1977]; 11. Rasmussen *et al.* [1981]; 12. Rasmussen & Khalil [1986]; 13. Rasmussen *et al.* [1976]; 14. Pierotti & Rasmussen [1978]; 15. Singh *et al.* [1977]; 16. Rasmussen *et al.* [1978]; 17. Tyson *et al.* [1978]; 18. Brice *et al.* [1977]; 19. Goldan *et al.* [1978]; 20. Roy [1979]; 21. Goldan *et al.* [1980]; 22. Weiss [1981]; 23. Cicerone *et al.* [1978]; 24. Cohen & Gordon [1978]; 25. Kaplan *et al.* [1978]; 26. Hirota [1978]; 37. Singh *et al.* [1979]; 28. Matthias *et al.* [1979]; 29. Lemon & Lemon [1981]; 30. DeLuisi [1981]; 31. Cronn *et al.* [1982]; 32. Muramatsu *et al.* [1981]; 33. Khalil & Rasmussen [1981]; 34. Khalil & Rasmussen [1983]; 35. Connell *et al.* [1980]; 36. Pierotti & Rasmussen [1980]; 37. Hiraki *et al.* [1980]; 38. Yoshida [1984]; 39. Hirota & Muramatsu [1982]; 40. Hiraki *et al.* [1983]; 41. Cronn *et al.* [1986]; 42. Oudot *et al.* [1990]; 43. Butler *et al.* [1989]

electrically excited singlet oxygen, (2) and (3).

$$N_2O + h\nu \rightarrow N_2 + O(^1D) \qquad < 240\ \mathrm{nm} \qquad \ldots (1)$$

$$N_2O + O(^1D) \rightarrow N_2 + O_2 \qquad \ldots (2)$$

$$N_2O + O(^1D) \rightarrow 2\ NO \qquad \ldots (3)$$

The reaction (3) is the major source for the stratospheric nitric oxide (NO) that controls the photochemical destruction of the stratospheric ozone (O_3) via the NO_x catalytic reaction [McElroy and McConnell, 1971; Crutzen, 1976; Liu *et al.*, 1976]. NO is once generated, stratospheric O_3 is decomposed via a cyclic reaction, (4) and (5). The net reaction is given as an equation (6).

$$NO + O_3 \rightarrow NO_2 + O_2 \qquad \ldots (4)$$

$$NO_2 + O(^1D) \rightarrow NO + O_2 \qquad \ldots (5)$$

$$O_3 + O \rightarrow 2\ O_2 \qquad \ldots (6)$$

The stratospheric ozone is vital to all living things, because the gas absorbs the short-wave ultraviolet radiation that is harmful for life. It is estimated that the 25 % increase of the tropospheric concentration of N_2O will result in decrease of the stratospheric O_3 concentration by 0.7 to 3.9 % with an average value of 2.2 % [Nicoli and Visconti, 1982; Callis *et al.*, 1983; Owens *et al.*, 1985; Isaksen and Stordal, 1986; Kinnison and Jhonston; 1988]. While multiple consuming process of N_2O have been suggested, reactions (1), (2), and (3) are thought to be the biggest sink in the geochemical cycle of N_2O. The annual decomposition of N_2O in the stratosphere is estimated at 11 Tg (10^{12} g) $N_2O\text{-}N \cdot y^{-1}$ [Crutzen, 1983; Cicerone, 1987]. If the stratospheric decomposition is an only evaluative sink for the tropospheric N_2O, which is probably correct, the atmospheric life-time is calculated as long as over one hundred year (Table I-2). Temporal and spatial well mixing of tropospheric N_2O [Levy *et al.*, 1979; 1982] may be due to such long residence time of N_2O in the troposphere.

TABLE I-2 Atmospheric Life-time of N_2O

Investigators	year
Schutz et al. [1970]	70
Junge [1974]	8-12
McElroy et al. [1976]	10-20
Sze & Wu [1976]	10-100
Schmeltekopf et al. [1977]	100
Rasmussem & Pierotti [1978]	11-30
Levy et al. [1979]	150
Weiss [1981]	100-140
Levy et al. [1982]	131
Ko & Sze [1982]	159
Crutzen, P.J., & U. Schmailzl [1983]	155-240
Jackman & Guthrie [1985]	156-196
Jackman et al. [1988]	120-134

Role in Climate System

The Earth's heat budget is controlled by the balance between infrared solar radiation in short wavelength (0.3-4 μm) and long-wave infrared re-radiation (4-100 μm) from the surface of the Earth. Both planetary radiations shorter than 8 μm and longer than 12 μm are absorbed by the abundant water vapor. The atmospheric carbon dioxide absorb the re-radiation of wavelength in-between 12 and 18 μm. The planetary radiation that is trapped by the water vapor and carbon dioxide heat the Earth, which is so called "green house effect" preventing drastic drop of temperature in the biosphere during the night time. The remaining spectral region from 8 to 12 μm is called as the "atmospheric window" (Fig. I-1).

What is surprising is that growth in concentrations of trace atmospheric gases including CH_4, N_2O, O_3 and CFCs have serious affection on the climate system besides of their less abundances, because those gases have strong absorption bands in the atmospheric window region. It is expected that the 25 % increment of tropospheric N_2O will cause the elevation of tropospheric temperature by 0.04 to 0.11 K with the mean value of 0.09 K [Yung *et al.*, 1976; Wang *et al.*, 1976; Wang and Sze, 1980; Donner and Ramanathan, 1980; Lacis *et al.*, 1981; Nicoli and Visconti, 1982; Callis *et al.*, 1983; Ramanathan *et al.*, 1985; Dickinson and Cicerone, 1986].

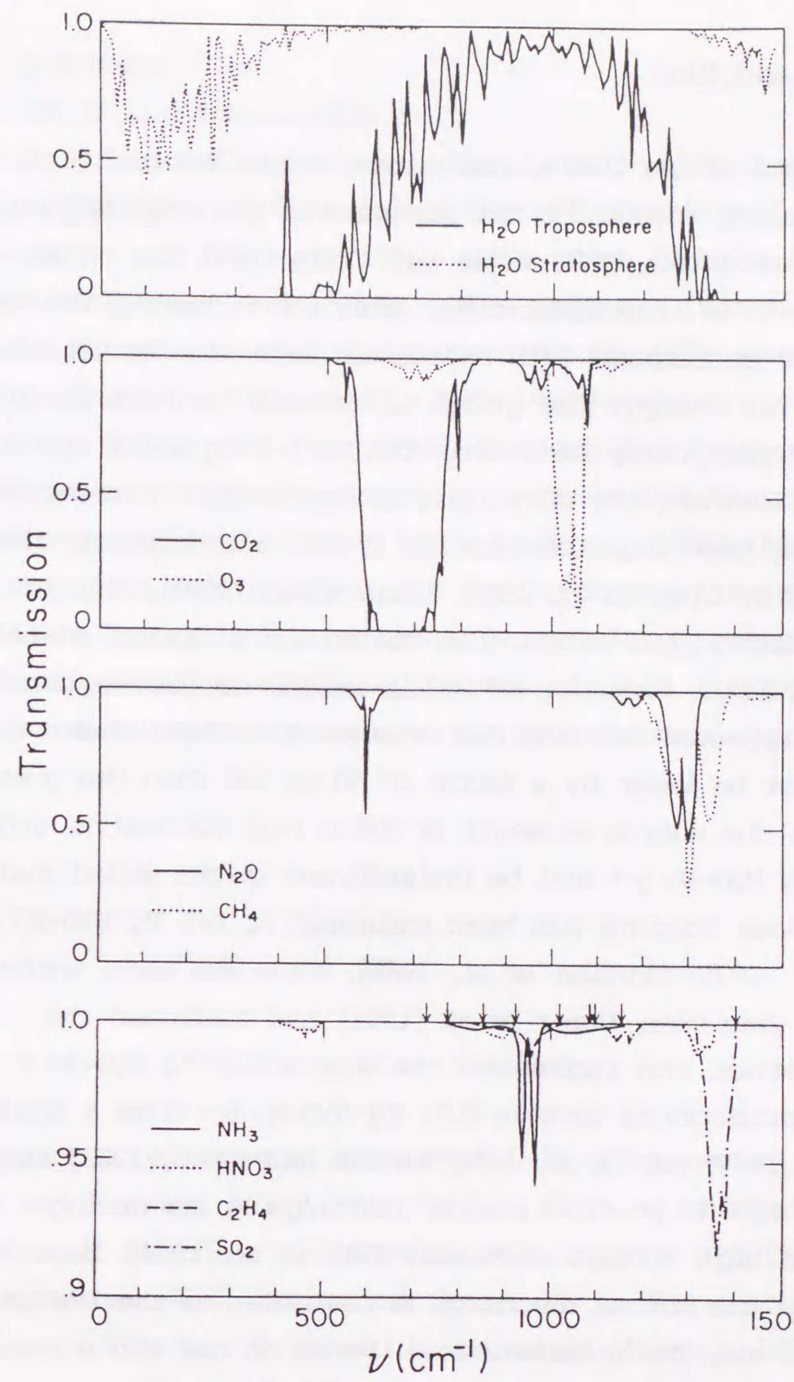

Figure I-1 "Atmospheric Window" and the transmission of thermal radiation by N_2O. From *W.C. Wang et al.* [1976] in *Science*. Water vapor effectively blocks the planetary radiation except for the "window", 700-1400 cm^{-1} (7-14 μm). Gases with absorption band within this region, making the window dirty, have significant effect on the global warming.

Abiotic Source and Sink

A. Source

Until the end of the 1980's, combustion origin N_2O had been considered as a top reason for the elevation in the troposphere [Pierotti and Rasmussen, 1976; Weiss and Craig, 1976, Hao *et al.*, 1987]. The growth of tropospheric N_2O after the Industrial Revolution indeed seems to correspond with a dramatic expansion in use of coal and petroleum for energy. The global N_2O release from the fossil fuel combustion had previously been estimated at 2-3 Tg $N_2O\text{-}N \cdot y^{-1}$ in the above papers. However, the surprising overestimation in these earlier assessments has been depicted recently [Muzio and Kramlich, 1988; Muzio *et al.*, 1989; Lyon *et al.*, 1989; Linak *et al.*, 1990]. This is due to an artifact in gas sampling in the earlier sutudies, which results in significant formation of N_2O in sample containers. Linak *et al.* [1990] suggested that the N_2O emission from fossil fuel combustion must be lower by a factor of 50 to 100 than the previous estimate. If so, the source strength of fossil fuel combustion should be 0.02-0.06 Tg $N_2O\text{-}N \cdot y^{-1}$ and be insignificant in the global budget. Flux from biomass burning has been estimated at 1-2 Tg $N_2O\text{-}N \cdot y^{-1}$ [Crutzen *et al.*, 1979; Crutzen *et al.*, 1985]. While the same artifact is expected in this case, Cofer *et al.* [1991] has confirmed the earlier assessments, and recommend the flux of ~1 Tg $N_2O\text{-}N \cdot y^{-1}$. Other abiotic productions involve 0.01 Tg $N_2O\text{-}N \cdot y^{-1}$ from a lightning [Levine *et al.*, 1979; Hill *et al.*, 1984; Levine and Shaw, 1983] and 0.02 to 0.5 Tg $N_2O\text{-}N \cdot y^{-1}$ from corona discharge in air during transmission of high voltage electricity [Hill *et al.*, 1984]. Because the number for the corona discharge is restricted in the United States alone, it may be underestimated. Based on the above estimates, the global abiotic source of 1-2 Tg $N_2O\text{-}N \cdot y^{-1}$ is probable.

B. Sink

Abiotic sinks for atmospheric N_2O excepting the stratospheric decomposition are photochemical and thermal decomposition of N_2O at surfaces of particulate matters in the troposphere [Rebbert and Ausloos, 1978; Pierotti *et al.*, 1978]. However, its global significance is not exactly known, but is probably negligible.

Microbial Aspects

A. Production and consumption

N_2O is produced by various kinds of microorganisms involving nitrifiers [Yoshida and Alexander, 1970; 1971; Richie and Nicholas, 1972; Bremner and Blackmer, 1978], denitrifiers [Delwiche, 1970, Knowles, 1982], non-denitrifying nitrate reducers [Yoshinari, 1980; Smith and Zimmerman, 1981; Kaspar and Tiedje, 1981; Kaspar, 1982; Bleakley and Tiedje, 1982; Smith, 1982; 1983; Anderson and Levine, 1986], fungi [Bollag and Tung, 1972; Bleakley and Tiedje, 1982], green algae [Weathers, 1984], cyanobacteria [Wethers and Niedzielski, 1986] and methane oxidizers [Hanson, 1980; Yoshinari, 1985]. Among these, nitrification and denitrification are the predominant routes.

N_2O is a by-product in an ammonia oxidation to nitrite and is never incorporated in nitrifying process anymore. Following reactions are believed as the mechanisms of N_2O production in nitrification.

$$\begin{tikzcd}
 & \text{(I)} & \text{(II)} & & \\
 & N_2O & N_2O & & \\
 & NOH \arrow[u] & NO \arrow[u] & & \\
NH_3 \arrow[r] & NH_2OH \arrow[r] \arrow[u] & NO_2^- \arrow[r] \arrow[u] & NO_3^- & \ldots\ (7)
\end{tikzcd}$$

There are two pathways to generate N_2O by *Nitrosomonas europaea* [Hynes and Knowles, 1984]. The reaction (I) is an abiological production of N_2O via a hypothetical precursor of NOH [Falcone *et al.*, 1963; Anderson, 1964; Hooper and Terry, 1979]. The reaction (II) is a reduction of NO_2^- to N_2O by nitrite reductase [Hooper, 1968; Richie and Nicholas, 1972; 1974]. While fairly constant yields of N_2O to NO_2^- in molar basis from 0.02 to 0.5 % are observed for *N. europaea* in an aerobic condition, low oxygen concentration ($< 56\ \mu M\ O_2$) enhances the production up to 5 % [Goreau *et al.*, 1980; Lipschultz *et al.*, 1981]. The yield increment is believed as a result of change in dominant pathway of N_2O production from (I) to (II). The reaction (II), which is occasionally called "nitrifier denitrification" [Poth and Focht, 1985], is recognized as an alternative utilization of NO_2^- as a terminal electron acceptor in place of O_2 under condition of oxygen stress. Consequently, N_2O yield in nitrification changes with availabilities of oxygen.

Denitrification is presented as a following sequential reaction [Knowles, 1982].

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \qquad \text{... (8)}$$

The N_2O produced as an obligate intermediate will eventually be reduced to N_2. Because a nitrous oxide reductase is an inducible enzyme, size of N_2O pool depends on the dynamics of the reaction governed by environmental factors, such as redox potential, pH value, temperature and availability of NO_3^- [Focht, 1974]. The final step in denitrification ($N_2O \rightarrow N_2$) is a possible biological sink for N_2O. Consumption of N_2O in soils [Brice *et al.*, 1977; Freney *et al.*, 1979; Ryden *et al.*, 1981], wetland sediment [Richey *et al.*, 1988], paddy rice field [Minami and Fukushi, 1984], and in aquatic systems [Cohen, 1978; Cohen and Gordon, 1978; Elkins *et al.*, 1978] must be attributed to thus microbial denitrifying activities. However, an unimportant sink strength of denitrification for the atmospheric N_2O is reasonable, because of too low concentration of N_2O in an ambient air for denitrifying bacteria to utilize. Additionally, there must be a competition in utilization by bacteria between the tropospheric N_2O and other electron acceptors in soil, such as nitrate, nitrite and elevated N_2O in the soil air as well.

In many of natural environments, nitrification and denitrification are seldom isolated in space, but commonly close to each other or even overlapped. Coupling of nitrification and denitrification was revealed in aquatic sediments [Jenkins and Kemp, 1984]. This indicates, in turn, the simultaneous contribution from these two processes on *in situ* N_2O pool. It should be noted that N_2O which is produced whether by nitrification or denitrification is involved as a terminal electron acceptor for denitrification. So that, it is hard to evaluate the relative contribution in N_2O production between nitrification and denitrification in nature. A conceptual model named "*Leaky pipes*" (Fig. I-2), which was originally

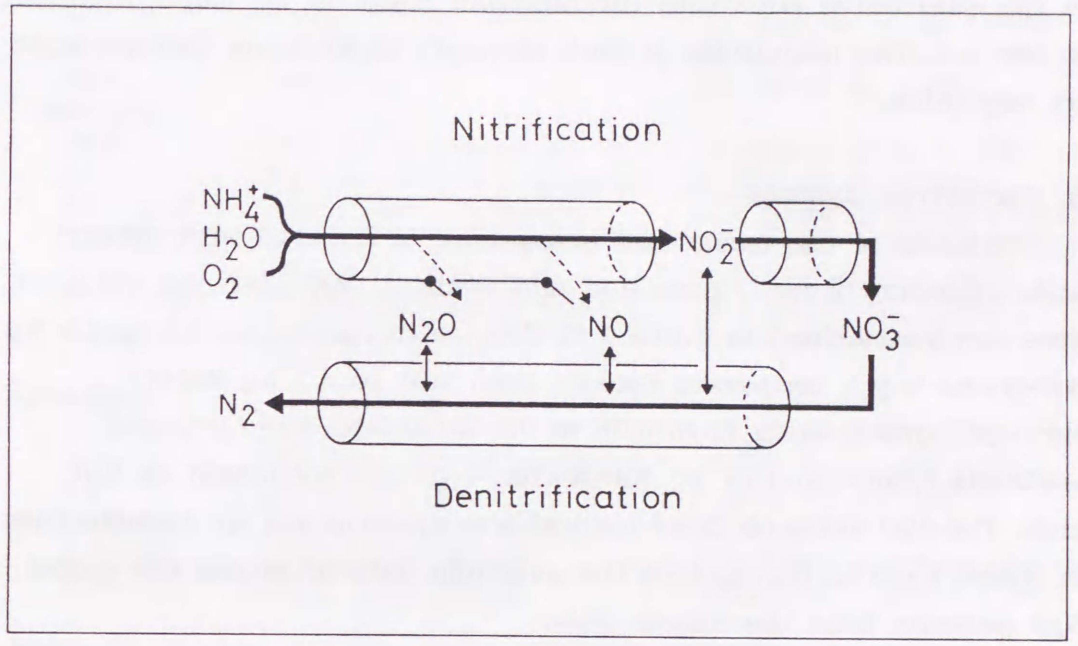

Figure I-2 "Leaky Pipes" conceptual model of *Mary K. Firestone*. The illustration from *O.C. Zafiliou* [1990] in *Nature* presents N_2O metabolism by nitrification-denitrification by three metabolically discrete types of microorganisms. It nicely demonstrates that the N_2O leaked by ammonia-oxidizing bacteria is possibly incorporated by denitrification reaction that also emits N_2O. Thus the source elucidation of natural environmental N_2O between nitrification and denitrification is commonly difficult.

presented by Mary K. Firestone, nicely demonstrates confusion in identifying mechanisms of N_2O production in natural environments. As a result, bulk N_2O emission from ecosystems is highly variable. Nitrogen fixing bacteria can incorporate N_2O ($N_2O \rightarrow NH_4^+$) [Hardy and Knight, 1966; Yamazaki *et al.*, 1987]. However, consumption of the atmospheric N_2O by nitrogen fixation is in question, because N_2 which is the alternative substrate for nitrogen fixers is far much abundant in the air. The magnitude of sink strength of nitrogen fixation must be negligible.

B. Terrestrial Systems

Majority of the terrestrial ecosystems is covered with natural soils including forest, grassland and wetland. Reported N_2O emission rate are summarized in Table I-3. Tropical forests from 1.1 to 2.5 kg $N_2O\text{-}N \cdot ha^{-1} \cdot y^{-1}$, temperate forests from 0.01 to 3.2 kg $N_2O\text{-}N \cdot ha^{-1} \cdot y^{-1}$, grass lands from 0.06 to 9.1 kg $N_2O\text{-}N \cdot ha^{-1} \cdot y^{-1}$, and wetlands from 0.06-13.1 kg $N_2O\text{-}N \cdot ha^{-1} \cdot y^{-1}$ are calculated as N_2O flux. The N_2O emission from natural soil systems are so variable that it seems hard to extrapolate the available data to assess the global N_2O emission from the native soils.

There are numbers of reports on N_2O emissions from fertilized soils. Table I-4, mainly after Eichner [1990], lists the percent N_2O release from fertilizer nitrogen. The ratios vary over three orders of magnitude according to the kind of fertilizer, but typically less than 1 %. Assuming the average emission ratio of 1 %, an annual N_2O production from a fertilizer nitrogen is estimated at 0.7 Tg $N_2O\text{-}N \cdot y^{-1}$, based on the worldwide consumption of fertilizer-nitrogen of 70 Tg $N \cdot y^{-1}$ in 1985/1986 [FAO, 1986]. Recent increase of cultivation lands accompanied with deforestation and burning of vegetations may directly enhance the emission of N_2O through this pathway.

TABLE I-3 Nitrous Oxide Emission Rates from Native Soils

$\mu gN_2O\text{-}N \cdot m^{-2} \cdot h^{-1}$	Location	Reference
Forest		
Tropical		
13± 28	Amazonia	*Livinglton et al.* [1988]
15.6	Amazonia	*Goreau & deMello* [1986]
28.5	Amazonia	*Keller et al.* [1986]
Temerate		
10.2	New York, hardwood	*Duxbury et al.* [1982]
1.7	New Hampsher, hardwood	*Keller et al.* [1983]
27.7-36.4	Wisconsin, coniferous	*Goodroad & Keeny* [1984]
5.4-15.5	Wisconsin, deciduous	*Goodroad & Keeny* [1984]
3-11	Germany, deciduous	*Schmidt et al.* [1988]
0.17± 0.25	Massachusets, coniferous	*Bowden et al.* [1990]
0.27± 0.28	Massachusets, hardwood	*Bowden et al.* [1990]
Grassland		
25.0-104	grass sward	*Denmead et al.* [1979]
0.7-2.5	Germany, grass sward	*Seiler & Conrad* [1981]
9.4	prairie	*Mosier et al.* [1981]
20.8-58.3	Denmark, grass sward	*Christensen* [1983]
2.2-2.5	Wisconsin, prairie	*Goodroad & Keeny* [1984]
2.3-66.2	UK, grass sward	*Egginton & Smith* [1986]
Wetland		
3.5	Louisiana, salt marsh	*Smith et al.* [1983]
5.5	Louisiana, brackish marsh	*Smith et al.* [1983]
6.3	Louisiana, fresh marsh	*Smith et al.* [1983]
30.6	Wisconsin, wet meadow	*Goodroad & Keeny* [1984]
0.7-1.1	Wisconsin, fresh marsh	*Goodroad & Keeny* [1984]
65-149	Wisconsin, drained-	*Goodroad & Keeny* [1984]

TABLE I-4 Production Ratios of Nitrous Oxide from Nitrogen Fertilizers

Fertilizer type	[1] Percent N_2O emission	Reference
NH_3	0.86 - 6.86	*Bremner et al.* [1981] *Breitenbeck & Bremner* [1986a,b]
NH_4^+	0.04 - 0.12	*Breitenbeck & Bremner* [1986a]
NH_4Cl	0.03 - 0.90	*Conrad & Seiler* [1980] *Seiler & Conrad* [1981] *Conrad et al.* [1983]
$(NH_4)_2SO_4$	0.08 - 0.18	*Breitenbeck et al.* [1980] *Conrad et al.* [1983]
NH_4NO_3	0.04 - 1.71	*Conrad & Seiler* [1980] *Mosier et al.* [1982] *Christensen et al.* [1983] *Slemer et al.* [1984]
$CaNO_3$	0.01 - 1.75	*Breitenbeck et al.* [1980] *Webster & Dowdell* [1982] *Breitenbeck & Bremner* [1986a]
KNO_3	0.02	*Conrad et al.* [1983]
$NaNO_3$	0.00 - 0.50	*Conrad & Seiler* [1980] *Seiler & Conrad* [1981] *Conrad et al.* [1983]
$CO(NH_2)_2$	0.07 - 0.18	*Breitenbeck et al.* [1980a] *Mosier et al.* [1981] *Slemer et al.* [1984] *Beitenbeck & Bremner* [1986]
manure	1.78 - 1.80	*Cates & Keeny* [1987]

[1] Nitrogen basis

C. Aquatic systems

Since the ocean occupies about 70 % of the Earth's surface, it is the biggest site for nitrogen metabolism among various types of hydospheric components. N_2O production from both nitrification [Yoshinari, 1976; Cohen and Gordon, 1979; Elkins *et al.*, 1978] and denitrification [Hahn, 1974; Singh *et al.*, 1979; Pierotti and Rasmussen, 1980], and consumption by denitrification [Cohen, 1978; Cohen and Gordon, 1978; Elkins *et al.*, 1978] were observed in oceanic systems.

Anthropogenic impacts affect freshwater environments more directly than the oceanic ones. Considerably high concentrations of dissolved N_2O, which associate with disposal of human waste, were reported in river systems [Kaplan *et al.*, 1978; Cicerone *et al.*, 1978]. Hemond and Duran [1989] reported that the high level N_2O in river water was produced mainly in the waste water treatment plant and partly in the river sediments. They estimated the N_2O production during treatment of waste waters from 50 billions of population as 0.02 Tg $N_2O\text{-}N \cdot y^{-1}$. N_2O supersaturated in discharge waters from agricultural lands was probably originated from fertilizer nitrogen [Dowdell *et al.*, 1979; Minami and Fukushi, 1984]. Forest clear-cutting resulted in elevation of N_2O concentration in seepage waters [Bowden and Bormann, 1986], which is probably a result of enhancement of nitrification.

Lake ecosystems can act as either sources or sinks for N_2O depending on those trophic states and other properties [Kaplan *et al.*, 1978; Knowles *et al.*, 1981; Lemon and Lemon, 1981]. Both productions by nitrification [Vincent *et al.*, 1981; Downes, 1988, Yoh *et al.*, 1988] and denitrification [Yoh *et al.*, 1983] are considered for the lacsturine N_2O.

Imbalance of Global Budget

Fig. I-3 illustrate a simplified biogeochemical pathways of N_2O and Table I-5 summarizes approximate fluxes for major pathways reported by WMO/UNEP, IPCC [1990]. One can see the deficient of the source strength with respect to the sum of the stratospheric decomposition and the tropospheric accumulation. Because the stratospheric reaction is an only predominant well-established sink of the tropospheric N_2O [Crutzen, 1983, Cicerone, 1987, WMO/UNEP, IPCC, 1990], this means underestimation of source strength.

Oppositely, there are some reports to show an excess in the total emission [Crutzen, 1983; Banin *et al.*, 1984; Cicerone, 1987; Yoshinari, 1991a]. According to the individual strengths of major sources presented in the previous section, the global emission should be 14-17 Tg N_2O-N · y^{-1}, by summing the annual accumulation of 3-6 Tg N_2O-N · y^{-1} and the stratospheric decomposition of 11 Tg N_2O-N · y^{-1}. On the other hand, the biogenic N_2O emission is counted as 1-3 Tg N_2O-N · y^{-1} in the ocean [Codispoti and Christensen, 1985; Cline *et al.*, 1987] and 4-29 Tg N_2O-N · y^{-1} in lands [Banin *et al.*, 1984]. The sum of these two, 5-32 Tg N_2O-N · y^{-1}, is severely unbalanced with the total biogenic source of 12-16 Tg N_2O-N · y^{-1} that is calculated by the total source (14-17 Tg N_2O-N · y^{-1}) minus total abiotic source (1-2 Tg N_2O-N · y^{-1}). The mismatch is due to the inaccurate estimation of emission from terrestrial ecosystems.

It has been suggested that some seepage and ground waters contain high level N_2O [Dowdell *et al.*, 1979; Bowden and Borman, 1986; Ronen *et al.*, 1988; Davidson and Firestone, 1988; Davidson and Swank, 1990; Ueda *et al.*, 1991a; 1991b]. These reports suggest worth while to determine a groundwater environment as a candidate for a forgotten compartment in biogeochemical cycle of N_2O. Details will be discussed in Chapter II.

TABLE I-5 Sources and Sinks of Nitrous Oxide

	TgN · year^{-1}
Source	
Oceans	1.4–2.6
Soils (tropical forests)	2.2–3.7
(temperate forests)	0.7–1.5
Combustion	0.1–0.3
Biomas burning	0.02–0.2
Fertilizer (including groundwater)	0.01–2.2
TOTAL	4.4–10.5
Sink	
Removal by soils	?
Photolysis in the stratosphere	7–13
Atmospheric Increase	3–4.5

From *WMO/UNEP, IPCC Report* [1990]

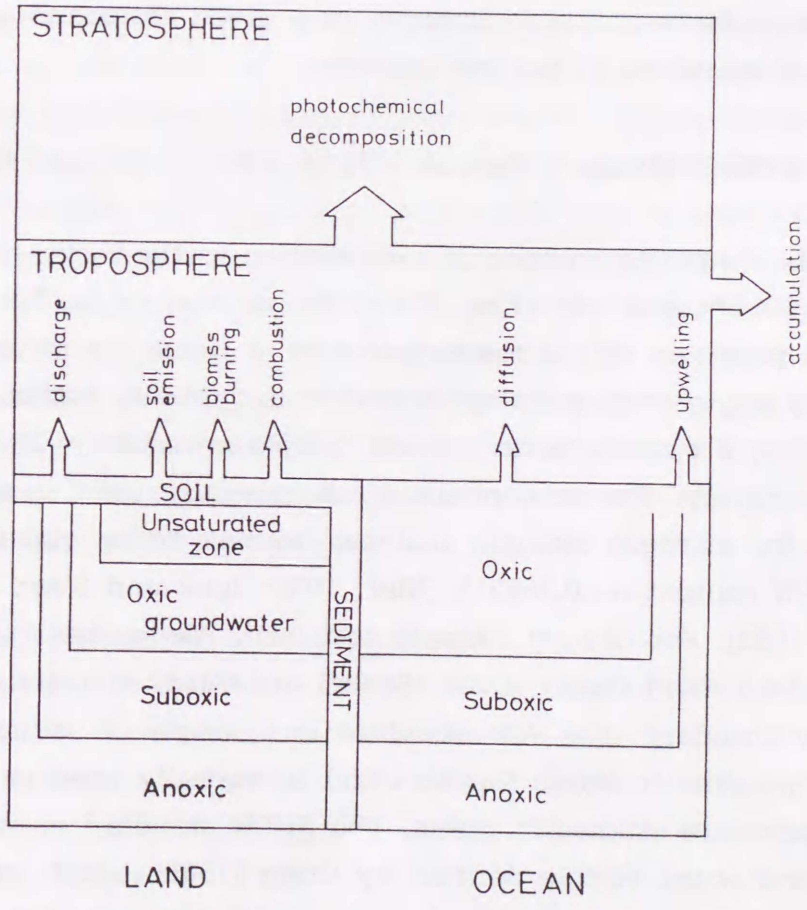

Figure I-3 Biogeochemical pathways of N_2O in the global cycle.

I-3. STABLE ISOTOPE STUDY

General Principles

Most of the bioelements consist of several isotopes with different atomic weight. It is widely known that a natural abundance of stable isotope in a given compound is used to identify its origin and to trace its fate in natural environment. The idea behind this is the fact that stable isotopic composition of an element is determined by the isotopic composition of source material and the degree of isotopic fractionation during its generation, then we can read the source of a given compound and its record of the past behavior. Lots of biogeochemical applications for various compounds can be found, which involve atmospheric gases, such as oxygen, argon, carbon dioxide and methane. General principles of the stable isotope study will be firstly discussed below.

The abundance of stable isotopes in a given compound is expressed in terms of δ-values in per mil notation:

$$\delta(‰) = (R_{sample} / R_{standard} - 1) \times 10^3 \quad \dots \ (9)$$

where R is a ratio in content of rare-isotope to the major-isotope. They are $^{15}N/^{14}N$ and $^{18}O/^{16}O$ or $^{17}O/^{16}O$ for nitrous oxide. The analytical precision of the measurement of δ-value for nitrogen and oxygen by using ratio mass spectrometer is typically within ± 0.2 ‰, involving preparations to convert sample materials in N_2 or CO_2 gas, respectively. The atmospheric N_2 is generally used as a primary standard for nitrogen isotopic analyses because of its globally uniform ^{15}N content as 0.3663 % [Nier, 1955; Junk and Svec, 1958; Mariotti, 1983]. For oxygen isotopic standard, Peedee belemnite (PDB) and Standard Mean Ocean Water (SMOW) are internationally accepted as a primary standard. The PDB standard is a sample of belemnite from Peedee Formation in North Carolina and is normally used in paleotemperature studies in ocean. The SMOW standard is originally a hypothetical water sample defined by Craig [1961], which isotope ratios of oxygen and hydrogen are similar to those of an average sample of ocean water [Craig, 1957]. The atmospheric O_2 is occasionally used for standard because of its isotopic uniformity in the surface atmosphere [Kroopnik, 1971]. Throughout this study, $^{15}N/^{14}O$ and $^{18}O/^{16}O$ are determined and expressed as deviation from the atmospheric nitrogen gas and the SMOW, respectively.

Difference in isotope composition between a substrate and a product of reaction is called "isotope effect" or "isotopic fractionation". To express the degree of fractionation, an isotopic fractionation factor (α) or an isotopic enrichment factor (ε) are commonly used. They are defined as follows:

$$\alpha = R_{substrate} / R_{product} \quad \dots \ (10)$$

$$\varepsilon = (1 - \alpha) \times 10^3 \quad \dots \ (11)$$

Since the isotopically lighter molecules react faster than the heavier ones, "α" is generally observed more than 1.000 and "ε" takes negative values. Such isotopic discrimination is commonly determined by two types of mechanisms, an equilibrium isotope fractionation and a kinetic isotope fractionation. The equilibrium isotope fractionation arises from the thermodynamic difference of isotopically substituted compounds. This mechanism is important in geological aspect, but less control of isotopic composition by this mechanism is expected in biological metabolic reactions. The other one, kinetic isotope effect is more determining the biological isotope fractionation in nature through the process of substrate uptake and enzymatic reaction. These kinetic fractionation are due to the difference in rates of transportation or reaction among isotopically substituted compounds.

Isotope Fractionation during N_2O Metabolism

The isotopic composition of environmental N_2O is mainly determined by a kinetic isotope effect because (1) N_2O is not at all in isotope exchange equilibrium with other nitrogenous compounds [Yoshida, 1984], (2) the nitrogen equilibrium isotopic enrichment factor during dissolution of N_2O in water is as small as -0.8 ‰ [Yoshida, 1984], and (3) oxygen isotope exchange between N_2O and water is negligible [Wahlen and Yoshinari, 1985]. Reports on nitrogen and oxygen kinetic isotope fractionation in nitrification and denitrification are summarized in Table I-6.

The nitrogen isotopic enrichment factors (ε_N) during denitrification ($NO_3^- \rightarrow N_2$) is in the -14 to -39 ‰ range, with a single exception of the smallest fractionation of Miyazaki *et al.* [1971]. Because the ε_N of -20 to -37 ‰ in the step of $NO_3^- \rightarrow N_2O$ is similar to the range of the entire denitrification ($NO_3^- \rightarrow N_2$), ε_N in the final step of $N_2O \rightarrow N_2$ in a course of denitrification should rival the value of $NO_3^- \rightarrow N_2O$ step. Because the ε_N of $N_2O \rightarrow N_2$ step should become negligible under low N_2O concentration [Yoshida, 1984], the fractionation of $N_2O \rightarrow N_2$ must be small in usual natural environments.

For nitrification, the author knows only one report about nitrogen isotope fractionation by Yoshida [1988]. In which, rather uniform ε_N from -60 to -68 ‰ were observed for $NH_4^+ \rightarrow N_2O$ with *Nitrosomonas europaea,* which was regardless the O_2 concentration. He explained such an extraordinary large discrimination by assuming a double stage fractionation during consequential process of $NH_4^+ \rightarrow NO_2^-$ followed by $NO_2^- \rightarrow N_2O$ (nitrifier denitrification). As referred to the fact that this type of N_2O production by nitrification dominates under the oxygen stress, some uncertainties still remain. Unfortunately, there is no report on ε_N in the other N_2O production via direct oxidation of NH_2OH in nitrification.

To the author's knowledge, there has been only three studies on the kinetic oxygen isotopic fractionation for nitrification and denitrification. Olleros [1983] obtained an oxygen isotopic enrichment factor of ε_O=-15 ‰ during nitrate reduction by laboratory experiments with isolated nitrate reductase. Bottcher *et al.* [1990] estimated the enrichment factor for nitrate reduction in groundwater by denitrification as -8.0 ‰. Wahlen and Yoshinari [1985] calculated ε_O for N_2O reduction as -37 ‰ (10 ℃) and -42 ‰ (26 ℃) by cultures of *Pseudomonas aeruginosa.*

TABLE I-6 Isotopic Enrichment Factors in Nitrous Oxide Metabolisms and Relating Proceses

Reaction	ε nitrogen (‰)	ε oxygen (‰)	Organism	Reference
<u>Nitrogen fixation</u>				
$N_2O \rightarrow$ cell	-17 – -34		*A. vinelandii*	*Yamazaki et al.* [1987]
<u>Nitrification</u>				
$NH_4^+ \rightarrow NO_2^-$	-18 – -36		*N. europaea*	*Delwich & Steyn* [1970]
	-21 – 0		*marine nitrifier*	*Miyake & Wada* [1971]
	-33 – -37		*N. europaea*	*Yoshida* [1984]
	-35		*N. europaea*	*Mariotti et al.* [1981]
$NH_4^+ \rightarrow N_2O$	-60 – -68		*N. europaea*	*Yoshida* [1984]
$(NO_2^- \rightarrow N_2O)$	-34 – -35		*N. europaea*	*Yoshida* [1984]
<u>Denitrification</u>				
$NO_3^- \rightarrow N_2$	-20		*P. sp.*	*Wellman et al.* [1968]
	-17		*P. denitrificance*	*Delwich & Steyn* [1970]
	-21 – 0		*S. marinorubra*	*Miyazaki* [1971]
	-40		*marine nitrifier*	*Cline & Kaplan* [1975]
	-14 – -23		*soil denitrifier*	*Blackmer & Bremner* [1977]
	-39		*S. marinorubra*	*Miyazaki et al.* [1980]
	-25 – -29		*soil denitrifier*	*Mariotti et al.* [1981]
$NO_3^- \rightarrow N_2O$	-33 – -37		*P. fluorescens*	*Yoshida* [1984]
$NO_2^- \rightarrow N_2O$	-20 – -30		*P. stutzeri*	*Mariotti et al.* [1982]
$N_2O \rightarrow N_2$	-1 – -27		*P. denitrificans*	*Yoshida* [1984]
		-37 – -42	*P. aeruginosa*	*Wahlen & Yoshinari* [1985]
<u>Nitrate reduction</u>				
$NO_3^- \rightarrow NO_2^-$	-29 – 0		*S. marinorubra*	*Miyazaki* [1971]
	-11 – -17		*natural soil*	*Blackmer & Bremner* [1977]
	-30	-15	*nitrate reductase*	*Olleros* [1983]

Previous Studies on Stable Isotopic Compositions in Environmental N_2O

The first measurement of nitrogen stable isotope ratio in environmental N_2O was made by Moore [1974]. He reported the $\delta^{15}N$ values of 5.2 ± 2.0 ‰ for the tropospheric N_2O and 19.2 ‰ for the stratospheric one. Yoshida and Matsuo [1983] reported the mean $\delta^{15}N$ value of 8.1 ± 1.0 ‰ for the tropospheric N_2O. Further data-base for $\delta^{15}N$ of N_2O was owing to Yoshida and his coinvestigators in the Pacific Ocean [Yoshida, 1984; Yoshida *et al.*, 1984; 1989]. They reported ^{15}N-depleted N_2O with respect to the atmospheric ones in the surface layers and ^{15}N-enriched N_2O in the deep ocean masses. They concluded that these N_2O in surface water and in deep sea were produced by nitrification and denitrification, respectively.

Wahlen and Yoshinari [1985] and Yoshinari and Wahlen [1985] emphasized the advancement of using oxygen isotope signature than nitrogen one in elucidating the source mechanism of N_2O. The idea behind this is based on the facts that oxygen isotopic exchanges of nitrite and nitrate with water are negligible [Hall and Alexander, 1940] and a larger variety in $\delta^{18}O$ than $\delta^{15}N$ of N_2O from different environments. Actually, the reported $\delta^{18}O$ range of environmental N_2O, +21 ‰ [Yoshinari and Wahlen, 1985] to +93 ‰ [Wahlen and Yoshinari, 1985], is larger than that of $\delta^{15}N$, -38.0 ‰ (see Chapter V) to +19.2 ‰ [Moore, 1974]. However, the measurements of $\delta^{18}O$ in N_2O falls behind those of $\delta^{15}N$.

Recently, the pioneering approaches to characterize natural N_2O by both nitrogen and oxygen stable isotope compositions have been tried in oceanic environments by Kim and Craig [1990] and Yoshinari *et al.* [1990]. Kim and Craig [1990] concluded by their two-isotope characterization that N_2O in deep ocean is produced from nitrification. It is arising as controversy which process provides the nitrogen and oxygen isotopically heavier N_2O in deep ocean; denitrification [Yoshida *et al.*, 1989], nitrification [Kim and Craig, 1990], or both. In order to answer the question, $\delta^{15}N$-$\delta^{18}O$ characterization of major terminal compounds in nitrogen metabolisms, such as nitrate (NO_3^-), nitrite (NO_2^-), nitric oxide (NO), and hydroxylamine (NH_2OH). However, stable isotopic information on these compounds are severely restricted.

It is expected that stable isotope signatures of N_2O must be a key to its source identification. The better way is to start the strategy at chemically simple systems, otherwise somewhat difficulties in preparations for the isotopic analyses may arise. A groundwater appears to be best suited for this criteria, because the preliminary survey suggests that N_2O concentrations in nitrate contaminated groundwaters should much exceed the atmospheric equilibria [Ueda, 1988], and because chemical compositions of groundwaters are simple and stable in general with respect to other surface waters. Such characteristics of groundwater environments make me free from complicated physical and chemical preparation of the sample for stable isotopic analyses. The goals of this dissertation are in item (1) to identify the source mechanisms of groundwater N_2O by the nitrogen and oxygen stable isotopic characterization, and (2) to examine the ability of the stable isotope study in evaluating the biogeochemical cycle of N_2O.

Concentrations of N_2O and other chemical constituents in groundwaters from various location are described in Chapter II. Origins of the groundwater N_2O are roughly discussed based on their chemical characteristics therein. A possible role of groundwater environments in biogeochemical cycle of N_2O is suggested by determining a possible charging of N_2O to an aquifer based on a long term observation at a natural spring. In Chapter III, the nitrogen stable isotopic characterization of the groundwater N_2O is carried out. Source mechanisms of the elevated N_2O are discussed form the comparison of nitrogen isotopic ratios of N_2O and NO_3^-. The new method to analyze nitrogen and oxygen stable isotopic compositions in N_2O are developed in Chapter IV. Elucidation of producing mechanisms of the groundwater N_2O is performed by determining $\delta^{15}N$ and $\delta^{18}O$ of N_2O and NO_3^- in groundwaters. Chapter V provides the two-isotope mapping for N_2O from different environments, including atmosphere, sea, and fresh water systems. Chapter VI depicts the future problems to develop the application of the two-isotopic diagram for re-evaluating the current global budget of N_2O.

CHAPTER II

CONCENTRATIONS OF NITROUS OXIDE IN GROUNDWATERS

ABSTRACT — Concentrations of N_2O in groundwaters were surveyed. Samples were collected from 61 plots in central Japan Islands and New York State during five years from October 1986. NO_3^-, O_2, NH_4^+, NO_2^- and dissolved organic carbon (DOC) were analyzed with a portion of the samples.

N_2O concentrations from 16 to 7060 nM (690 nM in average) corresponded 1.3 to 540 times of the atmospheric equilibria at *in situ* water temperatures. The studied groundwaters are chemically characterized by (1) sub-oxic to oxic conditions, (2) NO_3^- contaminations, (3) absences of NO_2^- and NH_4^+, and (4) depletion of DOC. These observations stronbly indicate low activities of nitrate respiration in the aquifers. $\Delta N_2O/NO_3^-$ molar ratio between 10^{-4} and 10^{-2}, where "Δ" presents the excess gas over that which would be in equilibrium with the atmosphere, did not contradict the N_2O yield in aerobic nitrification. Thus nitrification was suggested to be a likely mechanism of N_2O production, although ΔN_2O nor NO_3^- were not proportional to $-\Delta O_2$.

In a two-year frequent observation at one of the spring sites, J19, N_2O concentration depended on neither changes of $-\Delta O_2$ nor water discharge. This means independence of the N_2O production to the organic matter decay as well as imply an existence of N_2O pool free from dilution by the water recharge by rain falls within the soil-groundwater system. If the N_2O were derived from nitrification, in-between subsoil and unsaturated zone would be a probable site of nitrification utilizing ammonium supplied from shallower to deeper in the soil column as downward movement of the water. The assumption consists with the view from vertical profile of N_2O in soil column that the deep production was irrelevant to the N_2O emission from the soil surface. A possible N_2O charging to the J19 aquifer is estimated as 1.2-2.3 mg $N_2O\text{-}N \cdot m^{-2} \cdot y^{-1}$ by dividing annual N_2O discharges with the catchment area, which corresponds 0.5 to 15 % of the reported soil emission to the atmosphere. Whatever the true mechanism of N_2O production is, the result suggests meaningful role of groundwater in the biogeochemical cycle of N_2O, especially when the wide-spreading nitrate contamination of groundwater and the positive correlation between N_2O and NO_3^- concentrations are examined.

II-1. INTRODUCTION

Recent accumulation of N_2O in the troposphere is believed as a result of sn expansion of various kinds of human practices. Consider the global nitrogen budget, today's industrial nitrogen fixation is comparable or even exceed the biological one. A significant portion of such artificially fixed nitrogen from the atmospheric N_2 entirely reaches to fresh waters as un-incorporated chemical fertilizers or organic compounds through food webs, which eventually causes imbalance of nitrogen dynamics therein. Eutrophication of rivers, lakes, fresh reservoirs and groundwaters are the common examples. The nitrate contamination of groundwater is well known as a worldwide matter of environmental concern.

Through the preliminary survey, the author discovered a remarkable accumulation of N_2O in spring and well waters in the western part of Tokyo [Ueda, 1988]. Almost at the same time, Ronen *et al.* [1988] reported that nitrogen contaminated aquifers in Israel and Netherlands contained N_2O in extremely high concentration with respect to the atmospheric equilibria. Most recently, Smith [1990] and Smith *et al.* [in press] have observed elevated N_2O in the Cape Cod anoxic aquifer which is polluted with secondary treated sewage effluent. These observations suggest worth while to evaluate groundwater environments as reservoirs for N_2O. However, information on distribution of N_2O in groundwater environments is scarcely found excepting the above studies.

In this chapter, N_2O concentrations in groundwaters are surveyed along with those of some chemical constituents. Possible source mechanism of the groundwater N_2O and a biogeochemical role of groundwater environment as a pathway in the N_2O cycle are discussed.

II-2. MATERIALS AND METHODS

Study Area

Groundwater samples were taken at 31 plots in Japan (Fig. II-1) and 30 plots in the United States (Fig. II-2). Table II-1 and Table II-2 respectively list the sampling sites in Japan and US with their information. Yet the depths of surveyed aquifers range from 1 to 250 m below the land surface, their geological properties were not fully examined during the choice of the sites. Followings are the descriptions of the study area.

A. Japan

Samples were collected from wells and natural springs scattering in the central part of Japan Islands (Fig. II-1) from October 1986 to September 1991. Thirty-one plots locate in Tokyo Metropolis and Kanagawa, Saitama and Nagano Prefectures.

J01 - J02: *Two deep multilevel wells on the campus of Tokyo University of Agriculture and Technology at Fuchu, Tokyo.* Waters extracted from the two wells are mixed to be used as an only source for water supplement in the campus. Observation by the author's laboratory shows that NO_3^- concentration of these well waters has been increased by factor of three from 1976 to 1988 [Ogura, unpublished data], although the nitrogen source is not identified.

J03 - J18: *Shallow wells in Tokyo Metropolis and Kanagawa, Saitama and Nagano Prefectures.* J03-J06 wells were recently dug in parks on the Musashino Plateau by the city government of Kokubunji. They provide raw waters only for management of the parks. J07-J12 are private wells. J07 and J08 locate on the Musashino Plateau. J07 water is sometimes used only for a garden management. J08 daily supplies water for a Japanese style public bath. J09 and J10 are continuously used for both drinking and domestic purposes. J11 water is occasionally used only for a private garden management. J12-J14 are surrounded by rice paddy fields. J12 is extracted by a food industry manufacturing pickled vegetables. J13 and J14 are public wells to distribute water for drinking and domestic usage. J15-J18 wells locate in the City of Tokyo. Only J15 is offered to domestic purposes, whereas the others were terminated to be pumped up.

J19 – J25: *Natural springs in the western part of Tokyo.* Springs J19–J21 locate along the Kokubunji Cliff as the southern edge of the Musashino Terrace. The waters flow rapidly from a few meter thick Musashino Terrace Gravel Layer that underlies a 5-7 m thick Kanto Loam Layer composed of Andosol derived from volcanic ash materials, then the waters are discharged in to River Nogawa. Topographic and hydrological information suggests that each spring has own small catchment and that the spring is probably a only source of discharge for the shallow aquifers.

An intensive observation was performed at J19, spring site NKM, for two years from December 1986. Its recharge area of approximately 2.0 km^2 is a densely populated residential district [Ogura and Morikawa, 1985]. The aquifer is recharged mainly by a rain fall on the permeable surface because roughly two-three of the catchment is covered with impervious matters, mostly buildings and pavements. Since sewage produced in the catchment had been treated by septic tanks for a long period up to recently, significant contaminations by nitrate and chloride were demonstrated in these spring waters [Ogura and Morikawa, 1985].

Springs J22–J23 gush out beneath the Aoyagi Cliff which is the boundary between the Aoyagi Terrace and the Tamagawa Alluvial Bottomland. A part of their catchment is used as an agricultural land. These spring waters are discharged into Yagawa River. Springs J24 and J25 appear at the Tachikawa Cliff and the Fuchu Cliff, respectively, both divide the Tachikawa Terrace and the Tamagawa Alluvial Bottomland. Waters from J24 and J25 are respectively discharged to Yagawa River and to Fuchu-Yosui River.

J26 – J27: *Natural springs in Kanagawa Prefecture.* J26 water effluents at the foot of the Hayama Goyoutei. A portion of its probable catchment is offered as a golf course. J27 water was used by visitors to the Yadorogi Shrimp.

J28: *Seepage water from a forest in Rolling Land Laboratory, Tokyo University of Agriculture and Technology, Hachiooji, Tokyo.* J28 catchment of 2.15 ha is covered with planted forest composed of coniferous trees (0.6 ha) and deciduous trees (1.55 ha).

J29 – J30: *Drainage waters from cropping lands at Sugadaira, Nagano Prefecture.* J29 water was taken from a trench just beside the cropping fields, whereas J30 sample was collected directly from the drainage pipe buried beneath the cultivated soil.

J31: *Seepage water discharged into the Kokkai-Gijido-Mae station.* This is the deepest subway station in Japan (97 m below the ground level).

B. USA

Samples were collected through March to May in 1990 from twenty-nine wells providing raw waters without specific treatments and one natural spring. The sampling sites locate in Albany, Rensselaer, Oneida, Oswego and Nassau Counties in New York State, as shown in Fig. II-2. These plots are generally categorized as following three groups based on each type of land usage.

U01 – U16: *Private wells in Albany and Rensselaer Counties.* The waters extracted from U01, U02 and U03 are used for both drinking and domestic practices. Others provide water only for garden managements. Their surrounding land is residential districts.

U17 – U20: *Spring and well waters in agricultural regions in Oswego and Oneida Counties.* U17 is a natural spring whose catchment is used as a pasture. U18-U20 are private wells. U18 water is used only for domestic purposes, whereas the others are terminated to be extracted.

U21 – U30: *Public wells in Nassau County in Long Island.* These plots were selected because of the nitrate contaminations of underlying aquifers. Samples were collected from three aquifer layers from the uppermost, the upper glacial, Magothy, and Lloyd aquifers. The nitrate contamination due to a long term fertilization and an infiltration of a sewage from septic tanks is proceeding from the upper to the lower aquifers [Kreitler, 1978; Ragone *et al.*, 1981].

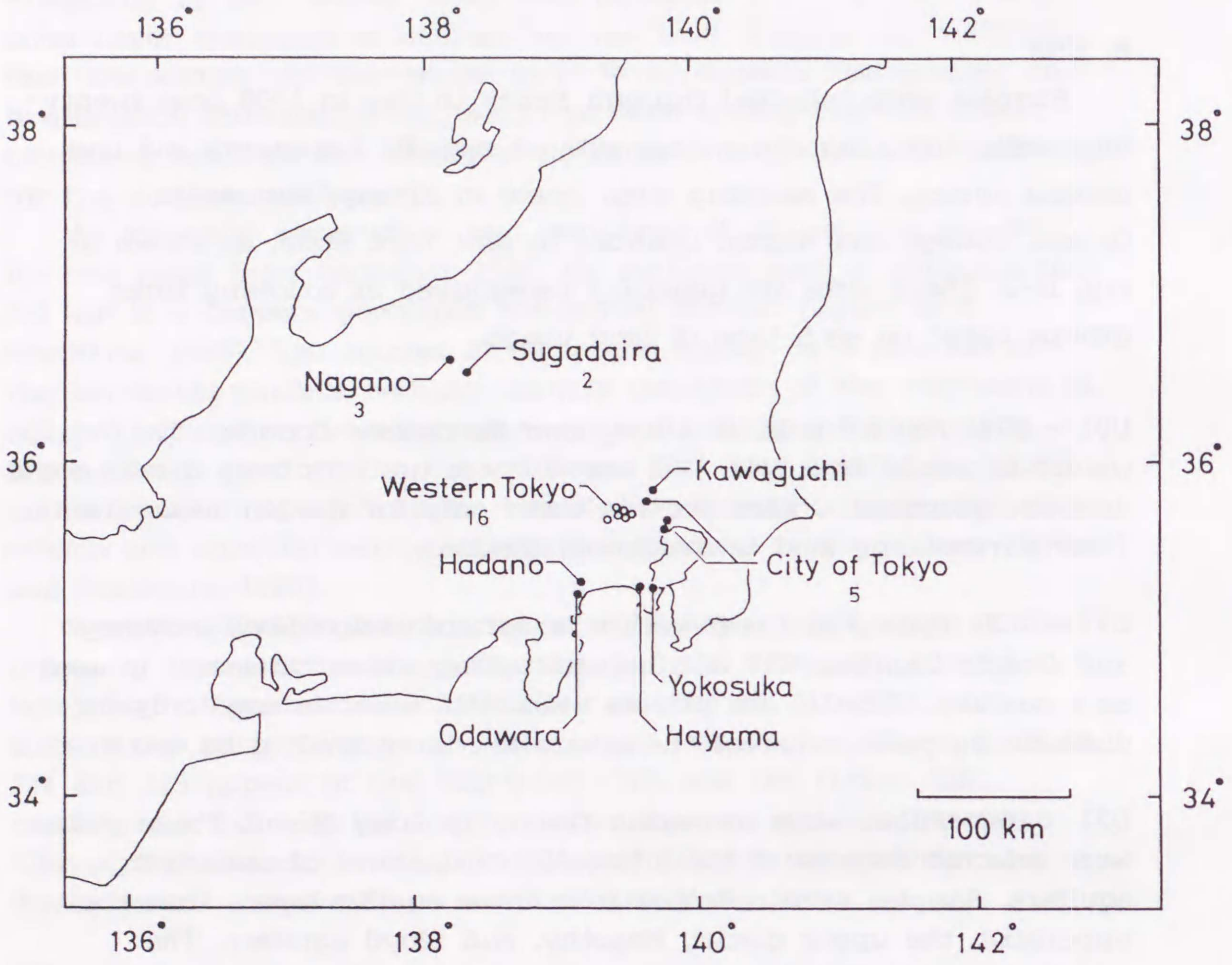

Figure II-1 Sampling locations of groundwaters in the mid-part of Honshyu-Island. Numbers are the count of wells and sprigs. Individual information appears in TABLE I-1.

TABLE II-1 List of Sampling Plots of Groundwaters in Japan

Code	Name	Type[1]	Depth[2]	Location	Land[3]	Pur[4]	Remark
J01	TUAT1	Pb	150	Fuchu, Tokyo	R	Dr	E-pump, multistrainer
J02	TUAT2	Pb	250	Fuchu, Tokyo	R	Dr	E-pump, multistrainer
J03	MUKA1	Pr	16	Kokubunji, Tokyo	R	Mp	H-pump, park facility
J04	MUKA2	Pr	19	Kokubunji, Tokyo	R	Mp	H-pump, park facility
J05	MUKA3	Pr	18	Kokubunji, Tokyo	R	Mp	H-pump, park facility
J06	MUKA4	Pr	21	Kokubunji, Tokyo	R	Mp	H-pump, park facility
J07	KKBJ1	Pr	NA	Kokubunji, Tokyo	R	Md	E-pump
J08	KKBJ2	Pr	60	Kokubunji, Tokyo	R	Md	E-pump, public bath
J09	POKA	Pr	30	Kawaguchi, Saitama	A	Dr	E-pump
J10	ATSU	Pr	43	Odawara, Kanagawa	R	Dr	E-pump
J11	YOKO	Pr	7	Yokosuka, Kanagawa	R	Md	E-pump
J12	NAGA1	Pr	15	Nagano, Nagano	A	Fm	E-pump, pikles
J13	NAGA2	Pb	12	Nagano, Nagano	A	Dr	E-pump
J14	NAGA3	Pb	12	Nagano, Nagano	A	Dr	E-pump
J15	ITBS1	Pr	<10	Itabashi-ku, Tokyo	R	Md	E-pump
J16	ITBS2	Pr	<10	Itabashi-ku, Tokyo	R	Md	H-pump
J17	MNTK1	Pr	<10	Minato-ku, Tokyo	R	Md	H-pump
J18	MNTK2	Pr	<10	Minato-ku, Tokyo	R	Md	H-pump
J19	NKM	Sp	0	Kokubunji, Tokyo	R	-	Kokubunji Cliff
J20	NKG	Sp	0	Koganei, Tokyo	R	-	Kokubunji Cliff
J21	NKK	Sp	0	Kokubunji, Tokyo	R	-	Kokubunji Cliff
J22	ASK2	Sp	0	Kunitachi, Tokyo	A	-	Aoyagi Cliff
J23	ASK4	Sp	0	Kunitachi, Tokyo	A	-	Aoyagi Cliff
J24	TC	Sp	0	Tachikawa, Tokyo	P	-	Tachikawa Cliff
J25	FC	Sp	0	Fuchu, Tokyo	R	-	Fuchu Cliff
J26	HAYA	Sp	0	Hayama, Kanagawa	F	-	golf cource
J27	YADO	Sp	0	Hadano, Kanagawa	F	-	secondary forest
J28	RLL	se	0	Hachiooji, Tokyo	F	-	secondary forest
J29	SGDR1	Se	0	Sugadaira, Nagano	A	-	arable land drainege
J30	SGDR2	Se	0	Sugadaira, Nagano	A	-	arable land drainege
J31	KKGD	Se	97	Chiyoda-ku, Tokyo	R	-	subway station

[1] Pb-public well, Pr-private well, Sp-natural spring, Se-seepage.
[2] Distance in meter from the ground level.
[3] R-residential district, A-arable land, P-park, F-forest
[4] Dr-drinking, Md-domestic managiments, Mp-park managements, Fm-food manufacturing

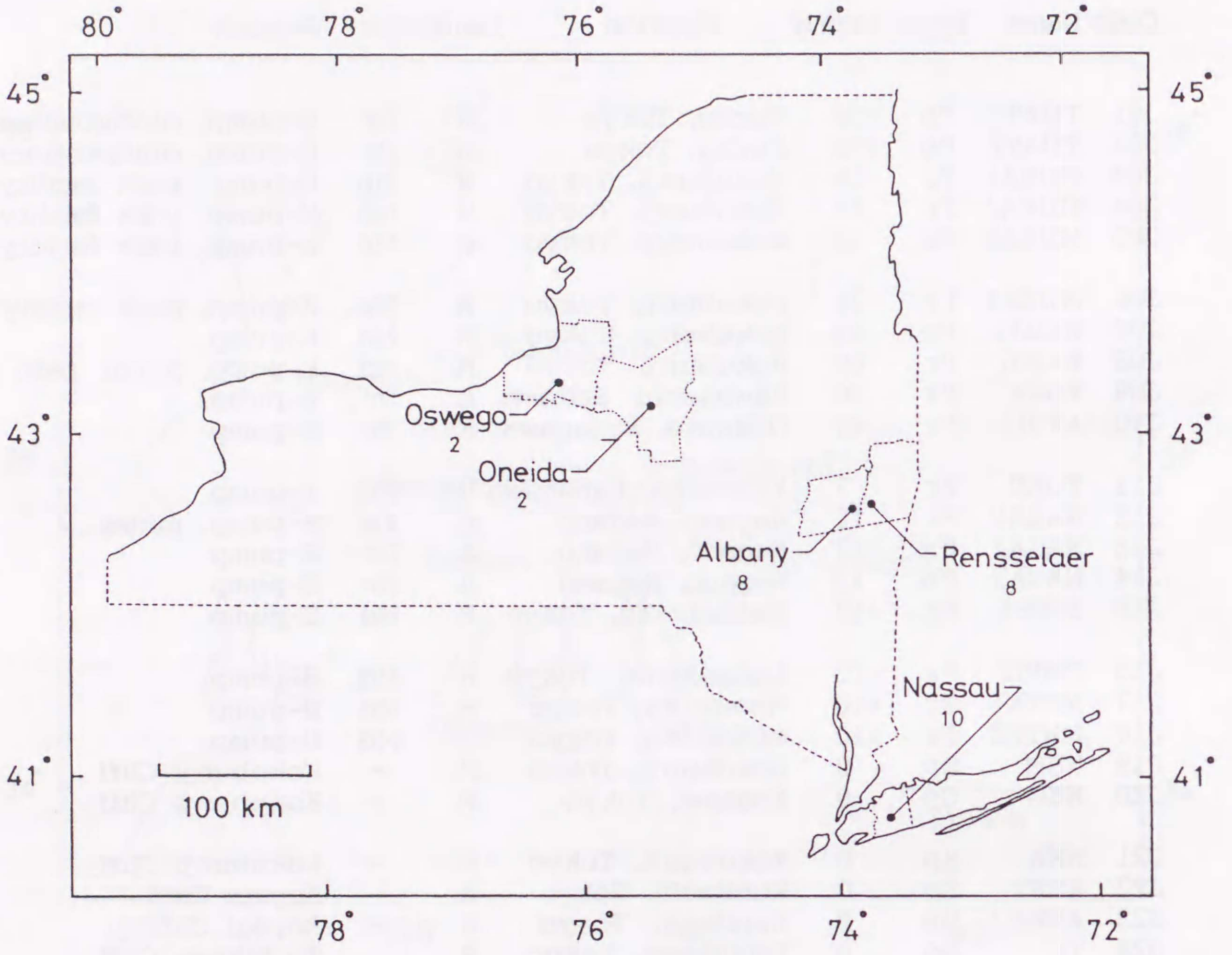

Figure II-2 Sampling locations of groundwaters in New York State. Names of counties where samples were collected and the number of sampling plots appear in the illustration. For an individual information, see TABLE I-2.

TABLE II-2 List of Sampling Plots of Groundwaters in New York State

Code	Name	Type[1]	Depth[2]	Location	Land[3]	Pur[4]	Remark
U01	ALB1	Pr		Guilderland, ALB	R	Dr	E-pump
U02	ALB2	Pr		Guilderland, ALB	R	Dr	E-pump
U03	ALB3	Pr		Guilderland, ALB	R	Dr	E-pump
U04	ALB3	Pr		Guilderland, ALB	R	Md	E-pump
U05	ALB4	Pr	9	Guilderland, ALB	R	Md	E-pump
U06	ALB5	Pr		Guilderland, ALB	R	Md	E-pump
U07	ALB7	Pr		Guilderland, ALB	R	Md	E-pump
U08	ALB8	Pr		Guilderland, ALB	R	Md	E-pump
U09	REN1	Pr		Schoback, RENS	R	Md	E-pump
U10	REN2	Pr		Schoback, RENS	R	Md	E-pump
U11	REN3	Pr	56	Schoback, RENS	R	Md	E-pump
U12	REN4	Pr	88	Schoback, RENS	R	Md	E-pump
U13	REN5	Pr	101	Schoback, RENS	R	Md	E-pump
U14	REN6	Pr	60	Schoback, RENS	R	Md	E-pump
U15	REN7	Pr	70	Schoback, RENS	R	Md	E-pump
U16	REN8	Pr	51	Schoback, RENS	R	Md	E-pump
U17	OSW1	Sp	0	Baldwinsvill, OSW	A	-	arable land drainege
U18	OSW2	Pr		Baldwinsvill, OSW	A	Md	E-pump
U19	ONE1	Pr		New Hartford, ONE	A	Md	H-pump, terminated
U20	ONE2	Pr		Winfield, ONE	A	Md	H-pump, terminated
U21	WL1	Pb	38	Wheeler Rd., NASS	R	Dr	E-pump, Glacial[5]
U22	WL2	Pb	123	Wheeler Rd., NASS	R	Dr	E-pump, Glacial[5]
U23	WL3	Pb	125	Wheeler Rd., NASS	R	Dr	E-pump, Magothy[5]
U24	WL4	Pb	115	Aheeler Rd., NASS	R	Dr	E-pump, Magothy[5]
U25	WI1	Pb	113	Wicks Rd., NASS	R	Dr	E-pump, Magothy[5]
U26	WI2	Pb	123	Wicks Rd., NASS	R	Dr	E-pump, Magothy[5]
U27	WI3	Pb	125	Wicks Rd., NASS	R	Dr	E-pump, Magothy[5]
U28	BE2	Pb	36	Bellmore, NASS	R	Dr	E-pump, Glacial[5]
U29	BE3	Pb	92	Bellmore, NASS	R	Dr	E-pump, Magothy[5]
U30	BE4	Pb	216	Bellmore, NASS	R	Dr	E-pump, Lloyd[5]

[1] Pb-public well, Pr-private well, Sp-natural spring.

[2] Distance in meter from the ground level.

[3] R-residential district, A-arable land

[4] Dr-drinking, Md-domestic managements

[5] The most upper Glacial aquifer, intermediate Magothy aquifer, and the deepest Lloyd aquifer.

N_2O Concentration

A. Sample Collection

After temperature and specific conductance of a well water had stabilized, the water was poured into a glass vial (100 or 50 mL) from the bottom without agitation through a tube attached to the tap. After overflowing at least equal volume of water to the bottle content, the vial was capped with a butyl rubber stopper without bubbles in the bottle by allowing them to escape through a syringe needle. The sample was poisoned with saturated $HgCl_2$ solution for Japanese one or 10 M NaOH for US one to cease microbial metabolism. The stopper was finally fixed with an aluminum cap then brought back to the laboratory and stored at the room temperature until analyses.

In case of spring water, a 250 mL glass syringe with two ports which is so-called "Nishimaki-type Syringe" (Fig. II-3) was used. One side was used for sucking water and the other for dispensing it. After repeat of sucking and discarding water for several times, the water was put into the vial from the bottom. Further treatments are as same as for well waters.

B. Purge and trap gas extraction method

The Japanese samples were analyzed by the method described here. Schematic diagram of the purge-trap extraction system is illustrated in Fig. II-4. All the pipes installed were made of stainless steel and grease-less Teflon valves were used. The apparatus was originally devised by Cohen [1977] and modified by Nishio [1982].

Water was transferred from the bottom of the vial through a syringe needle to two of volume known glass reservoirs connected in series by pushing the sample with air (Fig. II-5). The reservoir was equipped with two of 3-way Teflon cocks and a bypass for purging gas in the inlet system. Both ends of the reservoir were attached to the system by Teflon taper fittings. Air in the inlet part was first purged with pure nitrogen gas (> 99.999 %) for 5 minutes. By changing the 6-way valve (A) and subsequently turning the 3-way cocks at both ends of the reservoir simultaneously, the sample water was carried into a gas stripping chamber as the carrier gas flow. Dissolved gases were extracted by flushing the sample by the gas for 10 minutes.

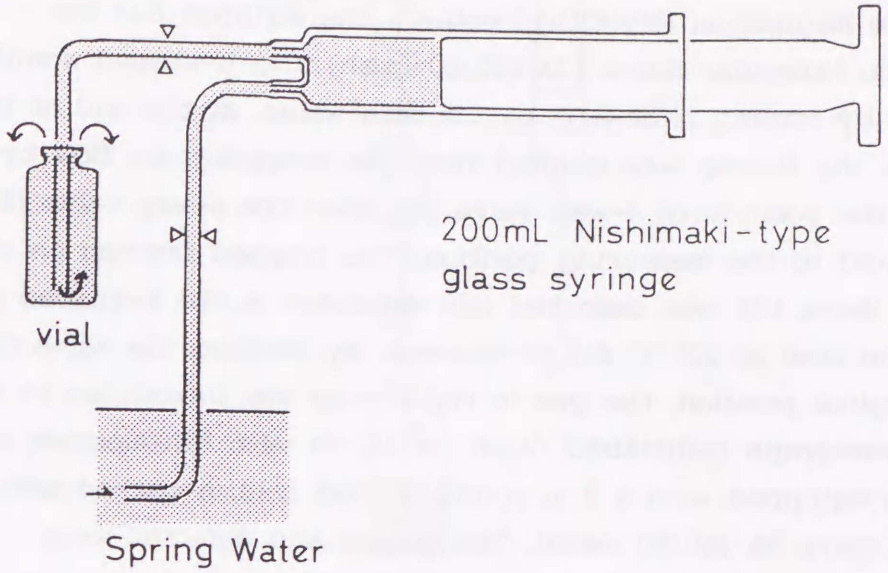

Figure II-3 Sampling device used for collecting spring water.

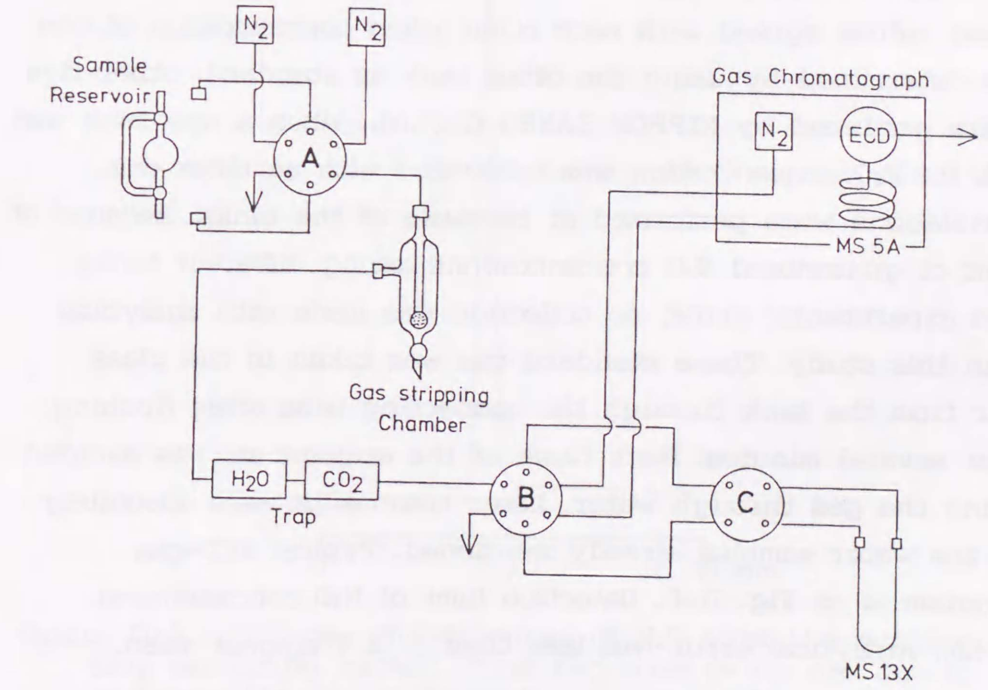

Figure II-4 Purge and trap extracting system used to measure dissolved N_2O concentration. For the operation, see text.

As water vapor and CO_2 were removed by silica gel-Ascarite-perchloride magnesium ($Mg(ClO_4)_2$) column, the stripped N_2O was collected on Molecular Sieve 13X (60/80 mesh) in a U-shaped stainless steel trap by cooling it at 0 ℃ by ice with water. At the end of the extraction, the U-trap was isolated from the stripping gas flow by changing the position of 4-way valve (C), then the 6-way valve (B) was adjusted to the measuring position. The trapped fraction on the Molecular Sieve 13X was desorbed and expanded in the U-shaped trap by heating the trap at 200 ℃ for 10 minutes. By backing the valve (C) to the original position, the gas in the U-trap was introduced to the gas chromatograph (SHIMADZU GC-7C or GC-8A with ^{63}Ni-electron capture detectors) equipped with a 2 m stainless steel column packed with Molecular Sieve 5A (60/80 mesh). The column and detector were maintained at 240 ℃ and 320 ℃, respectively.

As standard, seven commercial tanks with N_2O concentrations around 5 ppmv (4.4, 4.7, 5.2, 5.4) and 50 (52.7, 50.7, 50.4) ppmv were used. These tanks were balanced by ultra-pure grade N_2 or He gases. Two tanks with mixing ratios of 4.4 and 52.7 ppmv prepared by TAKACHIHO KAGAKU KOGYO Co.,Ltd. were firstly purchased. The guaranteed values agreed with each other when concentration of one tank was determined by using the other tank as standard. Other five tanks were produced by NIPPON SANSO Co.,Ltd.. When a new tank was provided, its N_2O concentration was calibrated with an older one. Such cariblations were performed at renewals of the tanks. Because of agreement of guaranteed N_2O concentrations among different tanks within an experimental error, no collection was made with analytical results in this study. These standard gas was taken in the glass reservoir from the tank through the connecting tube after flushing inside for several minutes. Back flush of the ambient air was avoided by venting the gas through water. Later treatments were absolutely same as the water samples already mentioned. Typical ECD-gas chromatogram is as Fig. II-6. Detection limit of N_2O concentration was 0.1 nM. Analytical error was less than ± 2 % against mean.

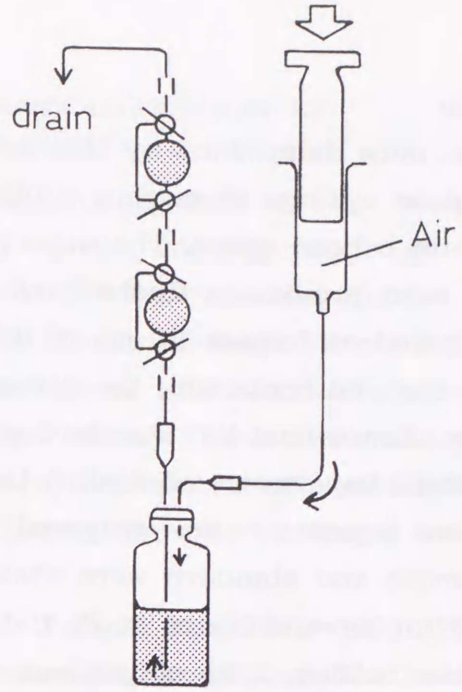

Figure II-5 Transference of sample water from a glass vial to reservoirs which are used to introduce water to the purge and trap extracting system.

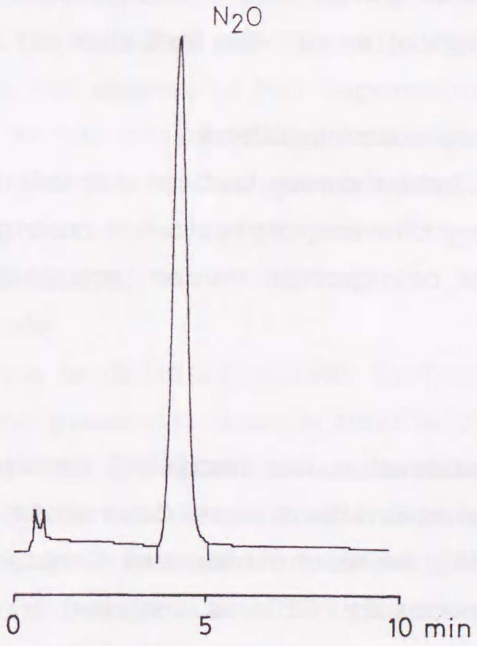

Figure II-6 ECD-gas chromatogram of N_2O after the purge and trap extracting method. First two small peaks are due to switching the 4-way valve. No oxygen peak appears in the chromatogram because oxygen is not adsorbed on MS13X at the ice-water temperature.

C. Headspace technique

All the US samples were determined by this method. 20 mL of water was withdrawn by a glass syringe through a rubber stopper as allowing an ambient air to flow in a head-space. The water was injected in a 50 mL vial which had been previously flushed and filled with He.

Standard was prepared as follows. 20 mL of N_2O-free distilled water was injected in the vial containing He with allowing equal volume of He to escape. Commercial N_2O standard gas (owned by Dr. T. Yoshinari, New York State Department of Health) taken in a gas tight syringe from a tank was injected to the prepared vial.

These vials for sample and standard were shaken vigorously by hand, then leave them for several hours at 25 ℃ to achieve gas-liquid equilibrium in the bottles. 1 mL of gas was sampled from the headspace by a gastight syringe, then injected to a gas chromatograph (SHIMADZU GC-2 mini) with ^{63}Ni-ECD. The sample gas was dehumidified in a dry-ice ethanol cold trap installed between the injection port and a MS5A column. Operating condition of the gas chromatograph was same as for the previous purge and trap extraction method. Detection limit was 0.3 nM. Analytical error was less than ± 3 %.

D. Calculation of atmospheric equilibria

Atmospheric equilibrium concentration was calculated after Weiss and Price [1980] taking the tropospheric N_2O mixing ratio of 305 ppbv, as a mean value of reported values (see Chapter I), and *in situ* water temperature.

Other Chemicals

Dissolved O_2 concentration was measured by Winkler's method. NO_3^-, NO_2^- and NH_4^+ concentrations were determined colorimetrically after Wood *et al.* [1967], Bendschneider and Robinson [1952], and Solorzano [1969], respectively. Cl^- was analyzed by mercury thiocyanate method. Dissolved organic carbon concentration was determined by the method of Menzel and Vaccaro [1964]. In regard to the US samples, NO_3^-, NO_2^-, and NH_4^+ concentrations were analyzed by Technicon Autoanalyzer II. DOC concentration was measured as carbon dioxide after ultraviolet-oxidation of the sample.

II-3. RESULTS AND DISCUSSION

N_2O Supersaturation in Groundwaters

Concentrations of O_2, N_2O, and NO_3^- in groundwaters are presented in Table II-3 and Table II-4 for Japanese and US samples, respectively. Some are results of single determinations and others are presented as mean values with standard deviation. According to a summary of the present survey (Table II-5), the groundwaters are characterized in item (1) oxic to sub-oxic condition, (2) significant NO_3^- contamination, (3) NO_2^- and NH_4^+ absences, and (4) low DOC concentration.

N_2O concentrations ranged from 16 to 7060 nM, corresponding to 1.3 to 540 times the calculated equilibria. The lowest concentration was observed in U24 well water extracted from 115 m deep Magothy aquifer on the Long Island, in which NO_3^- concentration was also quite low (4 μM). Surprisingly high concentrations were measured in J30, J14 and U05 waters. The J14 and J30 are a direct reflection of fertilization to the cropping field, whereas the U05 may be explained by considering that the location was formerly used as an arable land. However, as a whole, the degree of N_2O supersaturation does not directly correspond to the circumstantial land usage, which means multiple sources of nitrogen for the groundwater N_2O. In other words, the groundwater N_2O is derived not only from the fertilizer nitrogen as depicted by IPCC report (Table I-6) but also from variety of nitrogenous compounds.

N_2O concentrations in different aquatic systems were illustrated as Fig. II-7. The data presented include results of the author's preliminary survey [Ueda, 1988] and other investigators. Great attention must be paid to the elevation of N_2O in groundwaters as compared to the rest environments. It is important that the N_2O accumulations in groundwaters are widely observed in geographically isolated locations regardless the catchment concitions. The supersaturations were also measured in well waters collected at the central part of the Korean peninsula [Oh and Ueda, unpublished data]. These findings strongly suggest a possible concern of groundwater environments with the biogeochemical cycle of N_2O.

TABLE II-3 Concentrations of O_2, N_2O and NO_3^- in Japanese Groundwaters

Code	Tw (℃)	O_2 (μM)	N_2O (nM)	NO_3^- (μM)	$\Delta N_2O/NO_3^-$ ($\times 10^{-3}$)	$\Delta N_2O/-\Delta O_2$ ($\times 10^{-3}$)	$NO_3^-/-\Delta O_2$	n
J01	16.1±0.9	182±8	472±120	506±75	0.90±0.17	4.25±1.63	4.32±0.85	8
J02	16.4±1.0	85±47	873±321	222±10	3.92±1.13	4.50±1.79	1.30±0.35	8
J03	16.6	221	1240	373	3.31	16.6	5.02	1
J04	15.7	230	256	436	0.56	3.48	6.17	1
J05	16.2	246	287	701	0.40	5.38	13.6	1
J06	16.1	249	885	730	1.20	17.8	14.9	1
J07	17.2	259	434	493	0.86	12.9	14.9	1
J08	18.7	203	280	624	0.43	3.38	7.80	1
J09	17.8	165	69	ND	–	0.48	–	1
J10	16.2	220	119	ND	–	1.40	–	1
J11	16.6	25	81	ND	–	0.26	–	1
J12	16.5	42	690	213	3.20	2.68	0.84	1
J13	14.3	24	1032	274	3.72	3.58	0.96	1
J14	14.9	81	5280	1079	4.89	23.5	4.80	1
J15	16.3	293	800	615	1.28	198	154	1
J16	15.5	273	169	601	0.26	5.48	20.7	1
J17	15.5	186	361	814	0.43	3.03	7.02	1
J18	16.0	144	135	327	0.38	0.81	2.11	1
J19	15.6±0.2	260±19	316± 85	548±49	0.56±0.14	10.7±15.7	21.1±39.1	95
J20	15.7±0.2	262±10	435±113	709±92	0.60±0.12	12.1± 6.8	19.8± 8.5	9
J21	15.8±0.3	254±18	387±153	711±103	0.53±0.19	11.4± 6.7	20.3±14.6	7
J22	16.4±0.6	231±17	485±119	557±112	0.94±0.35	7.19±1.04	8.40±3.01	8
J23	16.2±1.3	262±12	335± 56	565± 54	0.60±0.04	8.07±1.05	13.5±0.9	4
J24	16.6±0.5	212±22	913±398	343± 83	2.80±1.34	14.1±12.8	4.86±3.12	8
J25	16.3±1.3	290±13	119±115	536± 72	0.21±0.26	12.6±7.99	100±47	5
J26	14.7	273	108	ND	–	2.84	–	1
J27	14.5	285	32	ND	–	0.91	–	1
J28	14.3	216	76	181	0.36	0.70	1.93	1
J29	11.4	252	490	1200	0.40	6.13	15.4	1
J30	12.7	288	7060	2143	3.29	214	64.9	1
J31	19.9	253	153	291	0.50	6.04	12.2	1

For Δ-value, see text. Numbers in the most right column present times of N_2O concentration analyses. "ND" means "not determined".

TABLE II-4 Concentrations of O_2, N_2O and NO_3^- in New York State Groundwaters

Code	Tw (℃)	O_2 (μM)	N_2O (nM)	NO_3^- (μM)	$\Delta N_2O/NO_3^-$ ($\times 10^{-3}$)	$\Delta N_2O/-\Delta O_2$ ($\times 10^{-3}$)	$NO_3^-/-\Delta O_2$	n
U01			160					1
U02	11.5	190	572	227	2.47	4.01	1.63	1
U03	11.5	150	155	886	0.16	0.80	4.93	1
U04	12.0	194	78	222	0.30	0.50	1.68	1
U05	11.7	36	4318	474	9.09	14.7	1.62	1
U06	11.4	147	1661	412	4.00	9.00	2.25	1
U07	13.5	243	182	1643	0.10	2.37	22.7	1
U08	10.8	230	701	1229	0.56	6.56	11.7	1
U09			570	542	1.03			1
U10			908	907	0.99			1
U11	12.6	308	781	292	2.64	56.8	21.5	1
U12	11.8	245	385	366	1.02	4.53	4.44	1
U13	12.4	289	243	186	1.25	6.79	5.45	1
U14	12.0	228	1294	561	2.29	13.1	5.73	1
U15	12.2	248	1194	519	2.28	15.5	6.80	1
U16	12.8	193	1013	299	3.35	7.89	2.35	1
U17	11.7	294	155	121	1.19	4.21	3.55	1
U18	10.6	73	125	621	0.18	0.43	2.36	1
U19	9.0	153	315	165	1.83	1.54	0.84	1
U20	8.5	198	149	96	1.42	0.87	0.62	1
U21	12.2	281	337	397	0.82	7.51	9.15	1
U22	12.5	234	254	217	1.12	2.75	2.46	1
U23	11.3	247	145	91	1.46	1.58	1.08	1
U24	10.8	125	16	4	1.03	0.02	0.02	1
U25	11.5	272	265	258	0.98	4.39	4.47	1
U26	11.6	253	450	392	1.12	5.76	5.15	1
U27	11.3	266	137	152	0.82	1.92	2.33	1
U28	12.7	125	81	60	1.17	0.36	0.31	1
U29	12.0	219	308	294	1.01	2.77	2.75	1
U30	11.8	31	55	n.d.	–	0.15	–	1

For Δ-value, see text. "ND" and "n.d." mean "not determined" and "not detected", respectively.

TABLE II-5 Summary of the Observation of Groundwaters

	min.	max.	mean	s.d.	n
Tw (℃)	8.5	19.9	14.0	2.6	55
pH	5.5	8.6	6.5	0.6	55
O_2 (μM)	24	308	209	75	55
N_2O (nM)	16	7060	688	1210	61
NO_3^- (μM)	4	2140	530	400	53
DOC (μM C)	n.d.	150	42	42	17

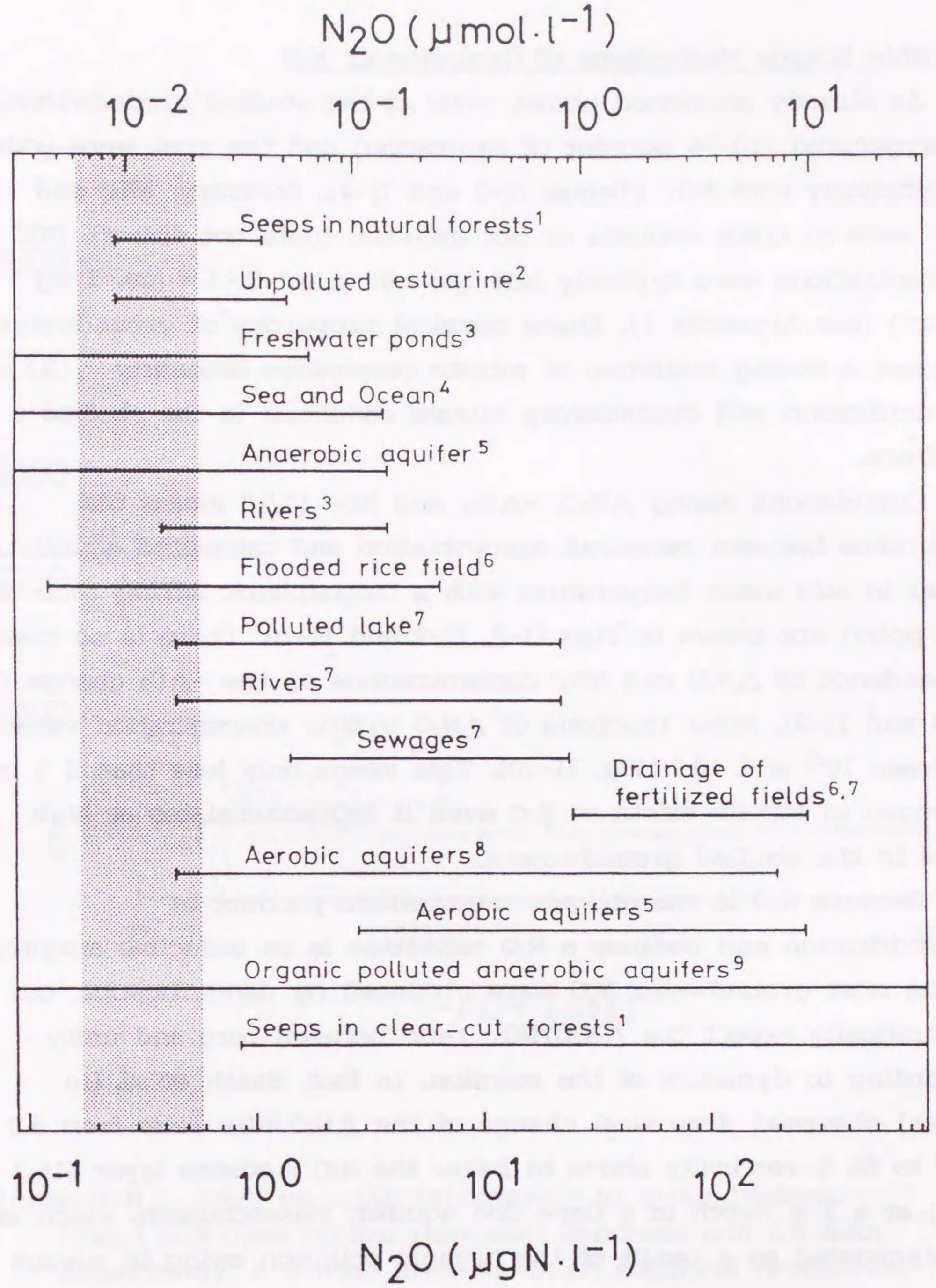

Figure II-7 N_2O concentrations in groundwaters with those in various aquatic systems. The shade presents equilibrium concentration with the ambient air (305 ppbv, *Butler et al.* [1989]) at 0-30 ℃ temperature range. References are 1. *Bowden & Borman* [1986]; 2. *Butler et al.* [1987]; 3. *Kaplan et al.* [1976]; 4. many; 5. *Ronen et al.* [1988]; 6. *Minami & Fukushi [1984]*; 7. *Ueda* [1988]; 8 This study; 9. *Smith et al.* [in press].

Possible Source Mechanisms of Groundwater N_2O

As already mentioned above, none of the studied groundwaters was deoxygenated (10-96 percent of saturation) and the most were polluted significantly with NO_3^- (Tables II-3 and II-4). Contrary, NH_4^+ and NO_2^- were in trace amounts or not detected (data not shown). DOC concentrations were typically less than 80 μmol C · L^{-1} (ca. 1 mg C · L^{-1}) (see Appendix 1). These chemical properties of groundwaters suggest a strong inhibition of nitrate respiration including denitrification and dissimilatory nitrate reduction in the studied aquifers.

Correlations among $\triangle N_2O$, $-\triangle O_2$, and NO_3^- ("△" means the difference between measured concentration and calculated equilibrium at an *in situ* water temperature with a tropospheric mixing ratio of 305 ppbv) are shown in Figs II-8, II-9 and II-10. There is no clear dependence of $\triangle N_2O$ and NO_3^- concentrations on the $-\triangle O_2$ change (Figs II-8 and II-9). Molar fractions of $\triangle N_2O$ to NO_3^- concentration varies between 10^{-4} and 10^{-2} (Fig. II-10). This means only less than 2 % of nitrogen in nitrate exists as N_2O even if N_2O accumulates at high level in the studied groundwaters.

Because N_2O is the obligate intermediate product in denitrification and because a N_2O reductase is an inducible enzyme, if the most groundwater N_2O were produced by denitrification, one can theoretically expect the $\triangle N_2O/NO_3^-$ ratio between zero and unity according to dynamics of the reaction. In fact, Smith *et al.* [in press] observed dramatical change of the $\triangle N_2O/NO_3^-$ ratio from 5.0×10^{-3} to 66 % vertically above to below the N_2O maximum layer (16.7 μM) at a 3 m depth in a Cape Cod aquifer, Massachusetts which is deoxygenated as a result of the organic pollution owing to sewage infiltration. According to their results of the laboratory incubation of the aquifer sediments, the N_2O is produced by the aquifer denitrification. Rather uniform $\triangle N_2O/NO_3^-$ in the present sub-oxic to oxic groundwaters as compared in the Cape Cod aquifer probably suggests minor contribution of *in situ* denitrification to the N_2O production.

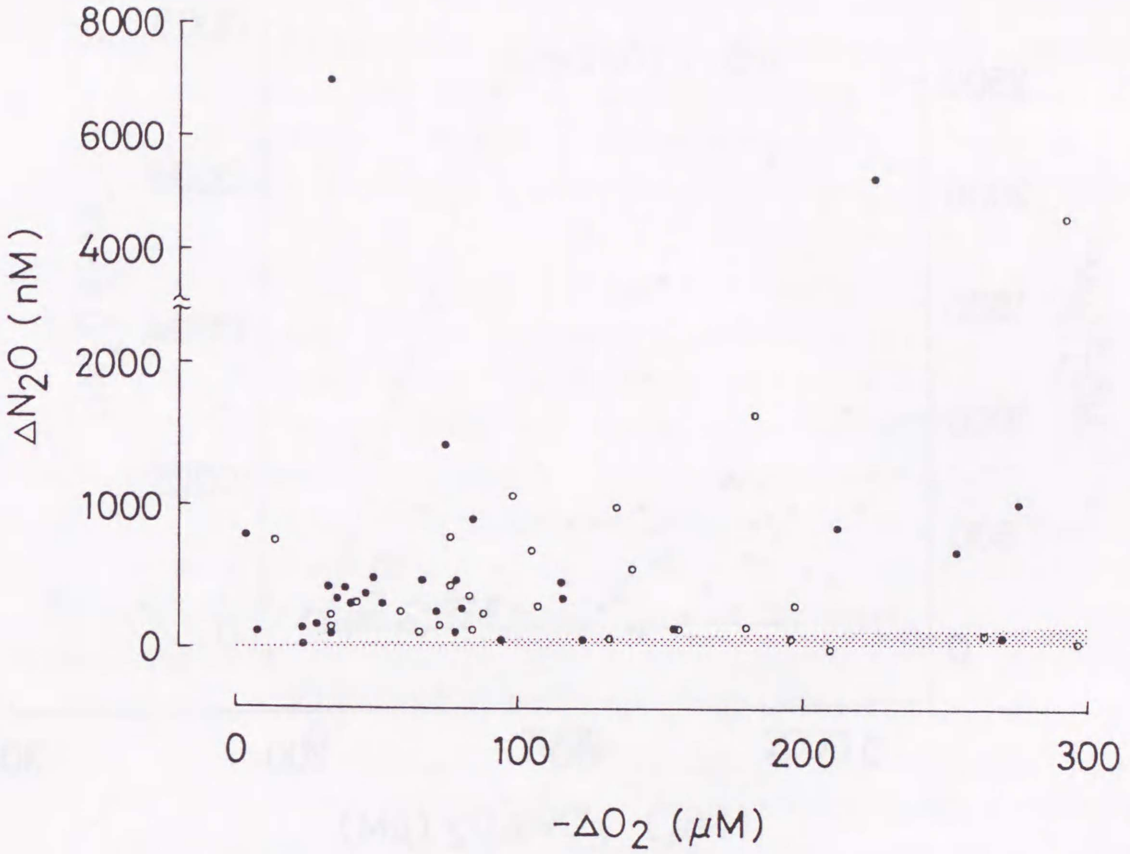

Figure II-8 $\triangle N_2O$ *vs.* $-\triangle O_2$ relationship in groundwaters. Closed and open circles represent Japanese and US data, respectively. A shaded area shows an empirical correlation between the two variables in ocean which was supposed by *Butler et al.* [1989]: $\triangle N_2O$ (nM) = $-13.5 + \{0.125 + 0.00993 \times T\ (℃)\} \times -\triangle O_2\ (\mu M)$, where "T" means water temperature. $0 < T < 30$ ℃.

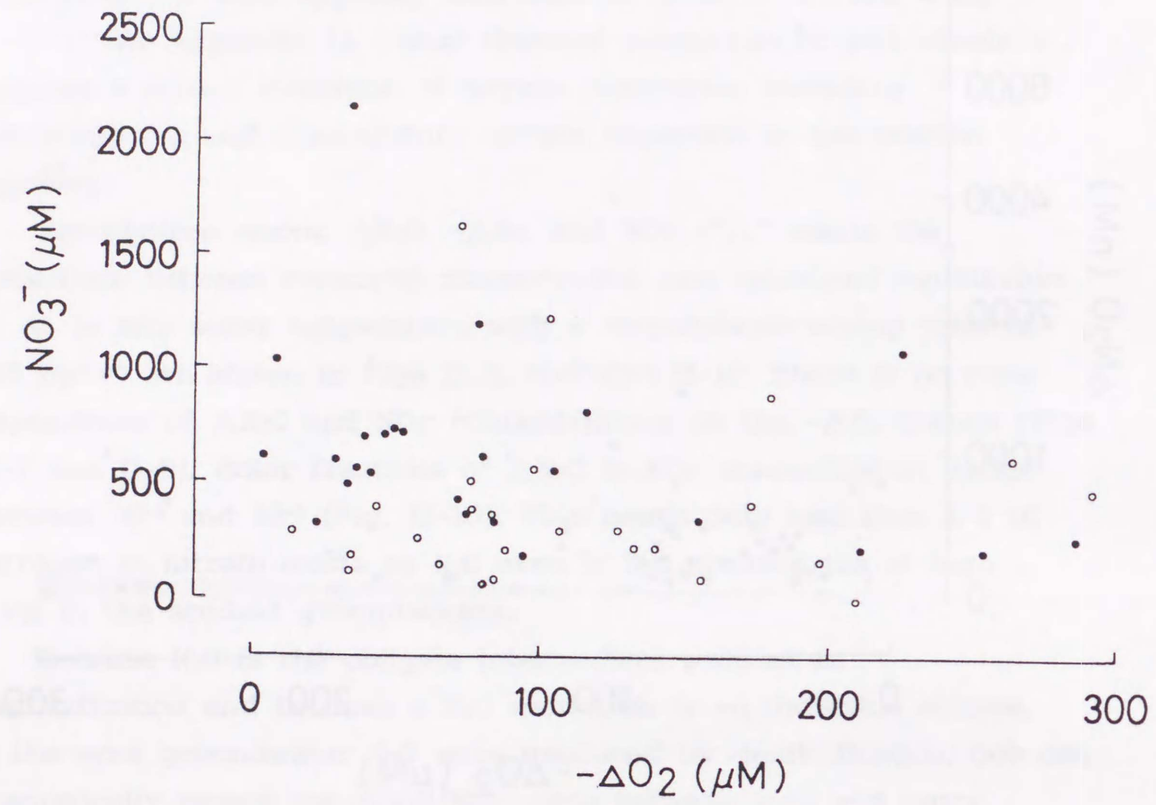

Figure II-9 NO_3^- *vs.* $-\Delta O_2$ relationship in groundwaters. Close and open circles represent Japanese and US data, respectively.

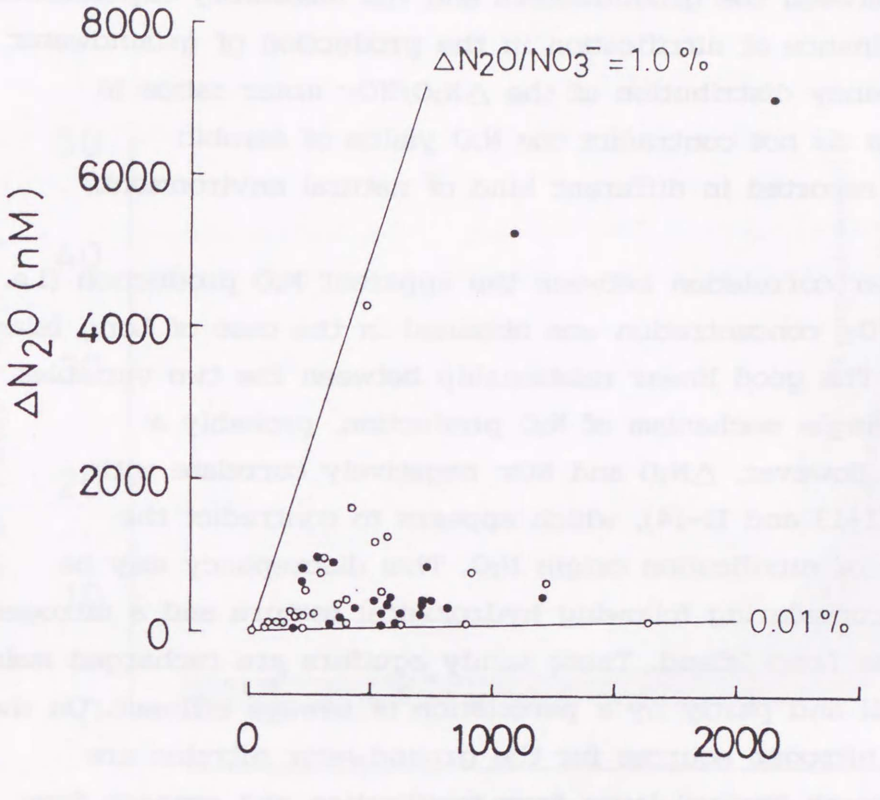

Figure II-10 ΔN_2O *vs.* NO_3^- relationship in groundwaters. Close and open circles represent Japanese and US data, respectively.

Fig. II-11 shows a relationship between $-\triangle O_2$ and $\triangle N_2O/NO_3^-$ molar ratios in the groundwaters with those obtained by cultures of *Nitrosomonas europaea* isorated from natural soil [Lipschultz *et al.*, 1981] and sea water [Goreau *et al.*, 1980]. The agreement of $\triangle N_2O/NO_3^-$ between the groundwaters and the laboratory experiments suggest dominance of nitrification in the production of groundwater N_2O. A frequency distribution of the $\triangle N_2O/NO_3^-$ molar ratios in groundwaters do not contradict the N_2O yields of aerobic nitrification reported in different kind of natural environments (Fig. II-12).

More clear correlation between the apparent N_2O production (i.e. $\triangle N_2O$) and NO_3^- concentration was obtained in the case of Long Island (Fig. II-15). The good linear relationship between the two variables suggests a single mechanism of N_2O production, probably a nitrification. However, $\triangle N_2O$ and NO_3^- negatively correlate with $-\triangle O_2$ (Figs II-13 and II-14), which appears to contradict the accumulation of nitrification origin N_2O. This discrepancy may be realized by considering following hydrological feature and a nitrogen budget in the Long Island. These sandy aquifers are recharged mainly by a rain fall and partly by a percolation of sewage effluent. On the other hand, nitrogen sources for the groundwater nitrates are supplied through the soil layer from fertilization and seepage from septic tanks. Since the aquifer water is an only source for water supply for the Long Island area, when the a shallower aquifer had been terminated to be pumped due to the nitrate contamination, a deeper aquifer was offered to public water supply instead. These facts may enhance an oxygen recharge to the nitrification site together with a rapid escape of the products (NO_3^- and N_2O) to a nitrification-free deeper zone. The lower concentrations of N_2O and NO_3^- with lower O_2 concentrations in the Long Island samples are thus explained by the rapid transportation of NO_3^- and N_2O to the aquifers in which O_2 concentration was originally low.

Thus nitrification is suggested to be a more likely route of N_2O production in the sub-oxic and oxic groundwaters as compared to the denitrification. This hypothesis will be further examined by the stable isotopic strategy in Chapter III and Chapter VI.

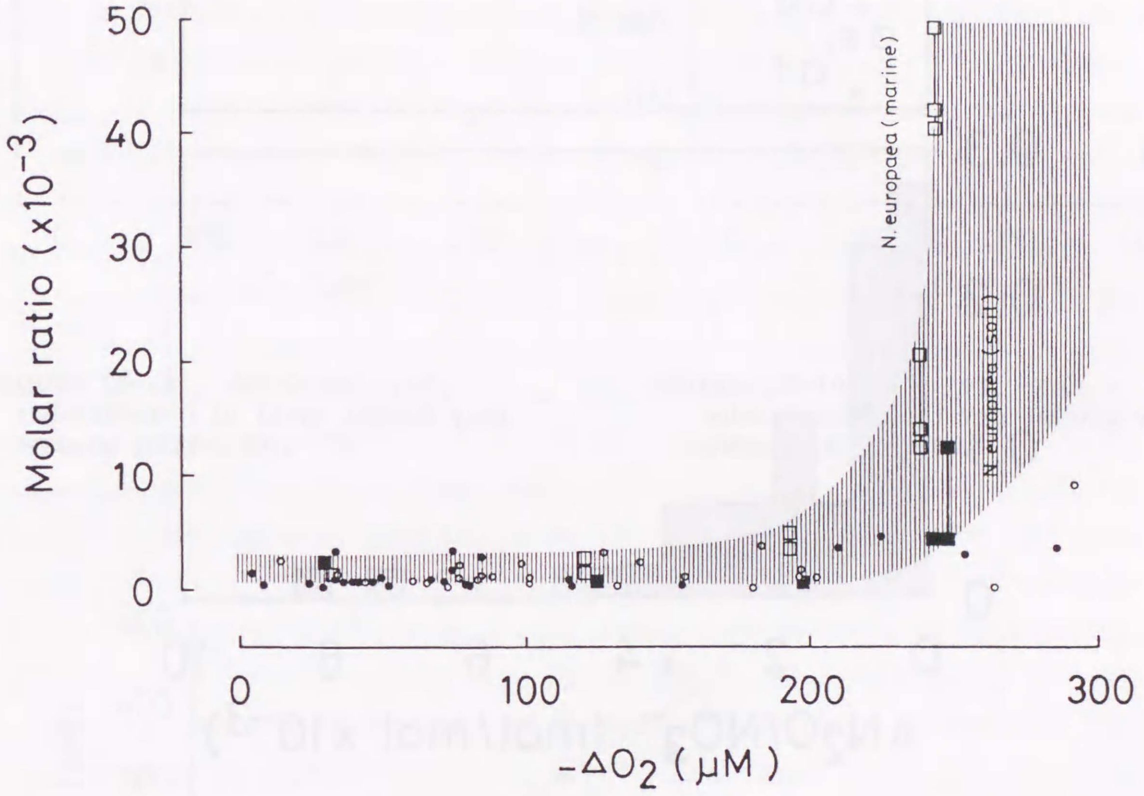

Figure II-11 Relationship between $\triangle N_2O/NO_3^-$ molar ratios and $-\triangle O_2$ in groundwaters. Close and open circles represent Japanese and US data, respectively. Change in N_2O yield of nitrifying bacteria under culture condition is together presented as a shaded area. Close and open squares show the results for *Nitrosomonas europaea* isolated from soil [*Lipschultz et al.*, 1981] and marine environments [*Goreau et al.*, 1980].

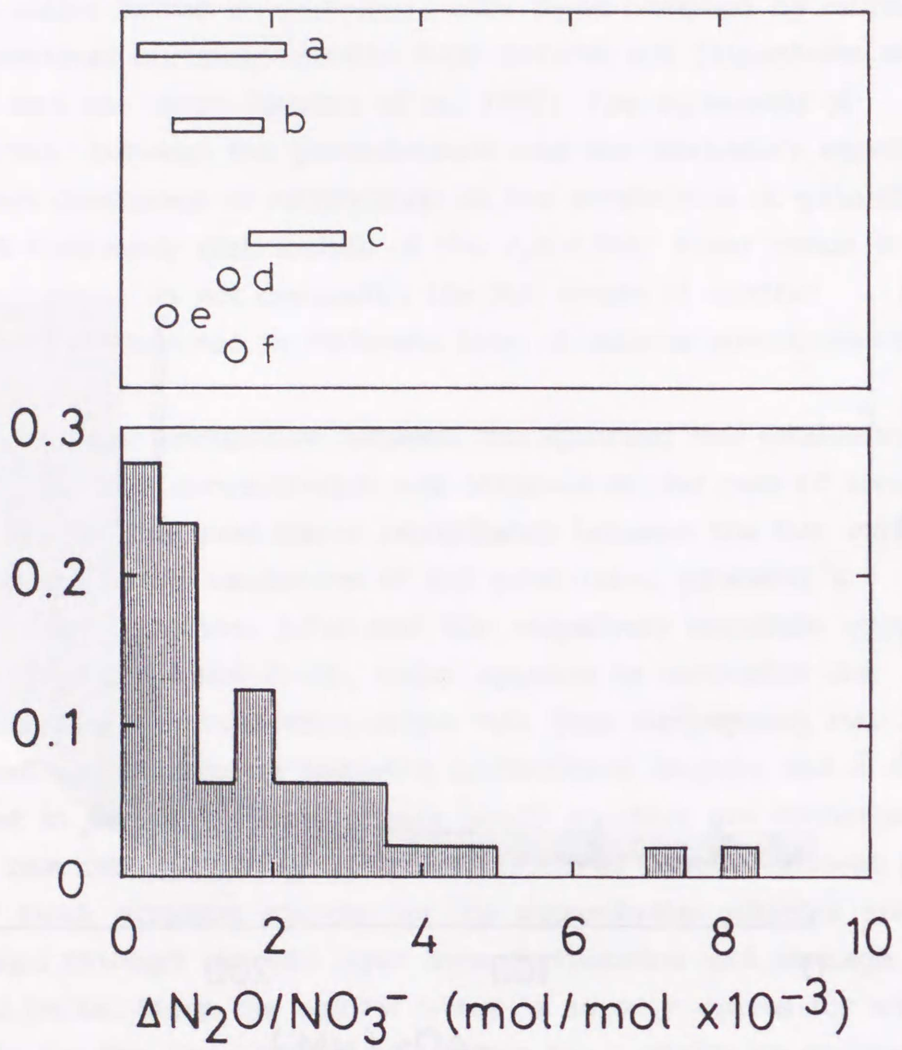

Figure II-12 Relative frequency distribution of $\Delta N_2O/NO_3^-$ molar ratios in groundwaters, n=61 (bottom), and its observations in other oxic environments where nitrification is believed as a predominant route of N_2O production (upper).

a–oxic soil: Bremner & Blackmer [1978]
b–ocean: Cohen & Gordon [1979]
c–oxic soil: Keeny *et al.*, [1985].
d–ocean: Elkins *et al.* [1978].
e–ocean: Yoshinari [1976]
f–nitrification facility: Yoshinari & Wahlen [1985]

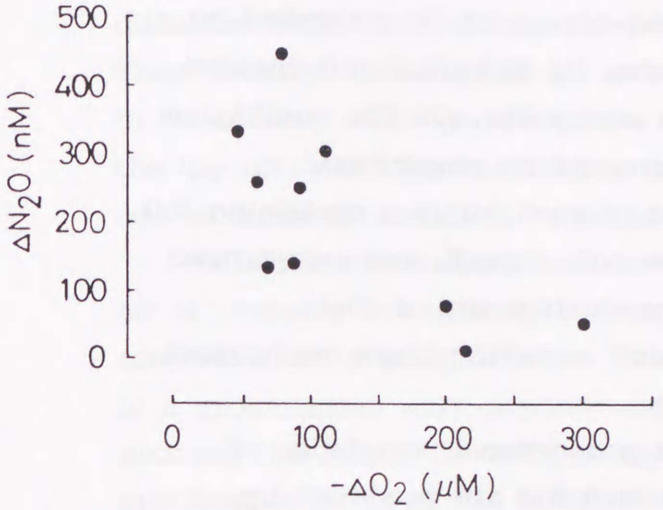

Figure II-13 ΔN_2O *vs.* $-\Delta O_2$ relationship in Long Island well waters (U21–U30).

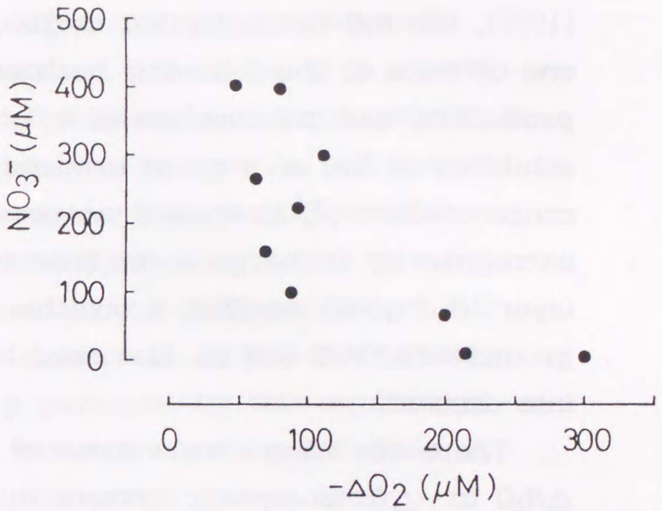

Figure II-14 NO_3^- *vs.* $-\Delta O_2$ relationship in Long Island well waters (U21–U30).

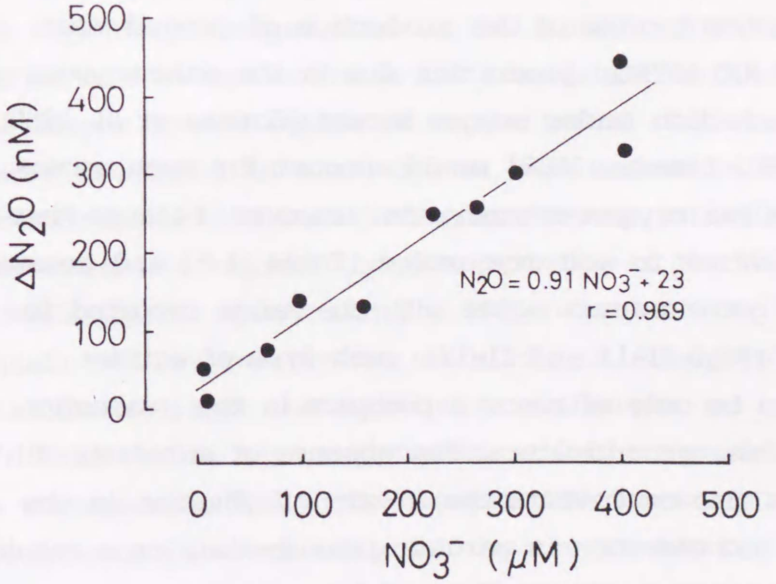

Figure II-15 ΔN_2O *vs.* NO_3^- relationship in Long Island well waters (U21–U30).

Production Site of Groundwater N_2O

According to the review for mechanisms controlling concentrations of common dissolved gases in groundwaters by Almon and Margaritz [1990], the N_2O concentration in groundwater must be controlled by one or more of the following mechanisms. (1) Biological and chemical production and consumption of N_2O in an aquifer. (2) The equilibrium solubility of N_2O at a given temperature and its atmospheric concentration. (2) Downward movement of small bubbles containing N_2O entrapped by recharge water from the soil, subsoil, and unsaturated layer to a given aquifer. A possible production site of the groundwater N_2O will be discussed below as taking these mechanisms into account.

There are many observations of a proportional correlation of ΔN_2O to $-\Delta O_2$ in aquatic systems in which N_2O are produced by nitrification [Yoshinari, 1976; Elkins *et al.*, 1978; Cohen and Gordon, 1979; Cline *et al.*, 1987; Yoh *et al.*, 1988; Butler *et al.*, 1989]. On the contrary, the most plots of ΔN_2O versus $-\Delta O_2$ in the groundwaters locate upper position than the line presenting an empirical relationship between the two variables in ocean water column at 0-30 ℃ temperature range (Fig. II-8). Assuming aquifer nitrification as a dominant route of the production of groundwater N_2O, higher yield of N_2O to NO_3^- production due to the enhancement of N_2O yield via NO_2^- reduction under oxygen stress [Goreau *et al.* 1980; Lipschultz *et al.*, 1981; Downes, 1988] would account for such excess production of N_2O to the oxygen consumption. However, because these groundwaters were rather to well oxygenated (Table II-5) and because $\Delta N_2O/NO_3^-$ ratios in groundwaters agree with the range reported for aerobic nitrification (Figs II-11 and II-12), such type of aquifer nitrification seems to be only of minor importance in the production of N_2O dissolving in the groundwaters. The absence of substrate NH_4^+ in the groundwaters also contradicts the *in situ* nitrification in the aquifers. Thus, the N_2O elevation in aerobic groundwaters as a result of an aquifer nitrification should be eliminated.

Seiler and Conrad [1981] observed that the N_2O mixing ratio elevated in the soil atmosphere as depth increase to 60 cm by factor of two as compared to the ambient concentration. They concluded that the N_2O emission from soil surface must be due to the N_2O production in the uppermost soil layer or at the soil surface, because the extent of N_2O production in deeper soil layers and its molecular diffusion to the soil surface did not affect the soil emission rate. Their findings also suggest an existence of N_2O source in a subsoil to an aquifer, which is apart from the production and consumption in the top soil. Such N_2O production in depper part of the soil column can be assumed as one of major sources for the groundwter N_2O.

An equilibrium between an aquifer waters and the N_2O in a soil air is probably not directly correspond to the determination of N_2O concentration in groundwaters. That is because, if N_2O concentration in a groundwater were predominantly governed by this equilibrium process, the $\Delta N_2O/NO_3^-$ ratio should deviated from the N_2O yield in nitrification owing to the substantial difference in solubility between N_2O and NO_3^-. According to the long term observation at J19, of which detail will be discussed in the next section, the N_2O concentration was independent from the fluctuation in water discharge corresponding the change in amount of precipitation. The observation indirectly suggests a N_2O production during percolation of the rain water as following description. Since NH_4^+ is commonly adsorbed on the negatively charged sites on the surface of soil particles, nitrification is also active in the boundary. The NH_4^+ supplement for the deeper zone is restricted in the period of the water movement, mostly downward, in a soil column. The undetectable NH_4^+ concentrations in the studied groundwaters probably reflect this mechanism and suggest complete oxidation of the NH_4^+ to NO_3^- and partially to N_2O accumulating in the groundwaters. The hypothesis can explain more reasonably the uniformity of $\Delta N_2O/NO_3^-$ in the aerobic groundwaters than the equilibrium mechanism.

Although the above discussion is fairly speculative, it may not be unreasonable to hypothesize that N_2O concentrations in the oxic groundwaters are not controlled by the production in aquifer waters, but by the production in deep soil layers, probably a nitrification as downward movement of rechage waters. Further study is awaited to determine this assumption.

Possible Charging of N_2O to an Aquifer

Periodical observation at the site J19, a natural spring site NKM, is presented in Fig. II-16. The $-\triangle O_2$ tends to be large in summer to fall and small in winter to early spring. This variation probably reflects seasonal fluctuation of aerobic organic matter decay in soil. On the other hand, such a trend was not clear in $\triangle N_2O$ nor NO_3^-. There is no significant correlation among $-\triangle O_2$, $\triangle N_2O$ and NO_3^- (Figs II-17, II-18 and II-19). The results suggest isoration of sites between minerarization and nitrification. Another possible reason for that is the fact found recently that the spring water is a mixture of two of different aquifer waters having each small catchment. The idea has been revealed by the deviated relative abundance in volatile chlorinated organic matters of each spring water [Miyazaki and Ogura, unpublished data].

The supersaturation of N_2O to the atmospheric equilibria was continuously observed irrespective of the significant seasonal fluctuation in water discharge. Because the water discharge changed as the amount of rainfall, this may be a reflection of a considerably big size of the N_2O pool in the soil-groundwater system or a production of the gas during infiltration of the recharge water as briefly mentioned in the previous section. The annual discharge of N_2O from the site J19 was calculated at 2.4 kg N_2O-N for 1987 and 4.7 kg N_2O-N for 1988, by summing up the products of $\triangle N_2O$ and water discharge. Assuming that a large proportion of N_2O in groundwater was produced in the overlying soil column, subsoil, or unsaturated layer then transported to the aquifer, N_2O charging rate can be estimated at 1.2 and 2.3 mg $N_2O\text{-}N \cdot yr^{-1}$ for 1987 and 1988, by simply dividing the annual discharges with the catchment area of 2.0 km^2 [Ogura and Morikawa, 1985]. When compared with reported N_2O emission from terrestrial ecosystem to the atmosphere of 28-190 mg $N_2O\text{-}N \cdot yr^{-1}$ [Banin *et al.*, 1984] and emission from temperate forest soils of 15-240 mg $N_2O\text{-}N \cdot yr^{-1}$ [Bowden *et al.*, 1990], the charging rate correspond to 0.5-15 %. When the fact that soil covers less than one-third of the catchment is taken into consideration, the ratio increases by factor of three. Although the soil emission rate in the J19 catchment was not determined in this study, the result implies

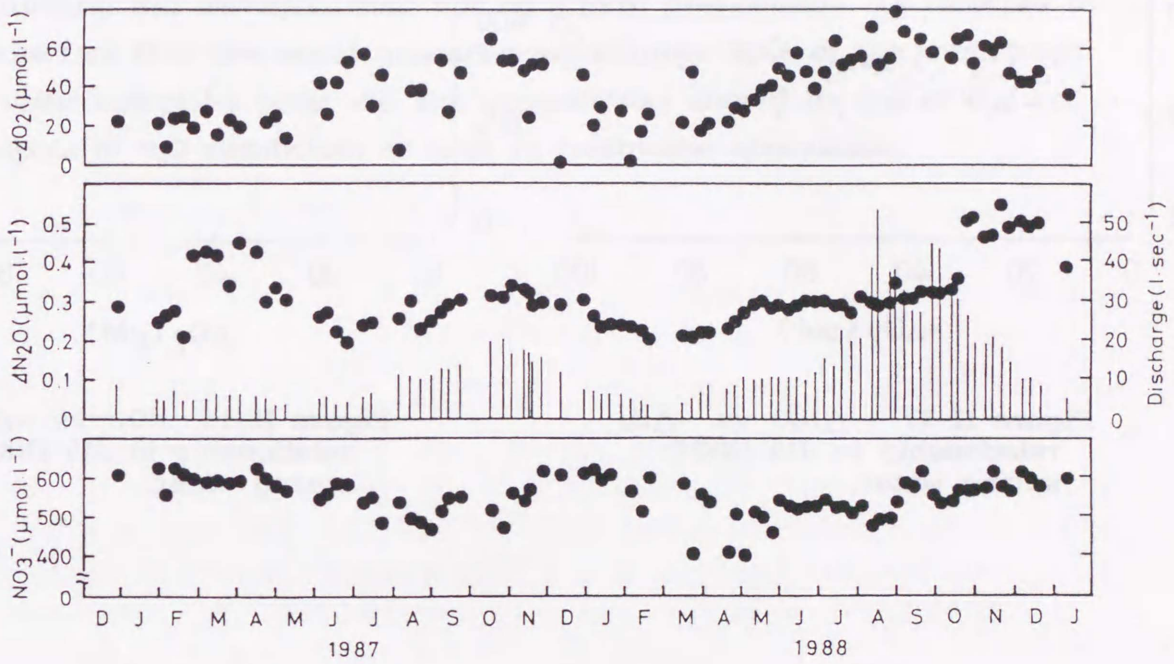

Figure II-16 Periodical observations of $\triangle N_2O$, $-\triangle O_2$, NO_3^- concentrations (closed circles) and water discharges (vertical bars) at the spring J19 (NKM) [*Ueda et al.*, 1991a]. Water temperature was quite constant at 15.6 ± 0.2 ℃. The change of water discharge corresponded to that in amount of rain fall.

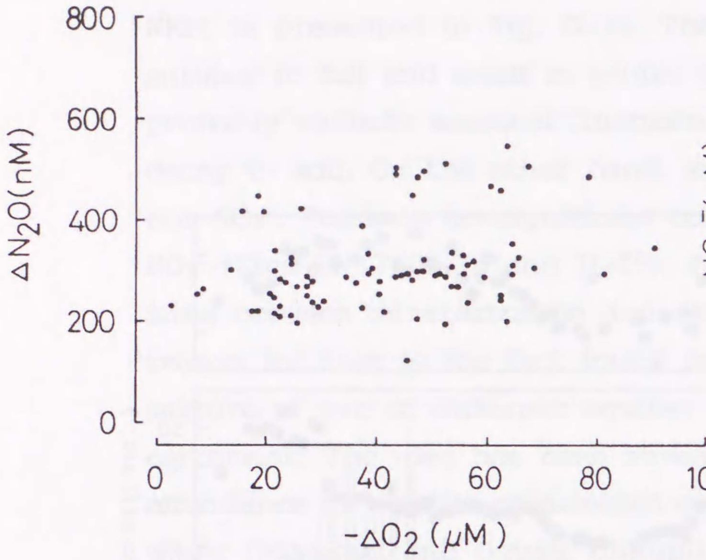

Figure II-17 ΔN_2O *vs.* $-\Delta O_2$ relationship in J19 (NKM) spring water.

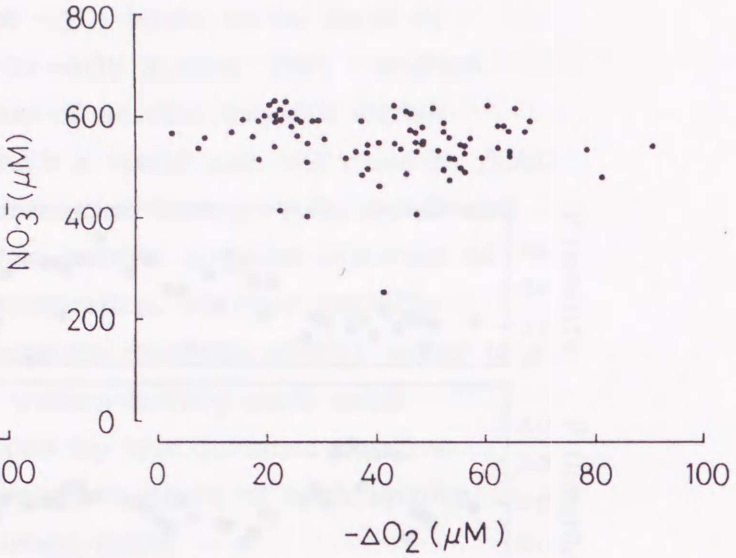

Figure II-18 NO_3^- *vs.* $-\Delta O_2$ relationship in J19 (NKM) spring water.

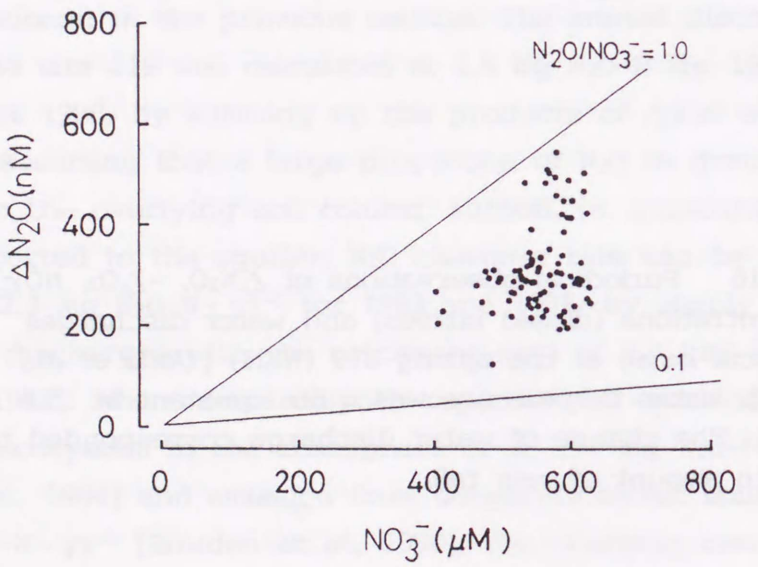

Figure II-19 ΔN_2O *vs.* NO_3^- relationship in J19 (NKM) spring water.

that groundwater may not be crucial but still be a significant reservoir in the global N_2O cycle.

Whatever the mechanism of N_2O production is, the degree of N_2O accumulation in groundwater can be recognized to some extent as a function of NO_3^- concentration. Because the nitrate contamination of groundwater is arising as a worldwide environmental issue, the relating N_2O elevation must not be a local phenomenon. As referred to the fact that the world groundwater occupies 98 % of the total fresh water excepting polar ice, the groundwater should be one of the hot spots in N_2O metabolism at least in freshwater ecosystems.

CHAPTER III

NITROGEN STABLE ISOTOPE RATIOS OF NITROUS OXIDE IN OXIC GROUNDWATERS

ABSTRACT — Nitrogen stable isotope ratios ($\delta^{15}N$) of N_2O and NO_3^- in 2 wells (J01, J02) and 6 natural springs (J19, J20, J21, J22, J23, J24) waters collected in the western part of Tokyo were determined by mass spectrometry to discuss the mechanisms producing the excess N_2O.

$\delta^{15}N$ values of the N_2O from -5.8 to -21.3 ‰ were significantly lower than those of coexistent NO_3^-, +3.8 to +8.3 ‰. It is widely known that $\delta^{15}N$ of groundwater NO_3^- is commonly close to that of source nitrogen, on condition that degree of nitrate consumption is negligible in an aquifer. Because plenty of dissolved O_2 and low DOC in these groundwaters suggest strong inhibition of nitrate respirations (Chapter II), the $\delta^{15}N$ NO_3^- must be nearly equal to that of source nitrogens. In that case, assuming nitrification as a producing mechanism of N_2O, the $\delta^{15}N$ difference between N_2O and NO_3^- is a result of a apparent isotope effect in N_2O production from ammonium. The ^{15}N-depletions in N_2O as compared to NO_3^- in the studied groundwaters consistent with the evidence that N_2O generated from nitrification is highly depleted in ^{15}N as compared to the substrate ammonium. The $\Delta N_2O/NO_3^-$ mole fractions in these groundwaters do not contradict the reported yield of N_2O in pure cultures of nitrifying bacteria and field observations (CHapter II).

In denitrification, on the other hand, the production of ^{15}N-depleted N_2O is restricted only in the period when the NO_3^- reduction to N_2O overwhelms the further reduction of N_2O to N_2. Because denitrification in natural environments tend to be in quasi-steady state, and because of similar isotope effects in both production and consumption of the N_2O, $\delta^{15}N$ in N_2O from denitrification is expected similar to that of the NO_3^-. The depletions in ^{15}N of the groundwater N_2O mean minor contribution of denitrification as a production route of N_2O accumulating in the present aquifers.

Thus, nitrogen isotopic findings suggest nitrification to be a major mechanism producing N_2O supersaturated in the studied groundwaters.

III-1. INTRODUCTION

Significant accumulations of N_2O in soil solutions [Davidson and Firestone, 1988], seepage waters from different soil systems [Dowdell *et al.,* 1979; Minami and Fukushi, 1984; Bowden and Bormann, 1986; Davidson and Swank, 1990, Ueda *et al.,* 1991b], spring waters [Ueda *et al.,* 1991a; 1991b], and aquifer groundwaters [Ronen *et al.,* 1988; Smith, 1990; Smith *et al.,* in press; Ueda *et al.,* 1991a; 1991b] have become known. These findings suggest significant role of groundwaters as one of reservoirs in the biogeochemical cycle of N_2O.

In Chapter II, it has been suggested by the following evidence that nitrification is a most likely source for the excess N_2O to the atmospheric equilibria in the studied groundwaters. (1) $\triangle N_2O/NO_3^-$ molar ratios overlap the reported N_2O yield for nitrification. (2) Low DOC concentration and oxygenated condition must inhibit an active nitrate respirations. (3) No detectable NH_4^+ and NO_2^- demonstrates little possibility of occurrence of dissimilatory nitrate reduction.

Nevertheless, N_2O yield during cultures of *Nitrosomonas europaea* [Goreau *et al.,* 1980; Lipschultz *et al.,* 1981] were so variable obeying an oxygen availability that it does not seem enough to identify nitrification as an dominant pathway of N_2O production by the parameter of $\triangle N_2O/NO_3^-$ ratio alone. If N_2O in the oxic groundwaters were produced in soil column and/or unsaturated zone then moved to an underlying aquifer as discussed in Chapter II, N_2O production by soil denitrification is expected as well as by nitrification. Also, even if the groundwaters appear to be well oxygenated, denitrification in micro anaerobic sites of aquifer sediments cannot be ruled out.

Stable isotope signatures may provide excellent information to think about origins and behaviors of targeting compounds in natural environments as discussed in Chapter I. The nitrogen stable isotope study is conducted here. The objective is to determine the hypothesis derived from the chemical characterization of groundwaters that the excess N_2O with respect to the atmospheric equilibria in the oxic groundwaters are mainly produced by nitrification.

III-2. MATERIALS AND METHODS

Sampling

Studies were conducted at 2 wells on the campus of the Faculty of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo (J01, J02) and 6 springs (J19, J20, J21, J22, J23, J24) in the western part of Tokyo. The sample identification is as presented in Table II-1. Waters were collected in polyethylene bottles (ca. 20 L in volume) after following manners.

Well waters were taken from taps which enable us to obtain raw waters before treatments with sodium hypochlorite. The water was introduced to the bottom of the bottle by a polyethylene tube connected to the tap. Since the wells are daily used, only several minutes of discharge prior to the collection was carried out. This period seemed adequate by monitoring the change in temperature and specific conductance of the effluent water. After allowing sufficient volume of water (usually almost half of the bottle content) to overflow, the bottle was capped by a rubber stopper with no head space. The stopper was equipped with a sintered glass ball (50 mm o.d., KINOSHITA RIKA KOGYO Co.,Ltd.) to facilitate gas dispersion during extraction. Twenty mL of 10 M NaOH was added per each bottle in the field to prevent the microbial metabolism and gasification of bicarbonate ions. Some samples were poisoned as soon as they were brought back to the laboratory. The bottles were stored at the room temperature until extraction.

In case of the spring sites, water was pumped up by a portable magnetic pump powered by an electric battery with a DC/AC invertor, and filled from the bottom of the bottle. Hereafter, handling of samples are as same as for the well waters.

Nitrogen Isotopic Analysis in N_2O

An outline of the measurement is shown in Fig. III-1. To extract dissolved N_2O, the sample container was connected in series to a system composed of a closed circulation line and a high vacuum line (Fig. III-2). The circulation line had been previously filled with pure He gas (> 99.999 %) which had been further purified by passing

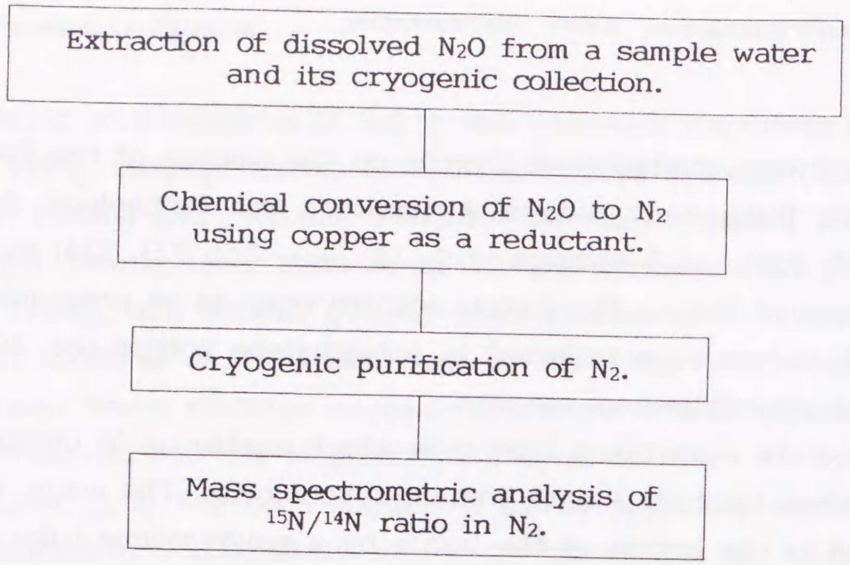

Figure III-1 Flow-chart of nitrogen isotopic analysis of N_2O in groundwater.

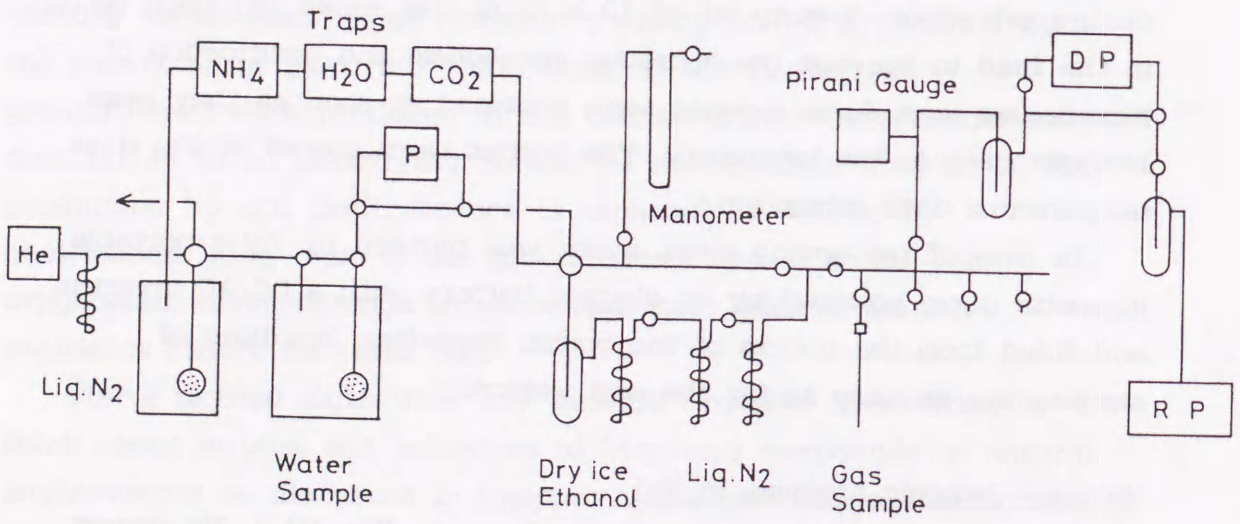

Figure III-2 System used to extract N_2O from a groundwater sample.

the gas in a glass coil chilled with liquid nitrogen. By opening two of 3-way cocks between the sample bottle and the system and subsequently starting a diaphragm pump installed in the system, about 2 L of the sample water was firstly pushed away from the top of the bottle and received by a glass reservoir, which make a head space in the sample bottle. Dissolved gases were stripped from the water by circulating He gas through the sintered glass balls in the sample bottle and the glass reservoir. Helium flow rate was 3 L・min^{-1}. The stripping gas was washed by diluted H_2SO_4 to eliminate NH_3. Water vapor and carbon dioxide in the gas was removed by Drierite and Ascarite column, respectively. After further dehumifying the gas in a dry-ice ethanol cold trap, N_2O was frozen out from the He carrier in the series of two glass coils chilled with liquid nitrogen. Because decline of N_2O recovery was observed when Molecular Sieve 5A was used as adsorber (data not shown), which happened in case that CO_2 and H_2O contaminations were significant, no molecular sieve type adsorber was used for the N_2O collection in this study. So that the system used in this study is absolutely free from a risk of isotope fractionation by using some adsorber to collect N_2O.

After 4 hours circulation of He gas, the two glass coils were isolated from the extracting system by turning a 4-way valve. The He gas and trapped gases in liquid phase in the coils were fully evacuated under liquid nitrogen temperature, then the cock to vacuum was closed. Trapped N_2O was expanded in the glass coils by changing the liquid nitrogen with a dry-ice ethanol slush. After waiting for several minutes, the N_2O was cryogenically collected in a Pyrex ampoule which had been previously filled with 1 g of porous copper (Cu^0) wires and been fully evacuated. The ampoule was pre-ignited at 550 ℃ for 3 hours to remove any organic compounds. After complete collection of the condensable fraction, the ampoule was evacuated again, then flame-sealed. N_2O was reduced to N_2 by heating the ampoule in an electric oven.

After gradually cool the ampoule to the room temperature in the oven, it was attached to the inlet system of the mass spectrometer (HITACHI RMU-6R, Mitsubishi Kasei Institute of Life Sciences) by two of Cajon fittings with a stainless steel bellows, then evacuated the air in a dead space. After full chilling of the ampoule with liquid nitrogen, N_2 was expanded in an inlet system to the cylinder for sample by breaking the ampoule. By controlling the mercury piston. The sample pressure was adjusted to that of the reference side which had been filled with standard. Commercial tank N_2 was used as the running standard, which was calibrated with the atmospheric nitrogen as the primary standard. The nitrogen isotopic abundances were expressed in terms of $\delta^{15}N$ (‰). Its definition was mentioned in Chapter I.

Nitrogen Isotopic Analysis in NO_3^-

$\delta^{15}N$ values in NO_3^- were measured after the method of Wada and Hattori [1976]. The preparation involves: (1) condensation of NO_3^- by distillation under basic condition, (2) Kjeldahl reduction of NO_3^- to NH_3, (3) steam distillation of the NH_3 into dilute H_2SO_4 to form $(NH_4)_2SO_4$, (4) oxidation of the ammonium by KOBr to obtain N_2.

Water containing 2 to 3 mg of nitrogen as NO_3^- was sampled from the 20 L tank which had been used for the N_2O extraction. The water was first reduced its volume to several milliliter in a glass beaker by evaporating it on an electric hot plate at 80 ℃. Since the water was poisoned with KOH solution at the time of sampling, NH_4^+ originally contained in a sample and derived from hydrolysis of labile organic matters should escape from the sample during the evaporation.

The sample was put in a Kjeldahl flask, then 2 g of Devarda's alloy was added. Immediately after adding 20 mL of 10 M KOH, NO_3^- began to be reduced to NH_3, then the generated NH_3 was steam-distilled into the receiving vessel containing 10 mL of 0.05 M H_2SO_4. Approximately 100 mL of recovered solution, acid ammonium sulfate, was concentrated to 5 mL by evaporating it with mild heat under vacuum condition.

The condensed acid $(NH_4)_2SO_4$ solution was transferred in one side of the Rittenberg's reacting container with a vacuum cock [Rittenberg, 1946]. The other side of the ampoule was filled with KOBr and KOH. The container was attached to the vacuum system, then degassed any ambient air from the two solutions, by freezing, evacuating, thawing, then evacuating again. After the confirmation of complete degassing by a Pirany gauge, the container was detached from the vacuum system. By mixing the two solutions, NH_4^+ was oxidized to N_2 after the following reaction.

$$2\ NH_3 + 3\ KOBr \rightarrow 3\ KBr + 3\ H_2O + N_2$$

Small amount of N_2O is co-produced during the above reaction. Because of the small nitrogen isotope fractionation in this branching reaction when the reactant NH_4^+ is sufficient, H_2O and N_2O produced were frozen out in a liquid nitrogen cold trap in this study. The remaining gas was further purified by circulating it through the furnaces packed with Cu and CuO which were heated at 400 ℃ and 600 ℃, respectively, using a mercury Toeplar pump for 15 minutes. Oxygen, carbon monoxide, organic gases, and any gaseous nitrogen oxides was converted to CO_2 or N_2, then CO_2 was frozen out in the liquid nitrogen cold trap installed in the system. Finally, N_2 gas was compressed in an evacuated Pyrex ampoule by a mercury Toeplar pump and flame-sealed. The measurement and notation of nitrogen isotope ratio are as same as stated in the previous section. The analytical error is within ± 0.2 ‰.

III-3. RESULTS AND DISCUSSION

Analytical Precision of Nitrogen Isotopic Composition in N_2O

Both quantitative treatment of an aimed compound and performance of stoichiometric reaction in chemical preparations are indispensable for a precious analysis of stable isotope ratio. In the present method, N_2O was reduced to N_2 by reacting it with metal copper (Cu^0) in a sealed Pyrex glass ampoule at 400 °C in place of the method of Yoshida [1984] who carried out the conversion in a vacuum system by circulating the gas through Cu (400 °C) and CuO (700 °C) furnaces by a mercury Toeplar pump. The arrangement aimed at simplifying the system and saving time by multiple treatment of samples.

Fig III-3 illustrates the temperature dependence of N_2 yields when 10 μmoles of N_2O were heated with 500 mg of Cu at given temperatures for 3 hours, then cooled overnight to the room temperature. One hundred percent N_2 yield was obtained in case that the temperatures were higher than 300 °C. Two of small illustrations clearly demonstrate that the condition to heat N_2O with 1 g of Cu at 400 °C for 3 hours followed by overnight gradual cooling ensure stoichiometric conversion of N_2O to N_2 when an initial N_2O is less than 100 μmoles. There is no systematic difference in $\delta^{15}N$ analyses between the sealed-tube method in this study and the dynamic conversion (Table III-1), which reveals no significant isotope effect in the preparation in this study. The reproducibility of measuring $\delta^{15}N$ in N_2O was ±0.1 ‰.

Efficiency of N_2O extraction from a water sample was determined. Fig. III-4 shows a typical change of dissolved N_2O concentration in a sample water with time of the gas extraction. Concentrations were measured by the purge-trap-GC method that was described in Chapter II. After 2 hours of circulating He gas, N_2O in sample water was below the detection limit of 0.1 nM. Similar time courses were obtained in other runs. For groundwater samples, 4-6 hours extraction was performed to make sure the one hundred percent collection of N_2O.

Possible nitrogen isotopic fractionation during the extraction was tested. Pure tank N_2O with known $\delta^{15}N$ value was spiked to the

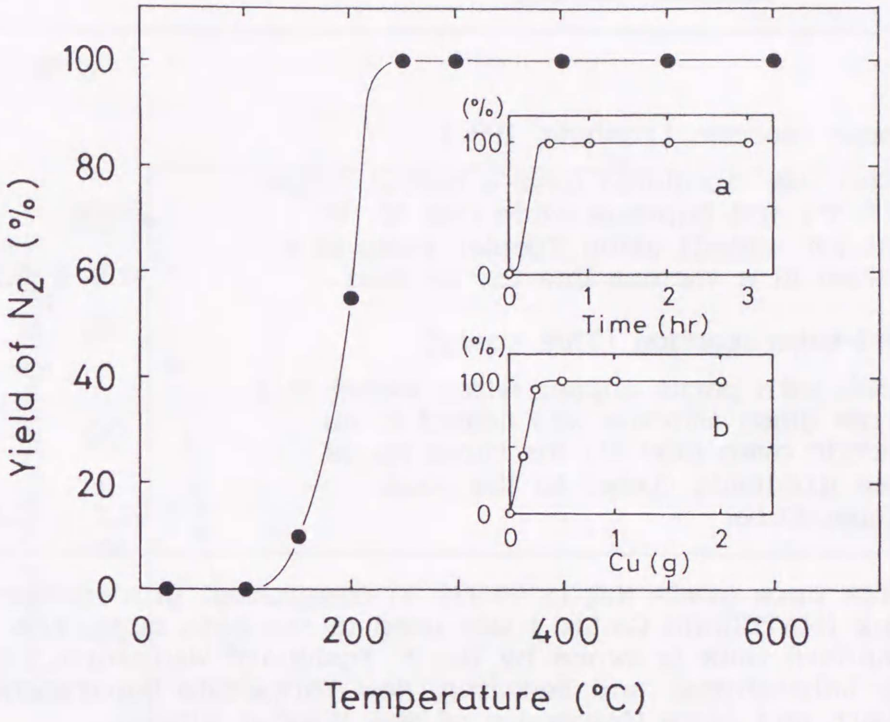

Figure III-3 Temperature dependence of the efficiency of $N_2O \rightarrow N_2$ reaction with copper as a reductant. About 10 μmoles of N_2O were measured volumetrically with precision of 0.1 μL (several nano-moles), then sealed in an ampoule with 500 mg of copper wires. After 3 hours heat of the samples at given temperatures, volumes of the produced N_2 were measured manometrically with the same precision for N_2O. Two of small boxes respectively illustrate, a) time cource of the reaction when approx. 10 μmoles of N_2O with 500 mg of copper wiers were heated at 400 °C, and b) approx. 100 μmoles of N_2O with 0, 0.25, 0.50, 1.0, 2.0 g of copper wiers were heated at 400 °C for 3 hours.

TABLE III-1 Comparison of the Nitrogen Stable Isotope Ratio in Standard N_2O after the Dynamic and Sealed-tube Reaction Methods

	$\delta^{15}N$
A. Dynamic reaction [*Yoshida*, 1984]	
1) N_2O was circulated over a heated copper (400 ℃) and cuprous oxide (700 ℃, Pt catalyst added) using Toepler pump in a system in a vacuum line for an hour.	2) +2.5 ± 0.1 ‰
B. Sealed-tube reaction [This study]	
1) N_2O with porus copper wires sealed in a Pyrex glass ampoule was heated in an electric oven (400 ℃) for three hours, then gradually cooled to the room temperature.	3) +2.5 ± 0.1 ‰

[1] Ultra pure grade N_2O (> 99.999 %) compressed in a commercial tank (MATHESON Co.Ltd.) was used in the both runs. The standard tank is owned by Dr. T. Yoshinari, Wadsworth Center for Laboratories and Research, New York State Department of Health and State University of New York at Albany.

[2] Triplicate measurements were carried out by Dr. M. Minagawa, Mitsubishi Kasei Institute of Life Sciences. The data were kindly provided by Dr. T. Yoshinari.

[3] Measurements (n=7) in this study.

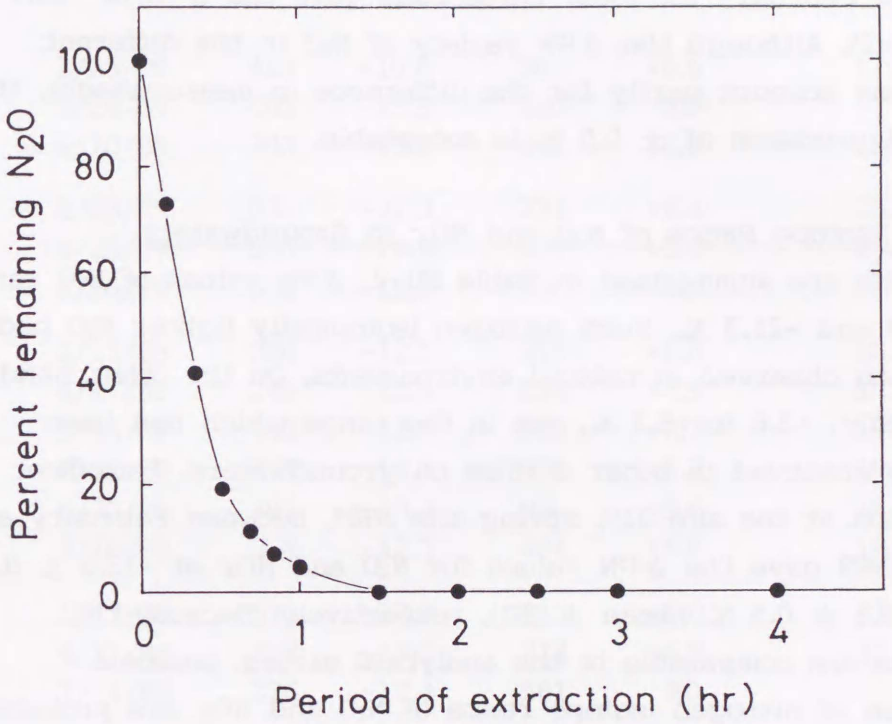

Figure III-4 Change of N_2O concentration in a sample water with time of extraction. Concentrations were measured by the purge and trap gas chromatography described in Chapter II. N_2O was not detected through 1.5 to 4 hours after the start of extraction. Similar results were obtained in other runs.

system through a rubber septum using gas tight syringe, then $\delta^{15}N$ of the collected N_2O was determined. $\delta^{15}N$ values of the tank N_2O and recovered N_2O were 1.8 ± 0.2 ‰ (mean ± SD: n=5) and 2.2 ± 0.2 ‰ (n=2), respectively.

Reproducibility in measuring $\delta^{15}N$ of N_2O in natural samples were tested with the J19 spring water samples collected in different bottles on February 23, 1989. Three runs gave the $\delta^{15}N$ of -13.0 ± 0.5 ‰ (n=3). Although the $\delta^{15}N$ variety of N_2O in the different bottles may account partly for the difference in measurements, the analytical precision of ± 0.5 ‰ is acceptable.

Nitrogen Isotope Ratios of N_2O and NO_3^- in Groundwaters

Results are summarized in Table III-2. $\delta^{15}N$ values of N_2O ranged from -5.8 and -21.3 ‰. Such nitrogen isotopically lighter N_2O had never been observed in natural environments. On the other hand, the $\delta^{15}N$ of NO_3^-, +3.6 to +8.3 ‰, are in the range which had been commonly observed in other studies on groundwaters. Periodical observation at the site J19, spring site NKM, between February and July in 1989 gave the $\delta^{15}N$ values for N_2O and NO_3^- at -13.0 ± 0.7 ‰ and +5.5 ± 0.5 ‰ (mean ± SD), respectively. Because the deviations are comparable to the analytical errors, seasonal fluctuation of nitrogen isotope ratios of N_2O and NO_3^- are probably small in the studied groundwaters. Thus, these groundwater N_2O are characterized by its much lower $\delta^{15}N$ values than those of the coexistent NO_3^-.

Producing Mechanisms of Groundwater N_2O

Nitrogen isotopic composition of groundwater NO_3^- is typically close to that of the source nitrogen on condition that NO_3^- consumption in an aquifer by denitrification and dissimilatory nitrate reduction is insignificant like the present groundwaters [Kreitler, 1975; Heaton, 1986, Mariotti, 1988]. Small nitrogen isotopic discrimination between the source nitrogen and the final product NO_3^- in natural environments can be explained by considering following facts; (1) natural nitrification activity tends to be determined by the ammonium availability, (2) the rate of nitrite

TABLE III-2 Nitrogen Stable Isotope Ratios of Nitrous Oxide and Nitrate in Groundwaters at the Western Part of Tokyo

Code	Date of sampling	N_2O		NO_3^-		Discrimination[1]
		conc (nM)	$\delta^{15}N$ (‰)	conc (μM)	$\delta^{15}N$ (‰)	$\Delta\delta^{15}N$ (‰)
J01	2/23/89	423	-10.6	561	+6.6	17.2
	4/24/89	495	-13.8	580	ND[2]	–
	6/20/89	393	-13.8	442	+6.0	19.8
J02	2/23/89	1031	-21.3	234	+4.4	25.7
	4/24/89	836	-19.9	218	+3.8	23.7
	6/20/89	676	-19.7	ND	ND	–
J19	2/13/89	286	-12.5	587	+6.0	18.5
	4/17/89	249	-12.4	534	+4.9	17.3
	5/15/89	340	-12.9	538	+6.3	19.2
	6/12/89	297	-14.1	531	+5.2	19.3
	6/20/89	297	-13.3	ND	+5.6	18.9
	7/18/89	318	-12.7	484	+5.6	18.9
J20	1/13/89	575	-17.0	815	+6.0	23.0
	4/17/89	318	-19.1	712	+6.9	26.0
	7/ 1/89	336	-17.4	561	ND	–
J21	4/17/89	115	-15.9	663	+7.3	23.2
	7/ 1/89	364	-18.1	682	–	–
J22	7/ 1/89	471	-11.3	622	+3.6	17.4
J23	7/ 1/89	399	-16.8	418	ND	–
J24	1/13/89	1493	- 5.8	414	+8.3	14.1

[1] Difference in $\delta^{15}N$ between N_2O and NO_3^-

[2] Not determined

oxidation is generally much faster than that of the oxidation of ammonium. So that, generally speaking, one can identify the source nitrogen of nitrate in a denitrification-free aquifer based on its $\delta^{15}N$ analysis; chemical fertilizer, -4 – +4 ‰, soil organic matters, +4 – +10 ‰, domestic sewage or animal manure, +10 – +20 ‰ [Kreitler, 1975; Heaton, 1986; Mariotti, 1988]. Concerning the studied groundwaters, because majority of the catchments is offered to residential districts, source contribution of agricultural practices is probably small. As referred to the pattern of land usage and the nitrogen isotopic categorization of nitrate, these groundwater NO_3^- derive largely from oxidation of soil organic matters which is the mixture of litters and domestic sewages from septic tanks previously used in this area [Ogura and Yoshida, 1981]. That is suggested by the high chlorine concentrations as well (see Appendix 1).

According to the $\triangle N_2O/NO_3^-$ molar ratios in groundwaters (see Chapter II), nitrification is suggested to be a major route of the N_2O production. On the assumption that N_2O is produced mainly by nitrification, the nitrogen isotopic discriminations between N_2O and NO_3^- are comparable to the difference in $\delta^{15}N$ between N_2O and nitrified nitrogen. A laboratory incubation of *Nitrosomonas europaea* showed that the N_2O from nitrification is extremely depleted in ^{15}N [Yoshida, 1988]. He reported that $\delta^{15}N$ of N_2O was lower than a substrate NH_4^+ by 60 ‰ and a end-product NO_2^- by 30 ‰, respectively. Concerning the soil nitrification, small apparent nitrogen isotopic fractionation between NO_2^- and NO_3^- is expected because NO_2^- is further oxidized to NO_3^- immediately after its production, which is revealed by the small NO_2^- pool in undisturbed natural environments. These facts suggest the nitrogen isotopic discrimination between the source nitrogen and N_2O in nitrification should be around 30 ‰. Significant depletions of ^{15}N in N_2O as compared to NO_3^- in the groundwaters are consistent with the results of laboratory experiments. However, the degree of ^{15}N-depletion of N_2O to NO_3^- in the groundwaters, 14.0 to 26.0 ‰, were smaller than the Yoshida's results. The less discriminations in the groundwaters may be explained as considering the fact that a magnitude of biological isotope fractionation is ordinary smaller in natural environments than that is obtained by a laboratory experiment because of a substrate limitation in nature. Small apparent nitrogen isotopic discriminations are also reported in case of groundwater denitrification [Mariotti *et al.*, 1988; Smith and Duff, 1988, Bottcher *et al.*, 1990; Smith, 1990; Wilson, 1990; Smith *et al.*, in press].

The less discrimination in nitrogen isotopes during N_2O productions from nitrification were also observed in other environments. In the oxic layers of eastern Tropical North Pacific Ocean, approximate $\delta^{15}N$ values of the net produced N_2O is predicted between -5 and -20 ‰ [Wada *et al.*, 1991], based on the mean observations of percent saturation of dissolved N_2O as 110-120 %, $\delta^{15}N$ in the dissolved N_2O as +5.2 ‰, and $\delta^{15}N$ in the atmospheric N_2O as +8.1 ‰ [Yoshida, 1984; Yoshida *et al.*, 1984]. $\delta^{15}N$ values of NH_4^+ and NO_3^- therein were reported as 6.5-7.5 ‰ [Miyake and Wada, 1967] and 4.8-7.5 ‰ [Miyake and Wada, 1967; Cline and Kaplan, 1975; Liu and Kaplan, 1989], respectively. At the 21 m depth of the mesotrophic alpine Lake Kizaki, Japan, where nitrification proceeded under low dissolved oxygen concentration, $\delta^{15}N$ of dissolved N_2O was -6.0 ‰, whereas those of NH_4^+ and NO_3^- were +0.2 ‰ [Wada *et al.*, 1991]. These two findings strongly suggest that the nitrogen isotopic discrimination in N_2O production by nitrification should be smaller in natural environments than that observed in laboratory experiments. Therefore, the assumption that a large part of the groundwater N_2O derives from nitrification is not unreasonable by the nitrogen stable isotopic view point.

If an aquifer denitrification occurs, the ^{15}N content in remaining NO_3^- should increase due to a favorable reduction of isotopically lighter nitrate [Mariotti *et al.*, 1988; Bottcher *et al.*, 1990; Smith, 1990; Fustec *et al.*, Smith *et al.*, in press]. Concerning the studied groundwaters, no ^{15}N enriched NO_3^- was observed, which consists with the conclusion based on the chemical characteristics of the groundwaters that NO_3^- reduction by denitrification and dissimilatory nitrate reduction are insignificant in the studied aquifers.

Even though low denitrification activity are expected in the present aquifers, reduction of only a small fraction of the NO_3^- pool is enough to sustain the N_2O pool in surveyed groundwaters. It should be noted that ^{15}N-depleted N_2O as compared to the substrate NO_3^- is produced from an early stage of denitrification because of predominantly governing by the isotope fractionation relating to the N_2O production. After the N_2O accumulation induce its further reduction to N_2 in denitrification, the $\delta^{15}N$ of N_2O depends upon the dynamics and the kinetic isotope effect in the production and consumption of N_2O. Namely, since nitrogen isotope fractionation factors during reductions of nitrate ($NO_3^- \rightarrow N_2O$) and nitrous oxide ($N_2O \rightarrow N_2$) are comparable order of magnitude as summarized in Table I-6, $\delta^{15}N$ of N_2O should become close to that of NO_3^- as the consumption of N_2O pool. Such phenomena were revealed by Wada *et al.* [1991] who traced $\delta^{15}N$ in N_2O during the anaerobic incubation of natural soils under the presence of sufficient nitrate in excess (Fig. III-5). The ^{15}N-depleted N_2O was initially observed during the period of high level of N_2O accumulation when the N_2O production rates were much higher than those of the N_2 production rates. Thereafter, the apparent nitrogen isotopic discrimination between N_2O and NO_3^- presented as a shaded area became smaller as a size of N_2O pool decreased. At the end of the incubation, $\delta^{15}N$ of N_2O was similar to that of the substrate NO_3^-, while the reaction had not yet achieved the steady state. The results clearly demonstrate that the ^{15}N-depleted N_2O than NO_3^- is accumulate only in the early stage of denitrification, and that the N_2O from a quasi-steady state denitrification gives a similar nitrogen isotope ratio to the substrate NO_3^-.

Because of well oxygenations of the studied groundwaters, anaerobic micro sites such as interior of soil aggregates or in cracks of sands and gravels in aquifers are probably the sites of denitrification, if it occurred. In such a condition, denitrifying activity must be determined by an availability of nitrate, which will result in a steady-state denitrification where most N_2O produced must be further reduced to N_2 [Mariotti, 1988]. On the basis of above consideration, the $\delta^{15}N$ of N_2O should be close to the substrate

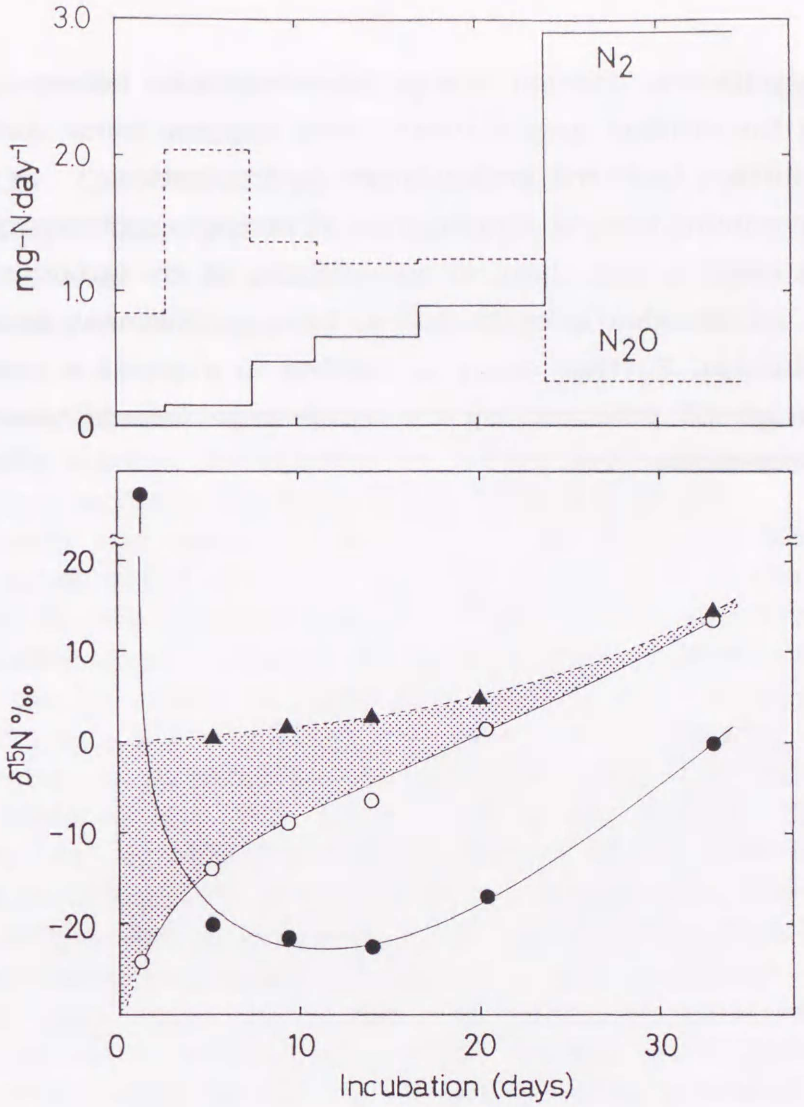

Figure III-4 Changes in $\delta^{15}N$ of N_2O (open circles) and chemical variables (NO_3^-: closed triangles, N_2: closed circles) during anaerobic soil incubation, from *Wada et al.* [1991] with partial modification. A shaded area presents an extent of apparent nitrogen isotopic discrimination between N_2O and NO_3^-. The large discrimination appeared at the initial stage of incubation, when a remarkable accumulation of N_2O was observed. As the N_2O production decreased, in other words the denitrification reaction closed to the steady state, the discrimination became smaller, and $\delta^{15}N$ of N_2O was nearly equal to that of NO_3^- at the end of the incubation. The results clearly demonstrate that ^{15}N-depleted N_2O as compared to the NO_3^- is produced by denitrification only in the period when N_2O production rate exceeds that of N_2.

NO_3^-. The significant nitrogen isotope discriminations between N_2O and NO_3^- in the studied groundwaters thus suggest minor contribution of N_2O production from the steady-state denitrification.

By determining natural abundances of nitrogen isotopes of N_2O and NO_3^- in this chapter and chemical compositions of the groundwaters in Chapter II, nitrification is concluded to be a predominant mechanism of N_2O production. Further study is awaited to evaluate a possible contribution of N_2O produced by the early-stage denitrification to the oxic groundwater pool.

CHAPTER IV

ORIGIN OF NITROUS OXIDE IN OXIC GROUNDWATERS: NITROGEN AND OXYGEN ISOTOPIC CHARACTERIZATION

__ABSTRACT__ — Analytical procedure to determine both nitrogen and oxygen stable isotopic compositions in N_2O has been developed. The new technique includes N_2O purification by means of gas chromatography and chemical conversion of N_2O to N_2 and CO_2 using a graphitic carbon foiled with gold as a reductant. $\delta^{15}N$ of the N_2 and $\delta^{18}O$ of the CO_2 are measured by mass spectrometry. Stable isotopic characterization of groundwater N_2O was carried out after this method to confirm the conclusion of Chapters II and III that N_2O in an oxygenated groundwater originates largely from nitrification.

Concerning 29 groundwaters in New York State, $\delta^{15}N$ and $\delta^{18}O$ values in dissolved N_2O were -2.4 to -32.0 ‰ and +30.8 to +41.6 ‰, respectively. For 14 Japanese groundwaters, $\delta^{15}N$ and $\delta^{18}O$ of N_2O were -28.4 to +2.3 ‰ and +22.0 to +50.4 ‰, respectively. The less contents of ^{15}N in N_2O as compared to that of the coexistent NO_3^- in oxic groundwaters as a result of Chapter III was confirmed again in the present observation. The lowest $\delta^{18}O$ value was measured with a seepage water N_2O from a cropping field, whereas the highest one with a shallow groundwater colored brown due to humic substance. When excepting these two distinct cases, the $\delta^{18}O$ values give smaller variation than $\delta^{15}N$. The $\delta^{18}O$ of N_2O are higher than that reported for NO_3^- in aerobic groundwaters, +3 to +10 ‰. The groundwater N_2O can be thus isotopically characterized by their ^{15}N-depletion and ^{18}O-enrichment as compared to the coexistent NO_3^-.

There was no concurrent enrichment of ^{15}N and ^{18}O in the groundwater N_2O, which means lack of significant modification of the stable isotope compositions in these groundwater N_2O through isotopic fractionation during N_2O consumption. The ^{18}O-enrichment of N_2O than NO_3^- contradicts to the nitrogen isotope fractionation of N_2O production by denitrification. Thus nitrification has been concluded to be a predominant mechanism to determine the isotopic composition of supersaturated N_2O in the nitrate contaminated aerobic groundwaters.

IV-1. INTRODUCTION

Through Chapters II and III, it has been concluded that soil nitrification is probably a major mechanism producing N_2O which is supersaturated in oxygenated groundwaters. The view is suggested by the following three findings. The first is the agreement of molar fractions of $\triangle N_2O$ to NO_3^- in the groundwaters with the reported N_2O yield during laboratory incubation of nitrifying bacteria and field experiments with various type of soils (Fig. II-5). The second argument is the significant depletion of ^{15}N in N_2O to the coexistent NO_3^-, which is consistent with the culture of *Nitrosomonas europaea* [Yoshida, 1989]. Chemical properties among the groundwaters, plenty of dissolved oxygen and little organic carbon, are the third basis suggesting strong inhibition of nitrate respirations in the aquifers (Table II-4 and Table II-5).

Yet, a possible contribution of N_2O production from denitrification on a pool in the oxic groundwaters cannot be ruled out. First, $\triangle N_2O/NO_3^-$ molar ratios vary over two order of magnitude, then the agreement of observations with the published ranges may be not enough to identify nitrification as an only source of the groundwater N_2O. Second, accumulation of ^{15}N-depleted N_2O was observed in the early stage of denitrification when a production of N_2O from NO_3^- overwhelms a reduction of N_2O to N_2 [Wada *et al.*, 1991]. Considering soil column as a major site of N_2O production, such type of denitrification with nitrogen isotopically lighter N_2O may happen immediately after a rainfall which should make soil column anaerobic temporarily. Also, even if a bulk groundwater seems well oxygenated, denitrification may proceed in micro anaerobic sites like cracks or slits of sands and gravels in the aquifers [Mariotti *et al.*, 1988].

An appropriate approach that may help elucidate producing mechanism of N_2O is to determine its oxygen stable isotope ratio together with nitrogen one. Wahlen and Yoshinari [1985] and Yoshinari and Wahlen [1985] firstly measured $\delta^{18}O$ of N_2O by IR-spectral method. Their data with large deviation among various environments exhibit the power of oxygen isotopic signature to be used in the source identification of N_2O. Recently, Kim and Craig [1990] performed nitrogen and oxygen isotopic characterization of the oceanic N_2O by means of mass spectrometry. While their study must be criticized because of lack of measuring N_2O concentration, there is no doubt that such a two-isotopic characterization must be the next stage of isotope study of N_2O [Zafiriou, 1990].

Even though, requirements of big size N_2O as much as 0.6 cc (STP) in Wahlen and Yoshinari [1985] and 1.0 cc (STP) in Kim and Craig [1990] for their $\delta^{18}O$ measurements were an obstacle to measure numbers of samples in natural environments. One of the objectives in this chapter is to develop a new method that enables us to measure $\delta^{18}O$ with a small amount of N_2O, less than 0.05 cc (STP). The goal is to re-examine the source mechanisms of the groundwater N_2O based on its two-isotope characterization.

The methodological development and the survey of the New York State groundwaters are the fruits of cooperative work with Dr. T. Yoshinari, New York State Department of Health and State University of New York at Albany.

IV-2. MATERIALS AND METHODS

Sample Identification

Groundwater samples were collected from the central part of Japan and New York State, USA. Serial code of each plot is the same that listed in Table II-1 and Table II-2, respectively. Collection of groundwater and extraction of N_2O from the sample were almost same as those described in Chapter III.

Analytical Methods

A. N_2O

$\delta^{15}N$ and $\delta^{18}O$ of N_2O were measured by mass spectrometry after chemically converting the gas to N_2 and CO_2, respectively. An outline of the analysis is shown as Fig. IV-1. The conversion of N_2O to N_2 and CO_2 was performed by reacting the N_2O with a hot graphite carbon rod (C^0) after the next formula.

$$N_2O + C \xrightarrow{\text{Au cat.}} N_2 + 1/2\ CO_2$$

Kim and Craig [1990] carried out the similar conversion. They chose a platinum wire coiled around a graphite rod as a catalyst, and performed the reaction by circulating the gas in a chamber containing the rod heated at 700 ℃ installed in a vacuum system using a Toeplar pump. CO produced due to high reaction temperature must be eliminated for $\delta^{15}N$ measurements in their method. In this study, on the contrary, gold was used as the catalyst to reduce the temperature of reaction, and the reaction was achieved in a sealed quartz ampoule. The reason for the choice of a gold catalyst is its unique character to absorb atomic oxygen (O) then release molecular oxygen (O_2). This property helps CO_2 generation from N_2O without CO formation, which results in an oxygen isotope fractionation and interference of $\delta^{15}N$ measurement because of the equal molecular weight of CO to N_2. Since the sealed-tube method does not require special apparatus for the gas circulation, the arrangements also achieve multiple sample treatments.

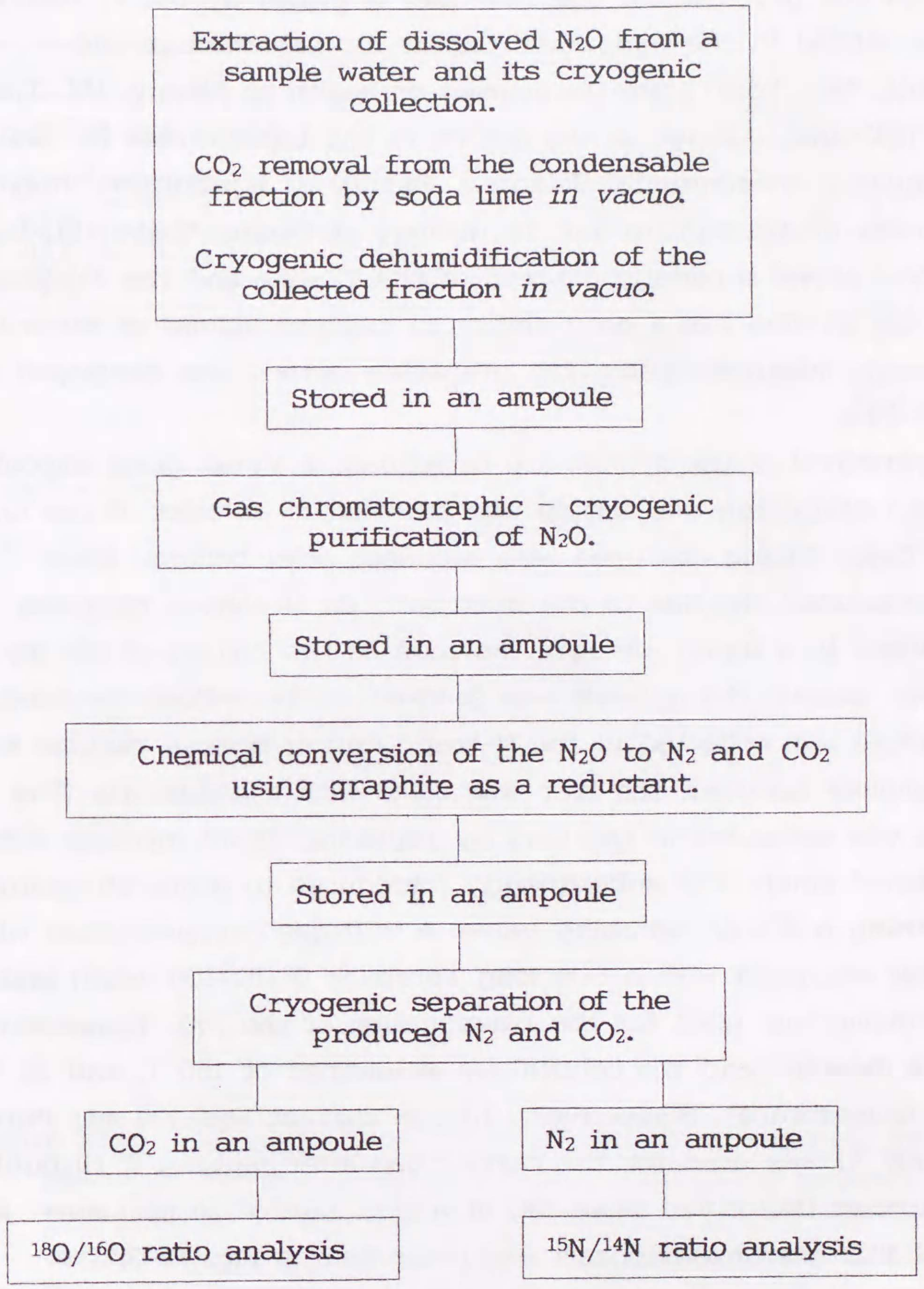

Figure IV-1 Flow-chart of nitrogen and oxygen stable isotopic analyses of N_2O in a groundwater.

Two vacuum systems with minor differences from each other were used for the preparation. One (TY-line) is owned by Dr. T. Yoshinari that is settled in the Wadsworth Center for Laboratories and Research, New York State Department of Health at Albany, NY. The other (SU-line) belongs to the author in the Laboratories for Soil and Aquatic Environmental Sciences, Faculty of Agriculture, Tokyo University of Agriculture and Technology at Fuchu, Tokyo (SU-line). Fig. IV-2 shows schematic diagram of the TY-line and the SU-line. Since the TY-line has a good ability to measure volume of micro-liter gas sample manometorically, the preparing method was developed using the TY-line.

Operations of the system are as follows. A Pyrex glass ampoule (6 mm o.d.) containing gas sample was attached to an inlet of the line using Cajon fitting equipped with stainless steel bellows. After fully evacuated the line to the inlet part, an U-shaped trap was submerged in a liquid nitrogen. As soon as the boiling of the liquid nitrogen ceased, the ampoule was cracked at the bellows by hand, then the sample was collected in the U-trap. Waiting several minutes for the complete transfer, the trap was filled with a helium gas. The sample was expanded in the trap by replacing liquid nitrogen with dry ice-ethanol slush, and subsequently introduced to a gas chromatograph by turning a 4-way switching valve. A TCD-gas chromatograph with an amplifier equipped with a 5 m long Porapack Q (80/100 mesh) stainless steel column was used for the fractionation of the N_2O. Temperatures for the detector and the column are maintained at 150 ℃ and 25 ℃ (room temperature), respectively. Bridge current was 120 mA. Pure He (> 99.999 %) was used for the carrier gas after passing it through a gas purifier (Molecular Sieve 5A) at a flow rate of 20 $mL \cdot min^{-1}$. A typical TCD-gas chromatogram was presented as Fig. IV-3.

After venting CO_2 fraction through mercury plug to prevent a counter flow of the ambient air, the N_2O fraction was introduced to a glass coil in a liquid nitrogen bath which had previously been evacuated and subsequently filled with the helium, by opening cock A and B then closing cock C. Because almost all CO_2 in a sample could be eliminated by a soda-lime trap under vacuum condition, no CO_2 peak usually appeared in the chromatogram. The purified N_2O was frozen out

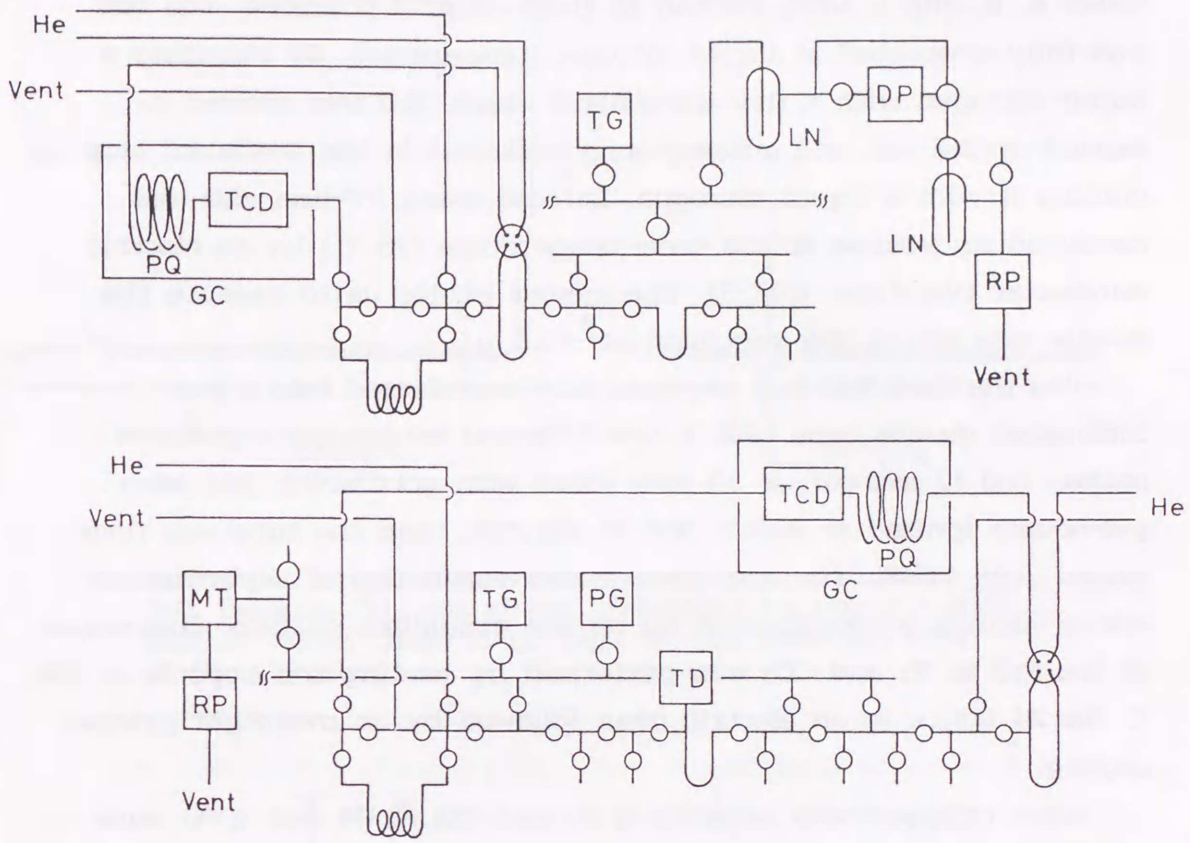

Figure IV-2 Schematic diagram of systems used to purify N_2O by means of gas chromatography. Major differences between the two systems, TY-line (above) and SU-line (below), are (1) a turbo molecular pump is used as a secondary pump in the TY-line, whereas a mercury diffusion pump with a liquid nitrogen cold trap in the SU-line, (2) ability of TY-line to measure μ-liter volume of gas.

Abbreviations: RP-rotary pump; MT-molecular turbo pump; DP-mercury diffusion pump; TG-thermistor gauge; PG-Penig gauge; TD-transducer; GC-gas chromatography; PQ-porapack Q; TCD-thermal conductivity detector; LN-liquid nitrogen.

from helium carrier in the chilled glass coil. After confirmation of full collection of the N_2O fraction by watching chromatogram, the cocks A, B, and C were backed to their original positions. The coil was fully evacuated at liquid nitrogen temperature. By changing a liquid nitrogen with a dry ice-ethanol slush, N_2O was allowed to expand in the coil and subsequently collected in the U-shaped trap by chilling it with a liquid nitrogen. In case using TY-line, N_2O was measured its volume at the room temperature (25 ℃) by an electric manometer (Validyne, CD233). The system enable us to measure the sample size within the precision of ±0.1 μL.

The purified N_2O was cryogenically transferred into a pre-combusted quartz tube (600 ℃ for 5 hours) containing a graphite carbon rod (2 mm o.d. × 13 mm) foiled with gold which had been previously ignited at 800 ℃ for 20 minutes, then the tube was flame sealed (Fig. IV-4). The activation allows elimination of impurities and a vacuum evaporation of Au on the graphite's surface. Conversion of the N_2O to N_2 and CO_2 was performed by heating the ampoule at 450 ℃ for 24 hours in an electric oven followed by an overnight gradual cooling.

After cryogenically separating N_2 and CO_2, $\delta^{15}N$ and $\delta^{18}O$ were determined by mass spectrometry. For US samples, measurements were performed with FINIGAN MAT Delta-S ratio-mass spectrometer equipped with micro cold-fingers at The Ecosystem Center, Marine Biological Laboratory, Woods Hole, Massachusetts. FINIGAN MAT 251 or HITACHI RMU-6R ratio-mass spectrometers, both belong to Mitsubishi Kasei Institute of Life Sciences, were used for measuring Japanese samples.

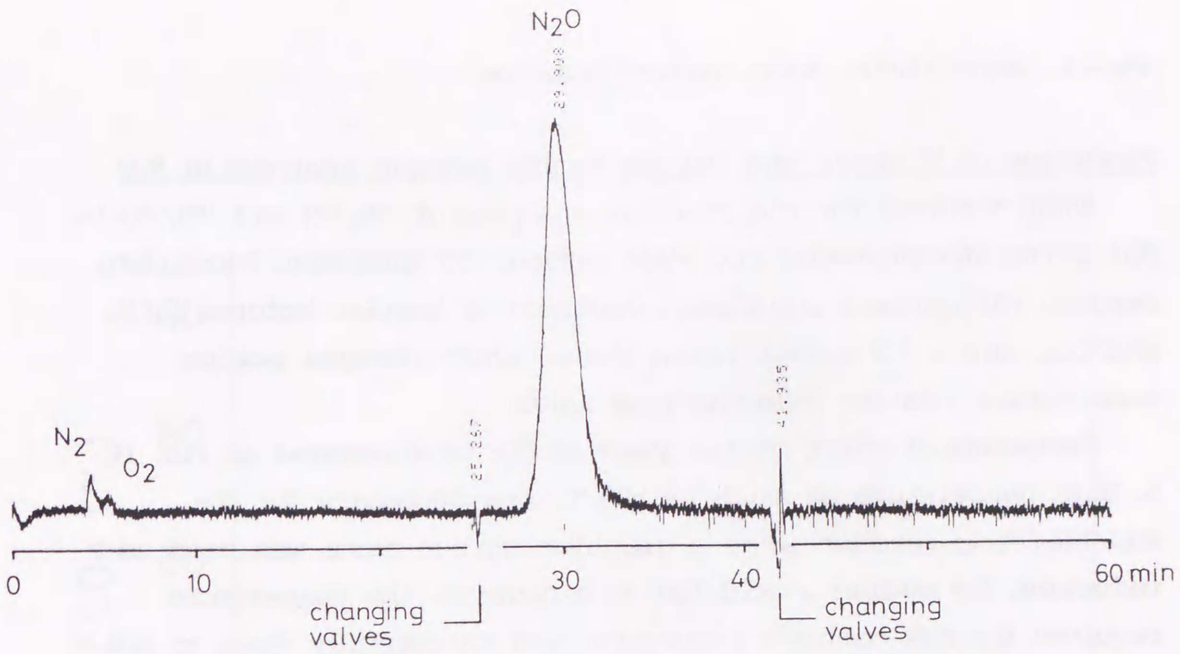

Figure IV-3 TCD-gas chromatograph during purification of N_2O. Because CO_2 was almost eliminated by using a soda-lime *in vacuo*, there is no peak at the retention time of CO_2 (20 min.).

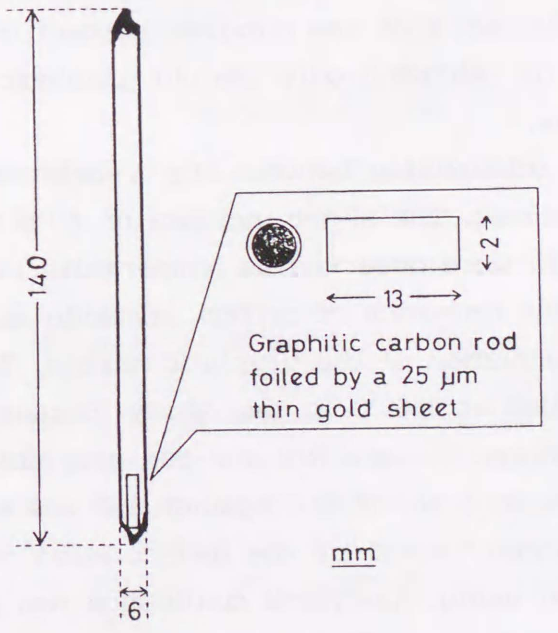

Figure IV-4 Schematic diagram of the sealed quartz ampoule to be combusted for converting N_2O to N_2 and CO_2.

IV-3. RESULTS AND DISCUSSION

Precisions of Nitrogen and Oxygen Stable Isotopic Analyses in N_2O

What required for the precious analyses of $^{15}N/^{14}N$ and $^{18}O/^{16}O$ in N_2O is the stoichiometric CO_2 yield without CO formation. Incomplete reaction will cause a significant depletion of heavier isotopes in N_2 and CO_2, and a CO contamination should shift nitrogen isotopic measurement heavier than the true value.

Temperature effect on the yield of CO_2 is illustrated as Fig. IV-5. High temperature as much as 600 ℃ was necessary for the stoichiometric reaction when a graphitic carbon alone was used as a reductant. By adding a gold foil as a catalyst, the temperature required for the complete conversion was successfully down to 420 ℃ in contrast with 700 ℃ by Kim and Craig [1990]. When samples were treated with a graphite rod foiled by gold above 500 ℃, the one hundred percent yield was always achieved. However, failures of stoichiometric conversion of N_2O were occasionally observed below 500 ℃. Such incompleteness were attributed probably to the surface deterioration of graphitic carbon rod after repeated use. The idea was supported by the fact that one hundred percent conversion could be performed again by replacing only the old graphitic carbon rod with a bland new one.

Fig. IV-6 shows relationship between the δ-determinations and the reaction temperatures. The slight increase of $\delta^{15}N$ and the dramatic drop of $\delta^{18}O$ were observed as temperature increased. This is due probably to the formation of carbon monoxide as a result of high temperature combustion of the graphitic carbon. The reaction temperature was settled at 450 ℃ in this study. Because of fragile situation for the reaction between N_2O and the graphite carbon rod foiled by the gold sheet, yield of CO_2 against N_2O was monitored to avoid incomplete reaction caused by the deterioration of graphitic carbon after repeated using. Analytical confidence was also checked by comparing carbon isotope ratio ($\delta^{13}C$) of CO_2 with that of graphite. If the CO_2 yield and the $\delta^{13}C$ indicated incomplete conversion, such sample was re-analyzed. Reproducibility of the measurements were ± 0.2 ‰ for $\delta^{15}N$ and $\pm$ 0.5 ‰ for $\delta^{18}O$.

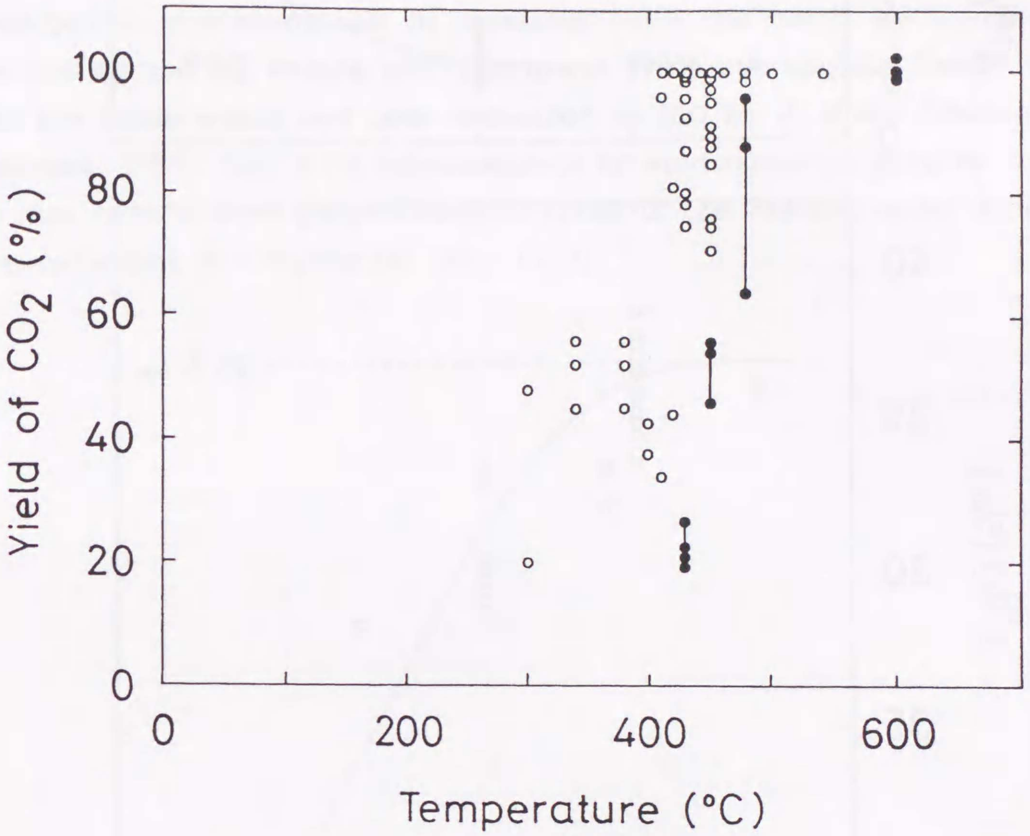

Figure IV-5 Temperature effect on a yield of CO_2 during conversion of N_2O to N_2 and CO_2. Open and closed circles are plots for the reaction of graphite with and without Au-catalyst. By using Au-catalyst, the reaction proceed stoichiometrically at 420 ℃. However, below 500 ℃, the yield sometimes dropped after repeated use of the graphite rod with Au-catalyst, which causes serious error in isotopic ratio measurements.

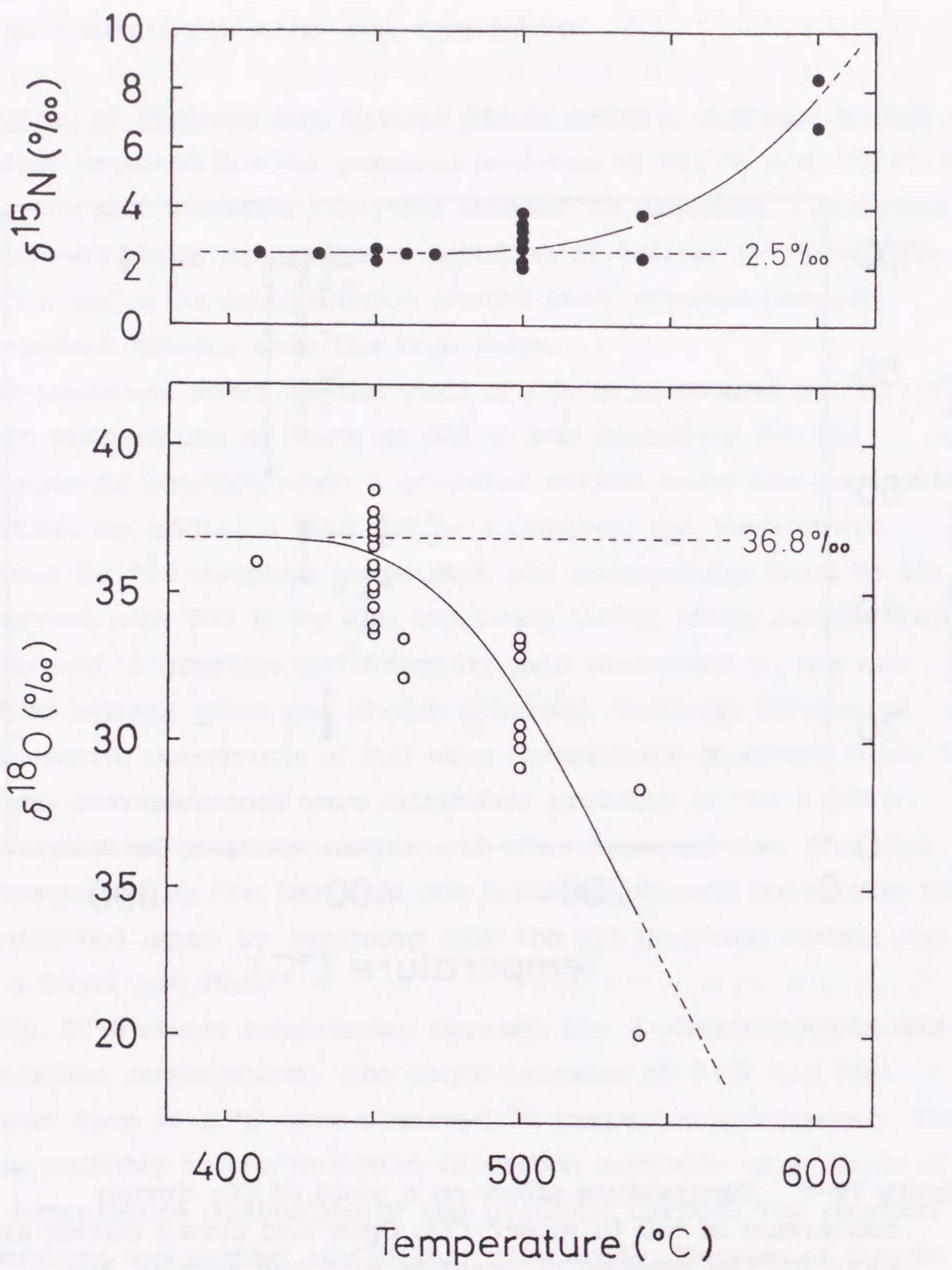

Figure IV-6 Temperature effect on measurements of nitrogen and oxygen isotope ratios in N_2O. Pure tank N_2O with $\delta^{15}N$ of 2.5 ‰ and $\delta^{18}O$ of 36.8 ‰ was used for the determination. At higher temperature than 500 ℃, dramatic drop of $\delta^{18}O$ with slight increase of $\delta^{15}N$ was obtained.

Reliability of the present preparation method was determined by comparing values measured by different preparations (Table IV-1). There is no systematic difference among the measurements by three different preparations. Oxygen isotope ratio measured by the method developed in this study was in agreement with the result by the IR-spectral method by Wahlen and Yoshinari [1985] but slightly lower than the value which had been measured as H_2O by J. White [Wahlen and Yoshinari, 1985]. The $\delta^{15}N$ measurements of environmental samples by the new method were proportional to those of the method using copper as a reductant in Chapter III (Fig. IV-9).

TABLE IV-1 Measurements of Nitrogen and Oxygen Stable Isotopic Compositions in Nitrous Oxide after Different Analytical Procedures

Std[1]	Gas[2]/ Preparation[3]/ Analysis[4]	Measurements[5]		Remark
		$\delta^{15}N$ (‰)	$\delta^{18}O$ (‰)	
#I	N_2, Cu-vacuum line, MS	2.5 ± 0.1	—	a
	N_2, Cu-sealed tube, MS	2.5 ± 0.1	—	b
	N_2 & CO_2, C/Au-sealed tube, MS	2.5 ± 0.1	36.8 ± 1.0	b
	N_2O, no chemical preparation, IR	—	31.0 ± 0.3	c
	H_2O, Cu-H_2, MS (CO_2-equi)	—	34.1 ± 0.2	d
#II	N_2, Cu-sealed tube, MS	1.7 ± 0.2	—	b
	N_2 & CO_2, C/Au-sealed tube, MS	1.8	—	b

[1] Standard — #I is an ultra pure grade tank N_2O (> 99.999 %) produced by MATHESON Co.Ltd., which is owned by Dr. T. Yoshinari. #II is an anesthetic grade gas provided from OSAKA SEITETU KOGYO Co.Ltd., which belongs to the author.

[2] Gas — $\delta^{15}N$ and $\delta^{18}O$ of N_2O were measured after converting it to the listed gas.

[3] Preparation — "Cu-vacuum line": N_2O was reduced to N_2 by circulating the gas in a vacuum system through furnaces of Cu (400 ℃) and CuO (700 ℃), which was developed by *Yoshida* [1984]. "Cu-sealed tube": N_2O was reduced to N_2 by reacting the gas with Cu in a sealed Pyrex tube at 400 ℃, for details see Chapter III. "C/Au-sealed tube": N_2O was converted to N_2 and CO_2 by reacting the gas with graphitic carbon under Au-catalysis condition in a sealed quartz tube at 450 ℃, which was developed in this chapter. "Cu/H_2: N_2O was first reacted with Cu to produce oxidized coppers, then the copper .lh12
oxides were reduced to H_2O by H_2.

[3] Analysis — MS-mass spectrometry. MS (CO_2-equi)-mass spectrometry tagged with CO_2 equilibrium method. IR-infrared spectral method.

[4] Remark — a. Measured by Dr. Minagawa, Mitsubishi Kasei Institute of Life Sciences; b. This study; c. *Wahlen and Yoshinari* [1985]; d. Measured by Dr. J. White [*Wahlen and Yoshinari*, 1985].

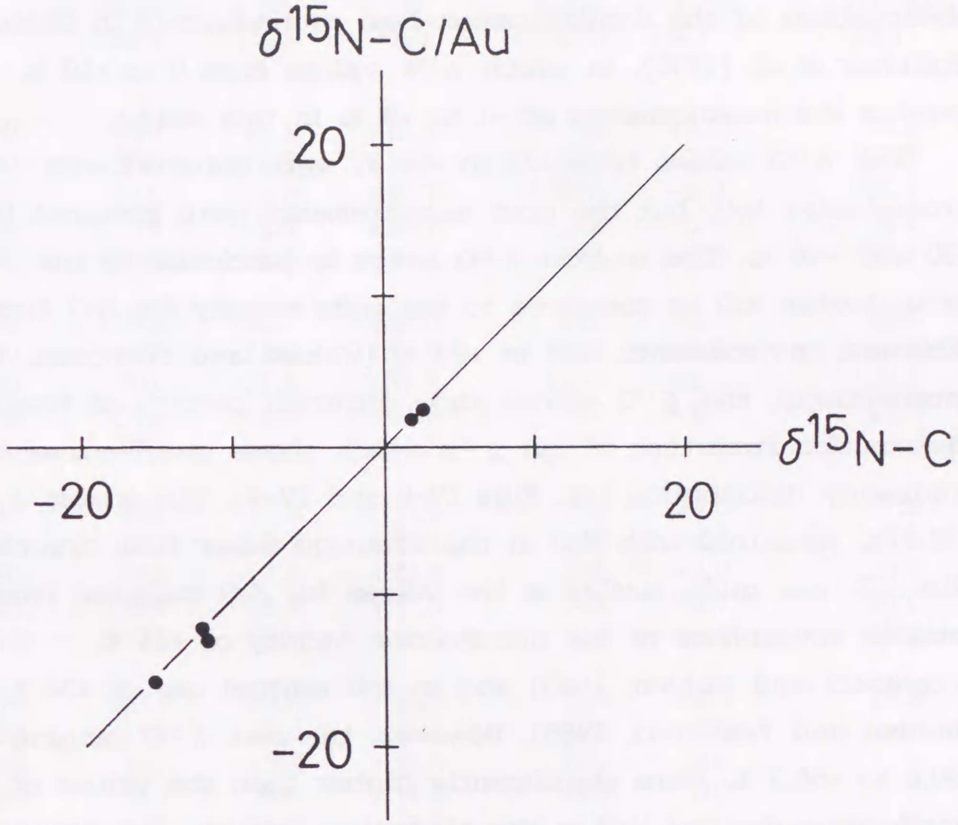

Figure IV-7 $\delta^{15}N$ measured by C/Au-sealed tube method *vs.* $\delta^{15}N$ by Cu-sealed tube method. Slope of the regression line is 1.006 and coefficient of correlation is 0.9999.

Stable Isotopic Compositions of N_2O and NO_3^- in Groundwaters

All results are listed in Tables IV-2 and IV-3, for the US and Japanese samples, respectively. Relative frequency distributions of $\delta^{15}N$ and $\delta^{18}O$ in the groundwater N_2O and NO_3^- are illustrated as Figs IV-8 and IV-9. $\delta^{18}O$ values of NO_3^- are cited from the observations of the denitrification-free groundwaters in Germany by *Bottcher et al.* [1990], in which $\delta^{15}N$ values from 0 to +10 ‰ overlap the measurements of -1 to +8 ‰ in this study.

The $\delta^{18}O$ values from +23 to +50 ‰ were obtained with groundwater N_2O, but the most measurements were centered between +30 and +40 ‰. The narrow $\delta^{18}O$ range is particular to the groundwater N_2O as compared to the wide variety for N_2O from different environments, +23 to +93 ‰ [Wahlen and Yoshinari, 1985]. Interestingly, the $\delta^{18}O$ values show different pattern of frequency distribution from that of the $\delta^{15}N$ which shows quasi-normal frequency distribution (cf. Figs IV-8 and IV-9). The lowest $\delta^{18}O$ of +22.0 ‰ measured with N_2O in the drainage water from cropping field, site J29, was quite similar to the values for N_2O collected from an interior atmosphere of the nitrification facility of +24 ‰ [Yoshinari and Wahlen, 1985] and in soil emitted gas of +24 ‰ [Wahlen and Yoshinari, 1985]. However, the rest $\delta^{18}O$ ranging from +30.8 to +50.3 ‰ were significantly higher than the values of the nitrification-derived N_2O in the above two studies. The differences seem go against the previous view that a large portion of the groundwater N_2O is produced by nitrification, which is based on the $\delta^{15}N$ determinations in N_2O and NO_3^-, the $\Delta N_2O/NO_3^-$ molar ratios, and the presence of dissolved oxygen without organic carbon in the groundwaters (see Chapters II and III). The highest $\delta^{18}O$ value (J11) was obtained with the brown-colored groundwater due to humic materials with the lowest dissolved O_2 concentration in the present study.

TABLE IV-2 Stable Isotopic Compositions of N_2O and NO_3^- in New York State Groundwaters

Code	Date	N_2O			NO_3^-		Discrimination
		conc (nM)	$\delta^{15}N$ (‰)	$\delta^{18}O$ (‰)	conc (μM)	$\delta^{15}N$ (‰)	$\Delta\delta^{15}N$ (‰)
U01	3/ 3/90	160	-15.1	+32.5	ND	+3.0	18.1
U02	5/ 2/90	572	-11.5	+34.5	227	+2.2	13.5
U03	5/ 2/90	115	-13.0	+32.7	886	-0.7	12.7
U04	5/ 2/90	78	-9.6	+36.1	222	-0.2	9.4
U05	5/ 2/90	4318	-20.6	+35.1	474	+7.5	28.1
U06	5/ 2/90	1661	-30.7	+32.0	412	+2.1	32.8
U07	5/ 2/90	182	-12.3	+32.4	1643	+1.2	13.5
U08	5/ 2/90	701	-27.8	+31.3	1229	+1.5	29.3
U09	3/14/90	570	-7.8	+41.6	542	+4.5	12.3
U10	3/14/90	908	-17.8	+31.1	907	ND	—
U11	5/10/90	481	-13.5	+35.0	292	+4.0	17.5
U12	5/10/90	385	-13.7	+34.1	366	+2.9	16.6
U13	5/10/90	243	-14.8	+32.8	186	+2.0	16.8
U14	5/10/90	1294	-13.9	+37.9	561	+6.7	20.6
U15	5/10/90	1194	-12.4	+41.6	519	+6.4	18.8
U16	5/10/90	1013	-10.6	+38.8	299	+4.0	14.6
U17	5/23/90	155	-2.4	+32.1	121	ND	—
U18	5/23/90	125	-15.3	+35.5	621	ND	—
U19	5/23/90	315	ND	+34.8	165	ND	—
U20	5/23/90	149	-16.8	+33.8	96	ND	—
U21	3/29/90	337	-14.3	+39.2	397	+4.4	18.7
U22	3/29/90	254	-12.6	+32.6	217	+3.8	16.4
U23	3/29/90	145	-11.8	+37.1	91	+3.2	15.0
U24	3/29/90	16	ND	ND	4	ND	—
U25	3/29/90	265	-11.0	+31.4	258	+2.1	13.1
U26	3/29/90	450	-11.2	+30.8	392	+5.1	16.3
U27	3/29/90	137	-5.7	+35.0	152	+2.9	8.6
U28	3/29/90	81	-7.8	+33.2	60	+3.7	11.5
U29	3/29/90	308	-25.2	+32.1	294	+4.5	29.7
U30	3/29/90	55	-32.0	+35.5	n.d.	ND	—

ND: not determined
n.d.: not detected

TABLE IV-3 Stable Isotope Compositions of N_2O and NO_3^- in Japanese Groundwaters

Code	Date	N_2O			NO_3^-		Discrimination
		conc (nM)	$\delta^{15}N$ (‰)	$\delta^{18}O$ (‰)	conc (μM)	$\delta^{15}N$ (‰)	$\Delta\delta^{15}N$ (‰)
J01	10/23/89	749	−12.9	+35.1	ND	+6.3	19.2
J02	10/23/89	1430	−12.2	+32.1	ND	+4.1	16.3
J09	9/16/89	69	−6.9	+38.2	ND	ND	—
J10	10/10/89	119	−11.1	+31.6	ND	ND	—
J11	10/ 8/89	81	−2.3	+50.4	ND	ND	—
J12	10/20/89	690	−3.2	+37.4	ND	ND	—
J13	10/20/89	1032	+1.0	+39.6	ND	ND	—
J14	10/20/89	5280	+2.3	+38.1	ND	ND	—
J19	9/21/89	397	−12.8	+31.7	ND	+5.6	18.4
J19	10/17/89	492	−12.6	+34.3	ND	+5.6	18.2
J26	10/ 8/89	108	+0.5	+34.9	ND	ND	—
J27	10/10/89	32	+2.7	+31.7	ND	ND	—
J29	10/20/89	490	−22.9	+36.8	ND	ND	—
J30	10/20/89	7060	−28.4	+22.0	ND	ND	—

ND: not determined

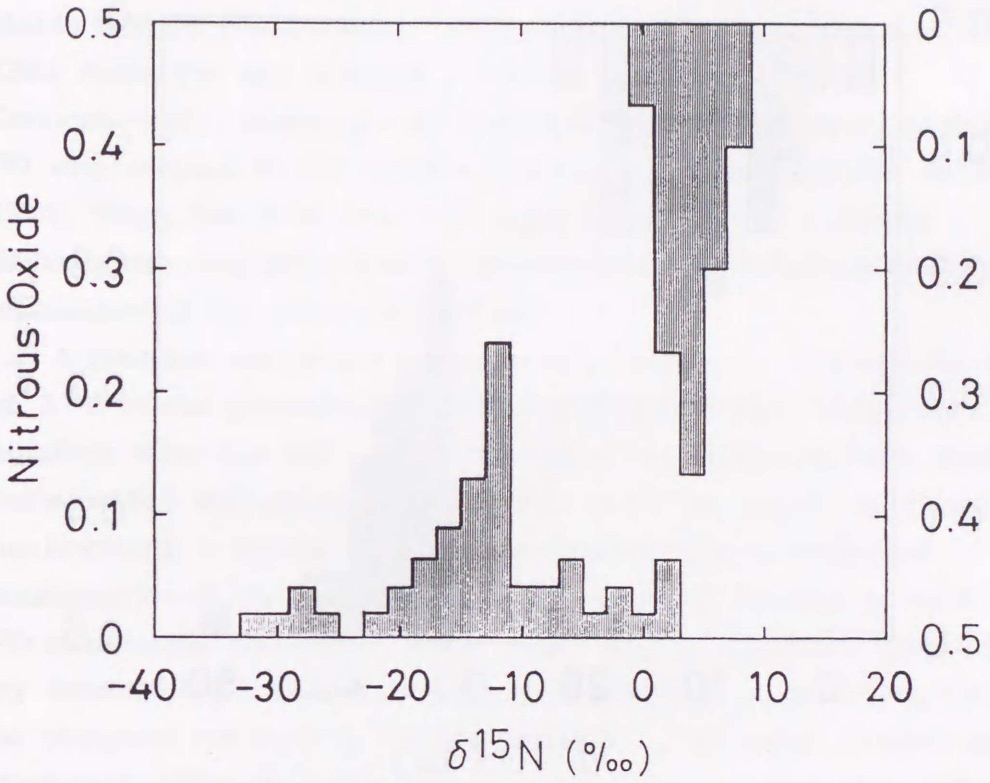

Figure IV-8 Relative frequency distribution of $\delta^{15}N$ in groundwater N_2O (n=46) and NO_3^- (n=30). Figures in the parentheses show numbers of the observation sites.

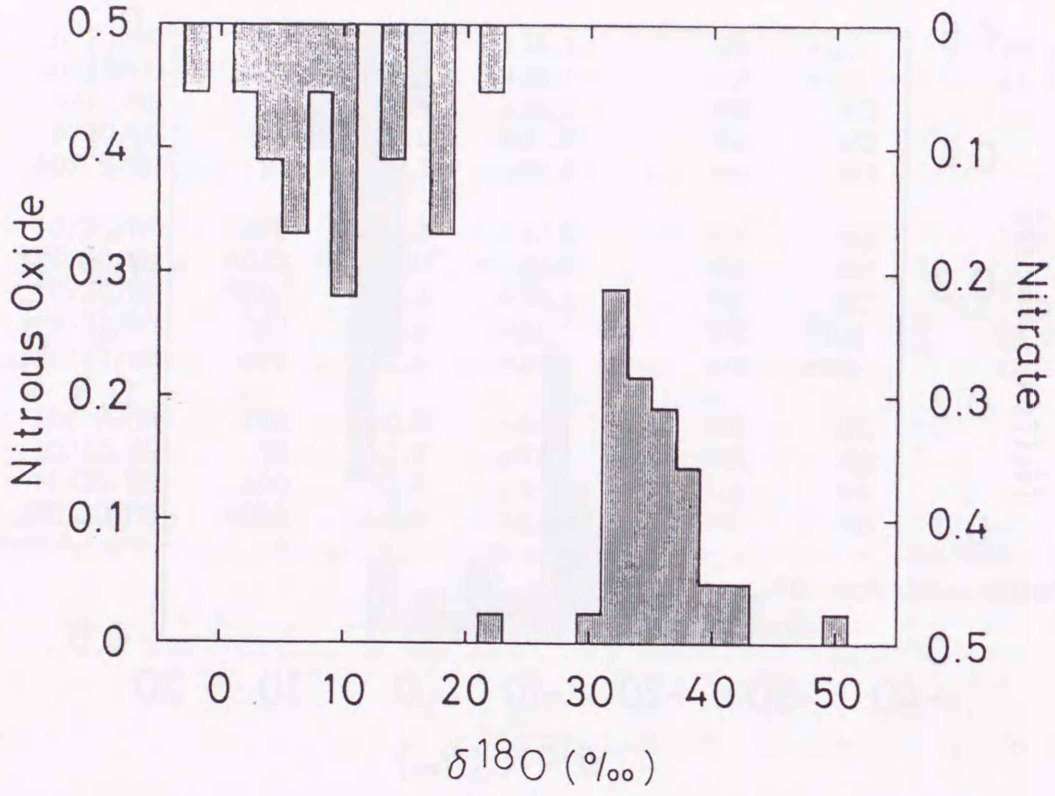

Figure IV-9 Relative frequency distribution of $\delta^{18}O$ in groundwater N_2O (n=42) and NO_3^- (n=18). Figures in the parentheses show numbers of the observation sites. Data for NO_3^- are cited from *Bottcher et al.* [1990]

Producing Mechanism of Groundwater N_2O

A. Denitrification

Dissolved N_2O in the studied groundwaters were depleted in ^{15}N and enriched in ^{18}O as compared to the NO_3^- produced by nitrification (Figs IV-8 and IV-9). If the groundwater N_2O with low $\delta^{15}N$ values were produced from the early stage of denitrification (see Chapter III), the $\delta^{18}O$ should be lower than that of NO_3^- due to an oxygen stable isotopic fractionation in the nitrate reduction [Olleros, 1983; Amberger and Schmidt, 1987; Bottcher *et al.,* 1990]. Contradictorily, these groundwater N_2O were significantly enriched in ^{18}O with respect to the nitrification-derived NO_3^- [Bottcher *et al.,* 1990]. Thus, the $\delta^{15}N$ and $\delta^{18}O$ measurements of N_2O clearly demonstrate negligible source contribution of denitrification as a mechanism of the groundwater N_2O.

A possible mechanism which is responsible for the heavier shift of $\delta^{18}O$ in the groundwater N_2O is some enrichment within the aquifers after the N_2O production. Since no oxygen isotopic exchange between N_2O and oxygenic compounds has been known in natural environment, a kinetic isotope fractionation due to biological consumption of the N_2O pool, probably a denitrification is such an ^{18}O-enrichment mechanism. When a N_2O pool is consumed significantly by denitrifying bacteria, isotopic enrichment in a remaining N_2O must be observed not only in oxygen but also in nitrogen [Wahlen and Yoshinari, 1985]. However, there was no linear relationship between $\delta^{15}N$ and $\delta^{18}O$ in the groundwaters (Fig. IV-10). Also the $\delta^{15}N$ values of neither N_2O nor NO_3^- depend on the degree of percent saturation of dissolved oxygen (Fig. IV-11). Therefore, the oxygen isotopic modification in the groundwater N_2O by denitrification is unexpected.

According to the above results, it can be concluded that denitrification is not an important mechanism controlling either size of N_2O pools and their stable isotopic compositions in the studied groundwaters.

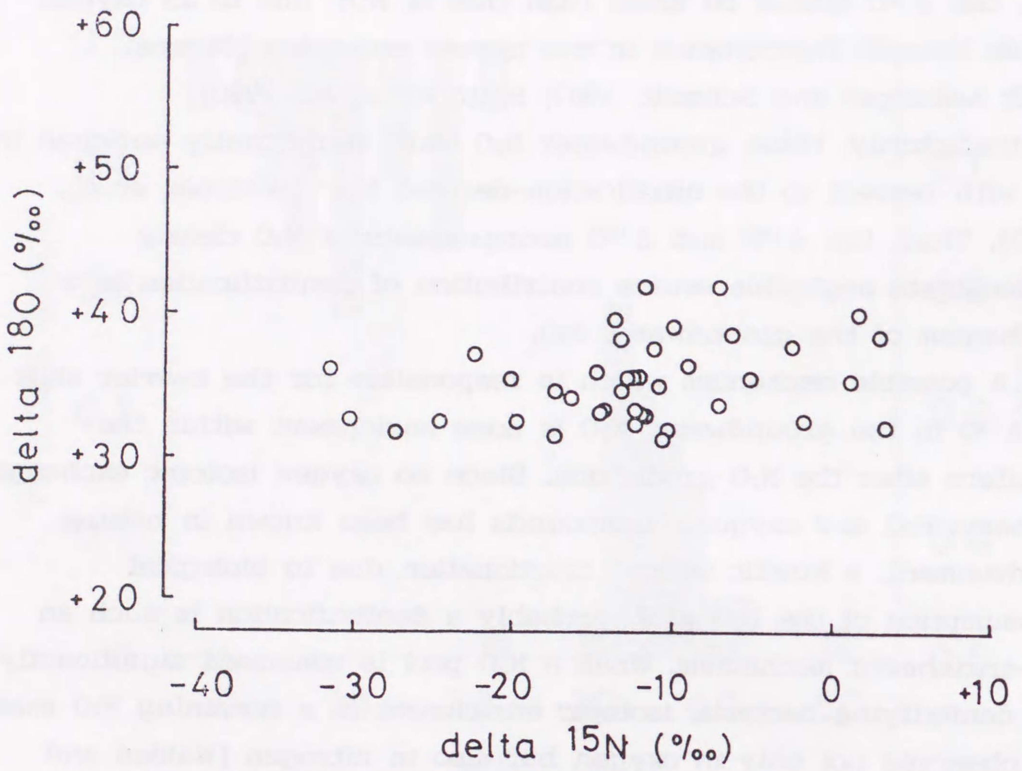

Figure IV-10 $\delta^{15}N$- $\delta^{18}O$ relationship in groundwater N_2O

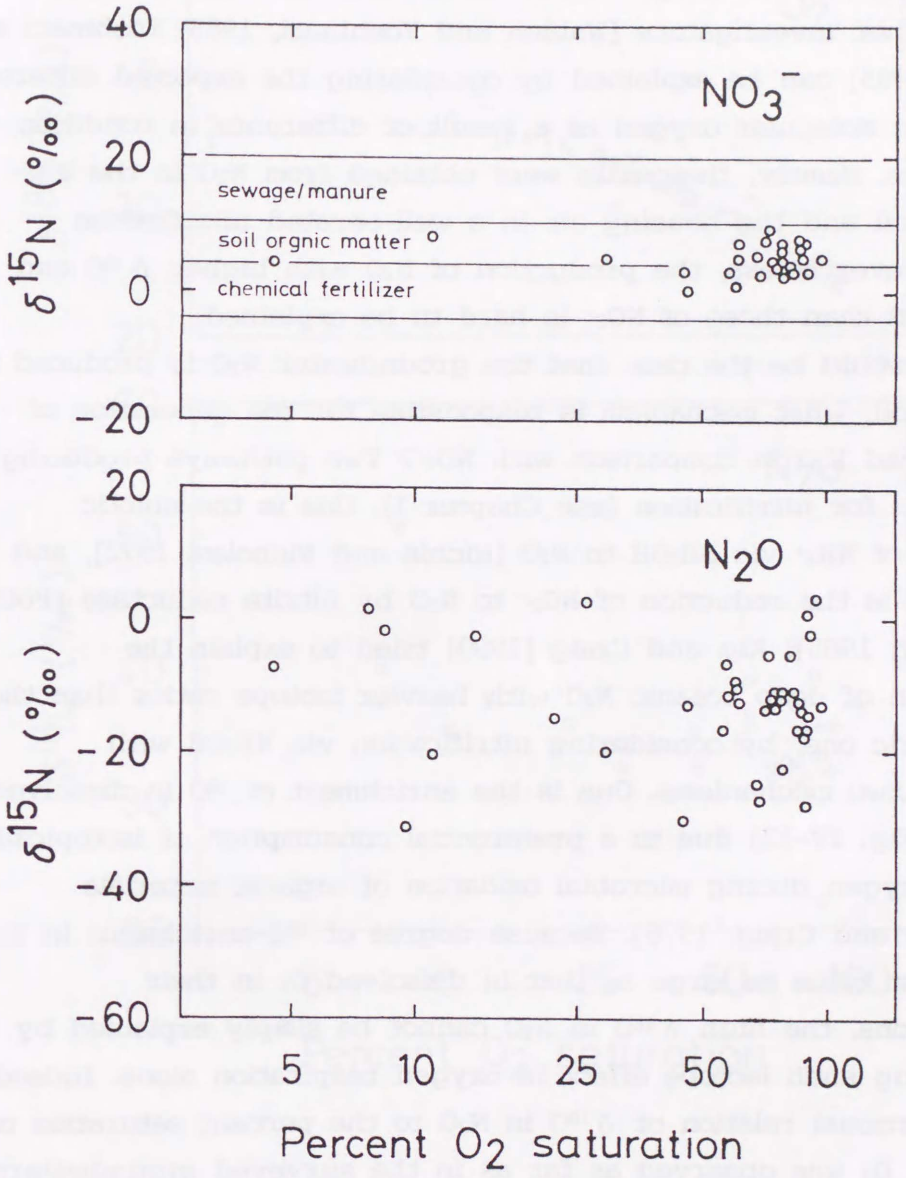

Figure IV-11 Dependence of the $\delta^{15}N$ values in NO_3^- and N_2O on the percent O_2 saturation in groundwaters.

B. Nitrification

Because of less governing the stable isotopic composition of the groundwater N_2O by denitrification, nitrification is suggested alternatively as a predominant source mechanism by the present study. The higher $\delta^{18}O$ of the groundwater N_2O than the nitrification-origin N_2O by other investigators [Wahlen and Yoshinari, 1985; Yoshinari and Wahlen, 1985] can be explained by considering the expected difference in $\delta^{18}O$ in molecular oxygen as a result of difference in condition of aeration. Namely, their data were obtained from N_2O in the top-surface soil and the housing air in a well-aerated nitrification facility. Nevertheless, the production of N_2O with higher $\delta^{18}O$ and lower $\delta^{15}N$ than those of NO_3^- is hard to be explained.

If it would be the case that the groundwater N_2O is produced from nitrification, what mechanism is responsible for the generation of ^{18}O-enriched N_2O in comparison with NO_3^-? Two pathways producing N_2O are known for nitrification (see Chapter I). One is the abiotic oxidation of NH_4^+ via NH_2OH to N_2O [Richie and Nicholas, 1972], and the other is the reduction of NO_2^- to N_2O by nitrite reductase [Poth and Focht, 1985]. Kim and Craig [1990] tried to explain the production of deep oceanic N_2O with heavier isotope ratios than the atmospheric one, by considering nitrification via NH_2OH with following two mechanisms. One is the enrichment of ^{18}O in dissolved oxygen (Fig. IV-12) due to a preferential consumption of isotopically lighter oxygen during microbial oxidation of organic materials [Kroopnik and Craig, 1976]. Because degree of ^{18}O-enrichment in N_2O was almost twice as large as that in dissolved O_2 in their observations, the high $\delta^{18}O$ in N_2O cannot be simply explained by considering such isotope effect in oxygen respiration alone. Indeed, no proportional relation of $\delta^{18}O$ in N_2O to the percent saturation of dissolved O_2 was observed as far as in the surveyed groundwaters (Fig. IV-12). The other mechanism is a predominant oxidation of NH_2OH to NO_2^- in nitrification, which will result in a pool of heavy-isotope-enriched NH_2OH to be oxidized to N_2O. In case of the groundwater N_2O, however, the enrichment of heavier isotopes occurred only in oxygen atom. The findings may suggest some other mechanisms to produce ^{18}O-enriched but ^{15}N-depleted N_2O in nitrification.

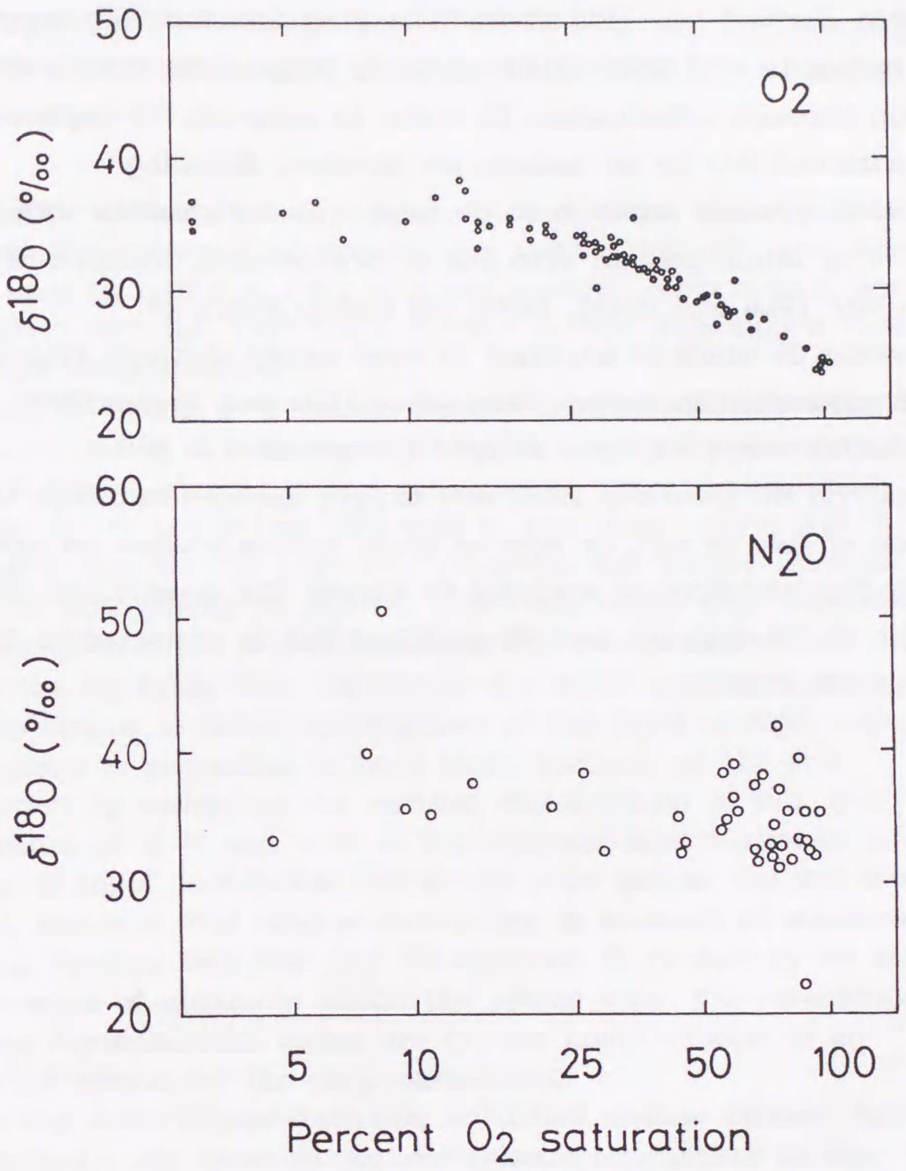

Figure IV-12 Dependence of the $\delta^{18}O$ values in O_2 and N_2O on the percent oxygen saturation. The upper illustration was made from data for dissolved oxygen in deep ocean by *Kroopnik & Craig* [1976].

Because of the less variety of $\delta^{18}O$ in the groundwater N_2O (Fig. IV-9) as compared with that of $\delta^{15}N$ (Fig. IV-8), and because there is no measurement of oxygen isotopically lighter N_2O than the nitrification-derived NO_3^- (Fig IV-9), it may be assumed that oxygen isotopic exchange with some other oxygenic compounds occur within N_2O production through nitrification. In order to generate ^{15}N-depleted and ^{18}O-enriched N_2O by an aerobic nitrification, following consequential process seems most probable: (1) Enrichments with both ^{15}N and ^{18}O in NH_2OH pool *in vivo* due to predominant oxidation of NH_2OH to NO_2^- [Kim and Craig, 1990], (2) incorporation of environmental O_2 which is enriched to some extent through kinetic isotope fractionation in oxygen respiration [Kim and Craig, 1990], and (3) double stage nitrogen isotopic fractionation in N_2O production via NO [Yoshida, 1988] and oxygen isotopic exchange within production of HNO or N_2O, or both of them. Future studies on isotope effects during nitrification are vital to answer the question on the production of ^{15}N-depleted and ^{18}O-enriched N_2O as compared to the NO_3^- by nitrification.

CHAPTER V

TWO-ISOTOPE MAP OF NITROUS OXIDE FROM DIFFERENT ENVIRONMENTS

ABSTRACT — Nitrogen and oxygen stable isotopic characterization of N_2O from the surface tropospheric air, combustion gas, and ocean water were carried out. Only nitrogen isotopic compositions were determined with N_2O from fresh surface waters including a river, lakes and sewage effluents.

Mean $\delta^{15}N$ and $\delta^{18}O$ values for the tropospheric N_2O were 6.5 ± 0.8 ‰ and 36.3 ± 2.7 ‰, respectively. It was firstly demonstrated that N_2O from a stack gas and car exhaust gases significantly depleted in ^{15}N and ^{18}O as compared to the tropospheric N_2O. A regression line for the plots of combustion N_2O passed through the values of atmospheric N_2 and O_2, which may imply high temperature combination of these two molecules as the source mechanism.

At the Monterey Bay, California, the depth profile of dissolved N_2O was similar to other observations in the North Pacific Ocean. Nitrification is suggested to be a major pathway of the N_2O production by examining the vertical distributions of N_2O, NO_3^- and O_2. Profiles of $\delta^{15}N$ and $\delta^{18}O$ in N_2O demonstrated enrichments of heavier isotopes just bellow the photic zone and in the N_2O maximum with O_2 minimum. The shallow enrichment is a result of nitrification utilizing ^{15}N-enriched NH_4^+ and ^{18}O-enriched O_2 caused by an active regeneration of nutrients within the photic zone. N_2O production via nitrifier denitrification under low oxygen concentration is an additional reason for the deep enrichment.

In the River Minami-Asakawa, an urban shallow stream, N_2O concentration was close to the atmospheric equilibrium in the headwaters, and the $\delta^{15}N$ (+10.7 ‰) was almost comparable to that is expected under the equilibrium with the atmosphere. On the other hand, significant increase of N_2O concentration with a dramatic change in $\delta^{15}N$ from -10.6 ‰ to -39.8 ‰ was observed in the lower reaches of the stream, where the mass balance study indicated an active oxidation of an externally loaded NH_4^+ to NO_3^-.

Sequential depletion of ^{15}N content from -3.1 ‰ to -5.6 ‰ with increase of N_2O concentration was also observed in a surface oxic layer of the Fayetteville Green Lake, a mesotrophic deep glacial

lake, in which almost all of the nitrogen ions presented as NO_3^-. In the Lake Teganuma, a hypertrophic shallow lake, $\delta^{15}N$ of -0.9 ‰ in dissolved N_2O was observed in the upper basin where increments of N_2O and NO_3^- with decrease of a riverine NH_4^+ were observed. The highest $\delta^{15}N$ of N_2O in study, +14.7 ‰, was obtained in the down basin where the NO_3^- concentration decreased by active denitrification in sediments.

Nitrogen isotopic findings in aquatic systems consist with the view that nitrification-derived N_2O is depleted in ^{15}N and denitrification-derived N_2O is more enriched with ^{15}N than that produced from nitrification. ΔN_2O-$\delta^{15}N$ diagram of the aquatic data gave a general trend of the lower ΔN_2O the higher $\delta^{15}N$. It was shown that one can elucidate major mechanisms controlling a size of N_2O pool in a given water system by tracing the plot in the diagram.

$\delta^{15}N$-$\delta^{18}O$ map for N_2O was compiled from the data obtained in this study and reported by other investigators. N_2O collected from different environments seemed to be classified roughly in three groups according to the $\delta^{15}N$-$\delta^{18}O$ comparison with those of the tropospheric N_2O; (1) N_2O with lower $\delta^{15}N$ and similar $\delta^{18}O$ in a groundwaters, (2) N_2O with similar $\delta^{15}N$ and higher $\delta^{18}O$ in an ocean, and (3) N_2O with lower $\delta^{15}N$ and $\delta^{18}O$ values in combustion gas. Future development of such two-isotope diagram for N_2O as shown in this study is believed to help in both discussing its origin and assessing the global budget.

V-1. INTRODUCTION

Complete understanding of the biogeochemical cycle of N_2O is vital to the awaited management of the tropospheric concentration. As discussed in Chapter I, because of the minor contribution of artificial processes on the total emission of N_2O, the imbalance between source and sink in the available global N_2O budget is owing mainly to the unreliable estimates of the natural source strengths. That is why most of the natural source strengths previously reported were estimated by simply extrapolating local emissions to the global scale, while representative emissions are hard to be obtained because large portion of the total emission is due to hot spots in the biosphere. Lack of the method to elucidate producing mechanisms of N_2O between two major processes, nitrification and denitrification, make it difficult to evaluate each source strength accurately under consideration of circumstantial factors affecting the production individually.

It has been revealed through the previous discussion on the groundwater N_2O that the stable isotope study is an excellent mean to discuss origins and behaviors of biogenic N_2O. The isotopic study will also assist the re-evaluation of the biogeochemical N_2O cycle. What urgently required for developing the strategy is to gather information on isotope signatures of N_2O in major sources and reservoirs.

In this chapter, isotopic characterizations of N_2O in different environments are performed. N_2O were collected from ambient air, combustion gas, sea, lake and river waters, and sewage effluent. Among these, studies on an atmospheric and combustion N_2O, and also a part of lake N_2O are due to the cooperative work with Dr. Yoshinari, Wadsworth Center for Laboratories and Research (WCL&R), New York Department of Health and State University of New York at Albany. Results for a sea water N_2O are the fruits of research with Drs. T. Yoshinari and L.A. Codispoti, Monterey Bay Aquarium Research Institute (MBARI).

V-2. ATMOSPHERE

Sampling and Analysis

A. Ambient air

Albany, NY: Atmospheric N_2O was collected near WCL&R using Molecular Sieve 5A pellets packed in a stainless steel tube which had originally been devised by Yoshida [1984]. The MS5A had been purified prior to use by heating the tube at 380 ℃ *in vacuo* until no desorbed gas presented. The tube was filled with pure helium above the ambient pressure, then kept until use. An ambient air was introduced to the tube using diaphragm pump after removals of water vapor and CO_2 by Aquasorb (P_2O_5, MALLINCKRODT) and Ascarite, respectively. The collected fraction was desorbed from the MS5A by heating the tube at 200 ℃ in helium gas flow (20 mL · min^{-1}), then re-collected in the glass coil chilled with liquid nitrogen. The gas was cryogenically purified between liquid nitrogen and dry ice-ethanol temperature, then sealed in a pre-ignited Pyrex glass ampoule after further eliminating CO_2 by a soda-lime trap *in vacuo*. The N_2O was converted to N_2 and CO_2 after the C/Au-reduction method (see Chapter IV), then $\delta^{15}N$ of N_2 and $\delta^{18}O$ of O_2 were determined with FINIGAN MAT delta-S ratio-mass spectrometer at The Ecosystem Center, Marine Biological Laboratory (MBL), Woods Hole.

Fuchu, Tokyo: Air samples were collected at the balcony (5 m above ground surface) of the building of Department of Environmental Science and Conservation, Faculty of Agriculture, Tokyo University of Agriculture and Technology. N_2O was collected by the following cryogenic mean. Air was sucked by a vacuum pump allowing gas flow rate and pressure in the glass coils at 2 L · min^{-1} and 100 mm Hg, respectively. Water vapor and carbon dioxide in the air were removed by Drierite-Ascarite-$Mg(ClO_4)_2$ column. Remaining water vapor was further eliminated in a dry ice-ethanol cold trap, then N_2O was frozen in two glass coils placed in liquid nitrogen baths. Oxygen and argon do not accumulate at the liquid nitrogen temperature (-196 ℃) under these operating conditions. Hereafter, treatments of the collected gas were as same as those for the Albany air samples.

B. Combustion gas

N_2O in car exhaust gases (n=2) and in a combustion gas of waste incinerator (n=1) were analyzed. These combustion gases were once collected in a Tedra bag using diaphragm pump in the field, then N_2O was separated by a cryogenic manner in the laboratory, which is almost same as that for the ambient air collected at Fuchu.

Recently, serious artifact was reported for measuring N_2O concentration in combustion gases [Muzio and Kramlich, 1988; Muzio *et al.*, 1989; Lyon *et al,*, 1989; Linak *et al.*, 1990] as discussed in Chapter I. Briefly, if water, nitrogen oxides and sulfur dioxide were present in a sample container simultaneously, huge amount of N_2O with respect to the original N_2O is produced during storage of the samples. In this study, acidic compounds including NO_x and SO_x in the combustion samples were removed by washing the gases with concentrated NaOH solution and also by Ascarite column. Water vapor was removed by Drierite and Aquasorb, respectively. A preliminary experiment revealed no N_2O production during a few days storage of combustion gas samples after these treatments. Isotopic compositions were measured after C/Au-reduction method with FINIGAN MAT delta-S at MBL.

Results and Discussion

A. Ambient air

Analytical results for the tropospheric N_2O are presented in Table V-1. The mean $\delta^{15}N$ in the surface continental air in this study of 6.5 ± 0.8 ‰ is higher than 5.2 ± 2.0 ‰ reported by Moore [1974] and lower than 8.1 ± 1.0 ‰ by Yoshida and Matsuo [1983], and comparable to 7.0 ± 0.1 ‰ reported by Kim and Craig [1990]. The average $\delta^{18}O$ values in the present study 36.3 ± 2.9 ‰ are lower than those obtained by Wahlen and Yoshinari [1985], 45.4 ± 2.1 ‰, and by Kim and Craig [1990], 44.7 ± 0.7 ‰. The average $\delta^{15}N$ and $\delta^{18}O$ for all the available data weighed by number of observations are +6.5 ‰ (n=98) and +40.9 ‰ (n=21), respectively.

Yoshida and Matsuo [1983] reported the general trend that N_2O in a maritime air mass was characterized by the lower $\delta^{15}N$ and higher concentration whereas N_2O in a continental air mass by the higher

$\delta^{15}N$ and the lower concentration. However, in this study, the $\delta^{15}N$ of land atmospheric N_2O are slightly lower than those reported for N_2O in the oceanic air. The results may reflect difference in the site of sampling and possibly in analytical procedure.

Fig. V-1 presents $\delta^{15}N-\delta^{18}O$ relationship in the atmospheric N_2O. $\delta^{18}O$ is more variable than $\delta^{15}N$, which agrees with the observation of surface atmosphere at Toyama Prefecture, Japan [Yoshida, personal communication]. These observations suggest advantage of $\delta^{18}O$ values to be used in kinetic implication of mechanisms determining tropospheric N_2O concentration.

B. Combustion gas

Measurements of combustion origin N_2O are shown in Table V-2. N_2O from combustion systems were characterized by the significant depletion in ^{15}N and ^{18}O with respect to the averaged values for the tropospheric N_2O. Interestingly, these plots of combustion-origin N_2O scattered around the line that brackets the values of tropospheric N_2 (0 ‰) and O_2 (23.5 ‰, Kroopnik, [1971]). Although further study is required, the findings may be not so unreasonable as considering the fact that a large portion of the combustion N_2O is produced from the chemical combination of N_2 and O_2 under high temperature condition.

It should be noted that the $\delta^{18}O$ values of these combustion N_2O were significantly lower than those previously reported by Wahlen and Yoshinari [1985]. Since they did not eliminate SO_2 and NO_x at the sample collection, their observation of high $\delta^{18}O$ values was probably due to N_2O production during the samples storage. In fact, N_2O concentrations in this study, 0.48-5.91 ppmv, were much lower than those measured by Wahlen and Yoshinari [1985], 10.1-50.0 ppmv. Accordingly, the δ-values in this study are probably correct.

Meanwhile, when the author tried to re-examine car exhaust N_2O in Tokyo, measurable N_2O was observed only for an hours from the start of the engine, then the concentration became lower than the atmospheric one and was finally not detected. Although combustion N_2O could be distinguished from the tropospheric N_2O by their stable isotopic compositions in this study, its representativeness is questionable.

TABLE V-1 Isotopic Compositions of Surface Tropospheric N_2O

		$\delta^{15}N$ (‰)	$\delta^{18}O$ (‰)	Remark
Albany	7/21/88	6.0	34.2	
	7/24/88	6.3	36.6	
	4/ 7/89	6.7	38.4	
	4/11/89	5.7	—	lost
	4/12/89	5.5	32.3	
	4/15/89	7.7	37.9	
	4/17/89	6.7	37.9	
	4/17/89	6.4	37.2	
	4/27/89	6.3	41.2	
	4/27/89	7.7	—	lost
	3/90	7.0	ND	
	3/90	5.9	ND	
	3/90	7.7	ND	
	3/90	7.2	ND	
	3/90	6.8	ND	
Fuchu	9/ 7/89	5.5	31.6	
	9/ 8/89	5.6	35.2	

ND: not determined

TABLE V-2 Concentations and Isotope Compositions of N_2O in Combustion gases

	conc (ppmv)	$\delta^{15}N$ (‰)	$\delta^{18}O$ (‰)
Stackgas from waste incineration plant*, Albany, NY	0.48	1.6	26.9
Car exhaust gas**			
SUBARU Sedan (2L)	1.85	-11.6	19.4
BUICK Sky Hawk (> 2L)	5.91	-22.6	15.2
OLDSMOBILE Catlas Supreme (>3L)	4.91	LOST	LOST

* Paper products are the major waste combusted in this plant.

** All cars are equipped with catalytic converters and consume gasoline. Numbers in parentheses represent approximate volumes of exhaust in liter.

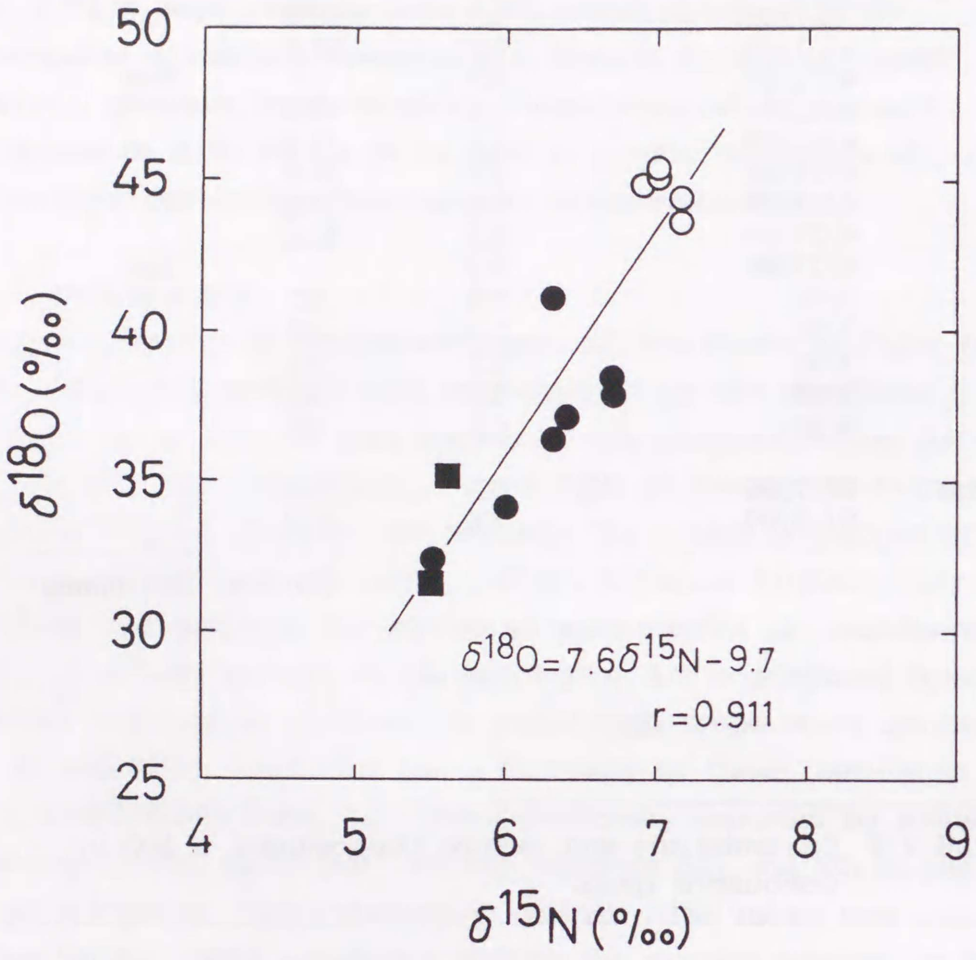

Figure V-1 $\delta^{15}N$ and $\delta^{18}O$ diagram of N_2O in ambient air. Close circle: Albany air; Close square: Fuchu air: Open circle: Pacific Ocean air [Kim and Craig, 1990].

V-3. SEA WATER

Sampling and Analysis

Sea waters were collected at different depths from surface to 1000 m in Monterey Bay, California (Fig. V-2) during a cruise of Point Sir on June 4, 1990 and a cruise of Point Lobos on June 14, 1990. Surface water was collected in a 100 L plastic barrel by a submersible pump. The barrel was brought back to the laboratory at MBARI, then N_2O was extracted. Deep water was collected by a 30 L Go-Flow bottles, which was equipped with a sintered glass at the bottom of the bottles to be used as a gas stripping chamber during on board N_2O extraction [Yoshinari *et al.*, in preparation].

Dissolved N_2O was extracted by circulating helium in a closed system. After removals of water vapor and carbon dioxide from the stripping gas by Aquasorb and Ascarite, N_2O was collected in MS5A tube. The gas desorption from the MS5A tube was carried out at MBARI. Subsequent isotopic preparations (see Chapter IV) were achieved at WCL&R. $\delta^{15}N$ and $\delta^{18}O$ were measured by FINIGAN MAT delta-S at MBL.

All the chemical analyses were carried out at MBARI. N_2O concentration was determined by ^{63}Ni-ECD gas chromatograph (HEWLETT PACKARD model 5890) after the head-space technique (see Chapter II) tagged with an auto-sampling system. Porapack Q was used for separation of the gas. Dissolved oxygen concentration was analyzed by Winkler titration. Nutrient concentrations were measured colorimetry.

Results and Discussion

Depth profiles of N_2O, NO_3^- and O_2 are illustrated in Fig. V-3. The N_2O concentrations were higher than calculated equilibria with atmosphere (305 ppbv) at *in situ* water temperatures and salinities throughout the water column. The N_2O and NO_3^- profiles are essentially mirror image of the O_2 profile with maximal N_2O concentration at 700 m depth in the O_2 minimum. These profiles are typical for the Monterey Bay water [L.A. Codispoti, personal communication] and quite similar to that of from the North Pacific [Cohen and Gordon, 1978; Yoshida *et al.*, 1989].

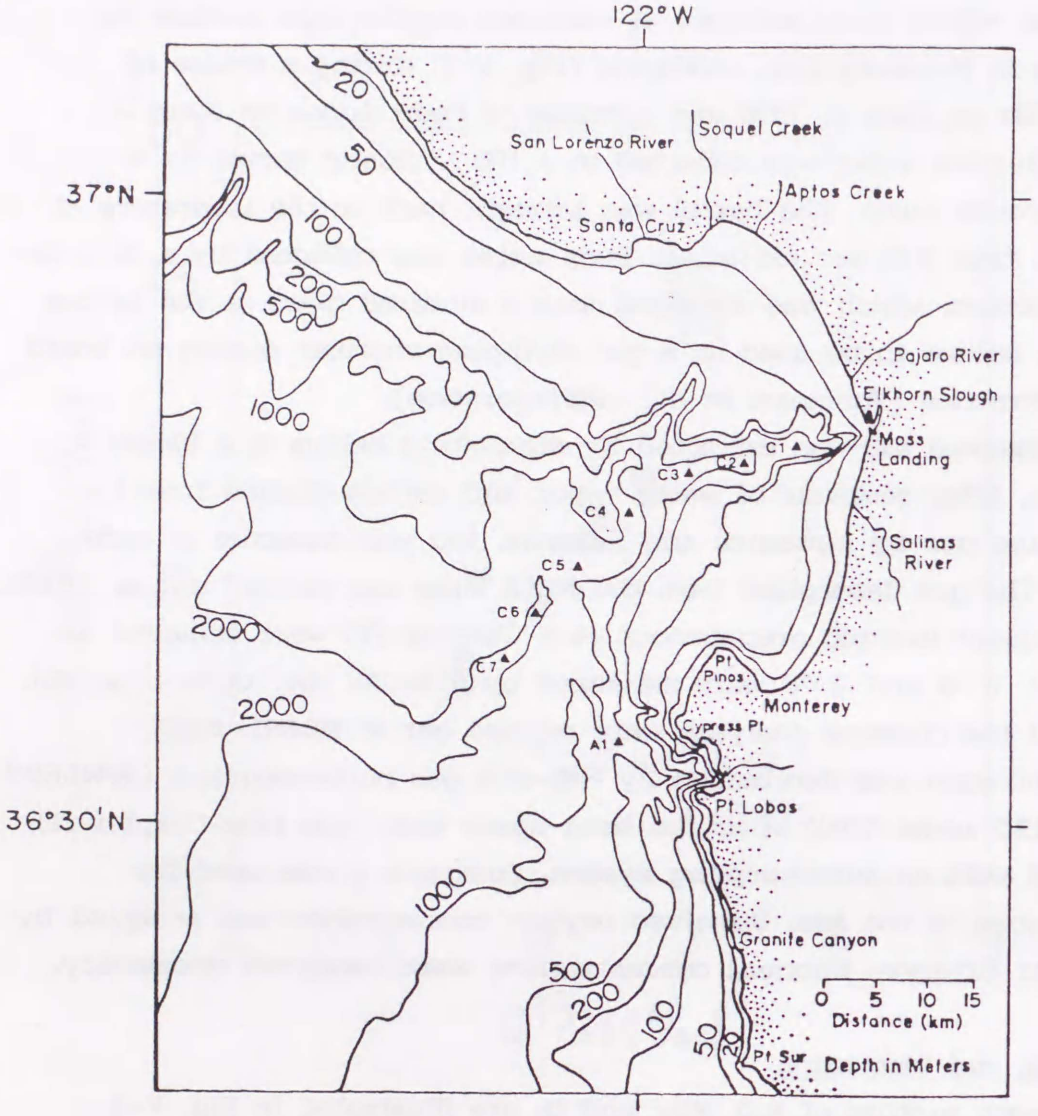

Figure V-2 Sampling locations at Monterey Bay, California. Vertical profile was taken from C5.

Positive correlations of NO_3^- and PO_4^{3-} to AOU ($-\triangle O_2$) through out the water column (Figs V-4 and V-5) strongly suggest regeneration of these nutrient elements at the expense of oxygen [Redfield, 1963]. A linear correlation between PO_4^{3-} and NO_3^- in an aphotic zone below 50 m in depth (Fig. V-5) indicates no significant consumption of NO_3^- via nitrate respirations therein.

N_2O increased with O_2 decrease in the water column (Fig. V-3) as firstly depicted by Yoshinari [1976] and later confirmed at various location by many of the past studies. However, $\triangle N_2O$ is incompletely expressed by an empirical equation for the oceanic $\triangle N_2O$-AOU relationship with inclusion of temperature as a variable which was reported by Butler *et al.* [1989] (Fig. V-7). As far as concerning the plots from 50 to 700 m in depth, which corresponds the bottom of a photic zone to the N_2O maximum layer, are well fitted on two regression lines (Fig. V-8). It should be noted that there is a change in gradient at the AOU value of 217-240 μM in 200-250 m in depth. This finding and a recent observation of the similar gradient change at an AOU value of 197 μM at the upwelling region in the northwest Indian Ocean [Law and Owens, 1990] suggest a single mechanism of the N_2O production, in which the N_2O production increase significantly under the oxygen stress. $\triangle N_2O$-NO_3^- plots in the aphotic zone are also divided in two groups of 50-200 m and 250-700 m (Fig. V-9), which indicates the higher yield of N_2O to NO_3- in the oxygen-depleted zone while NO_3^- production is still proportional to the AOU (Fig. V-4). According to the slopes of 1.20×10^{-3} and 1.86×10^{-3} in Fig. V-9, the apparent production ratios between N_2O and NO_3^- of 2.4 % and 3.7 % in atom-N basis for the 50-200 m and the 250-700 m were calculated for the aphotic zone. These ratios overlap the previously reported ranges of 0.13-0.37 % [Cohen and Gordon, 1979] and 0.18-0.36 % [Oudot *et al.*, 1990], and are higher than that of 0.12 % [Yoshinari, 1976]. The findings are well explained if nitrification were assumed as a production route of these N_2O in aphotic zone, because the N_2O yield elevates in lower O_2 concentration during a culture of marine nitrifying bacteria [Goreau *et al.*, 1980] as previously illustrated in Fig. II-11.

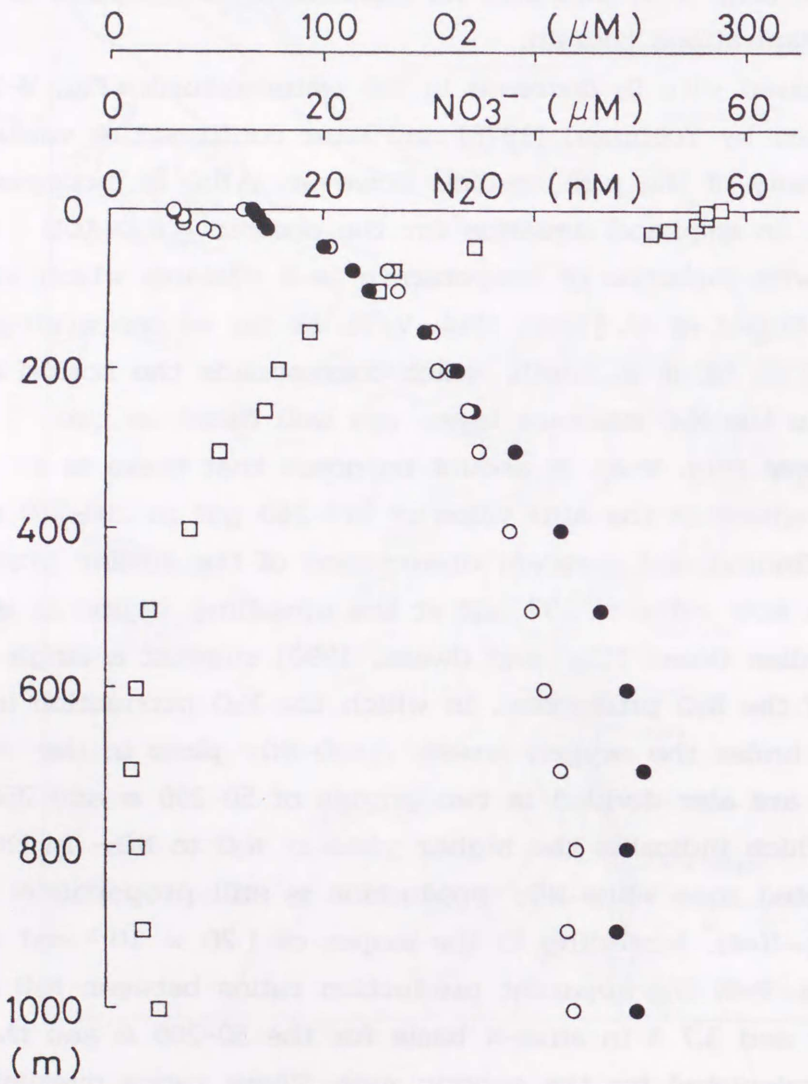

Figure V-3 Vertical profiles of N_2O (close circles), NO_3^- (open circles) and O_2 (open squares) from the Monterey Bay.

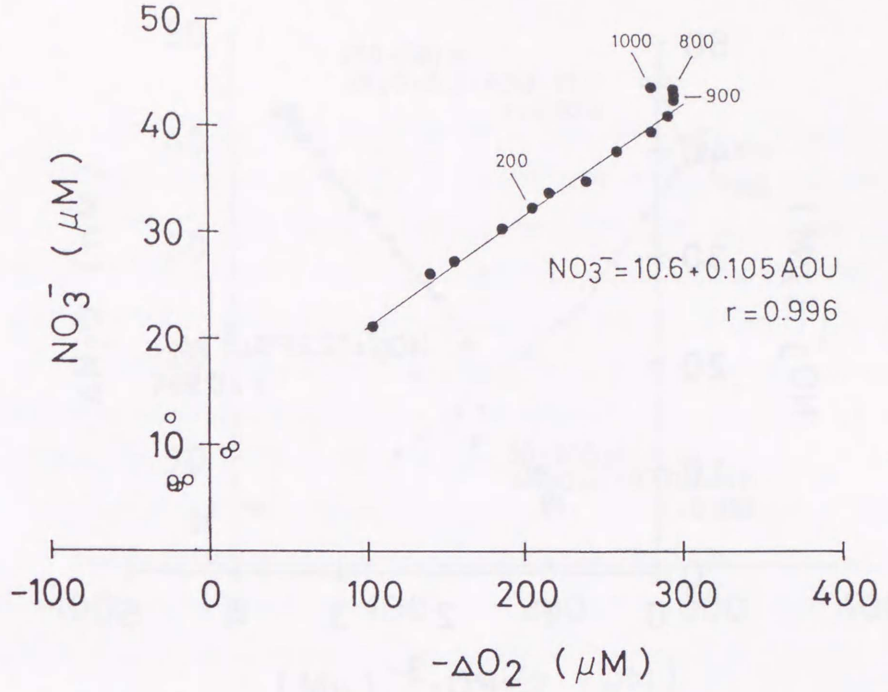

Figure V-4 Relationship between apparent oxygen utilization ($-\Delta O_2$) and NO_3^- concentration in the Monterey Bay water column. Opene and closed circles present data from photic and aphotic zone, respectively.

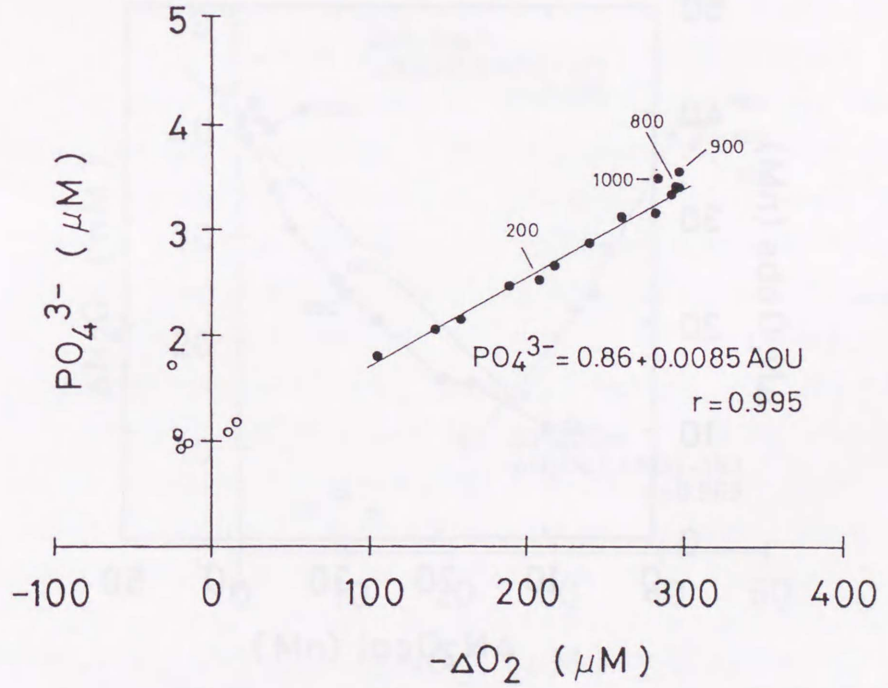

Figure V-5 Relationship between apparent oxygen utilization ($-\Delta O_2$) and PO_4^{3-} concentration in the Monterey Bay water column. Open and closed circles present data from photic and aphotic zone, respectively.

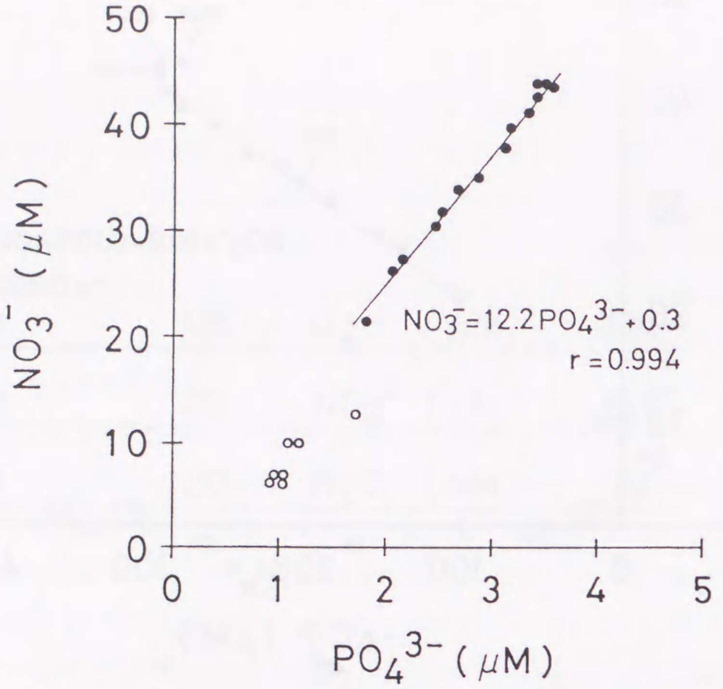

Figure V-6 Relationship between PO_4^{3-} and NO_3^- concentrations in the Monterey Bay water column. Open and closed circles present data from photic and aphotic zone, respectively.

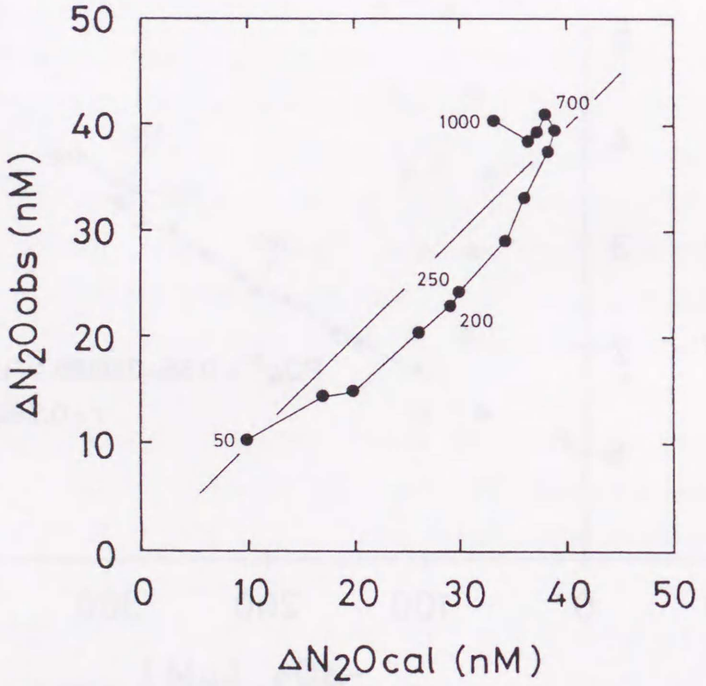

Figure V-7 Plots of ΔN_2O observed in an aphotic zone of the Monterey Bay water *vs.* ΔN_2O calculated with an oxygen utilization and water temperature after an empirical equation for the ocean, ΔN_2O (nM) = $-13.5 + \{0.125 + 0.00993 \times T\ (℃)\} \times AOU\ (\mu M)$ by *Butler et al.* [1989].

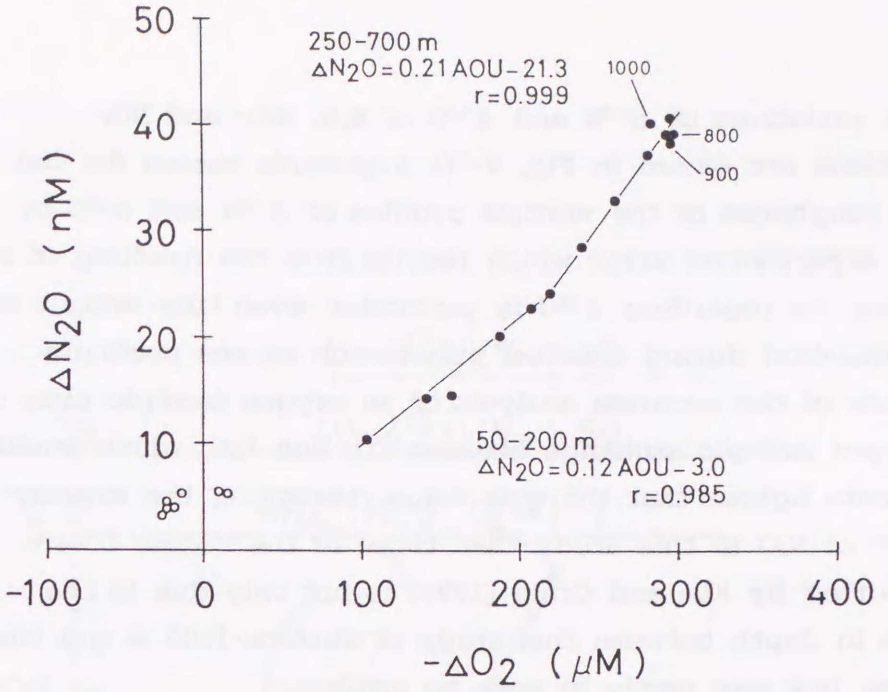

Figure V-8 Plots of apparent N_2O production (ΔN_2O) *vs.* apparent oxygen utilization ($-\Delta O_2$) in the Monterey Bay water column. Open and closed circles present data from photic and aphotic zone, respectively.

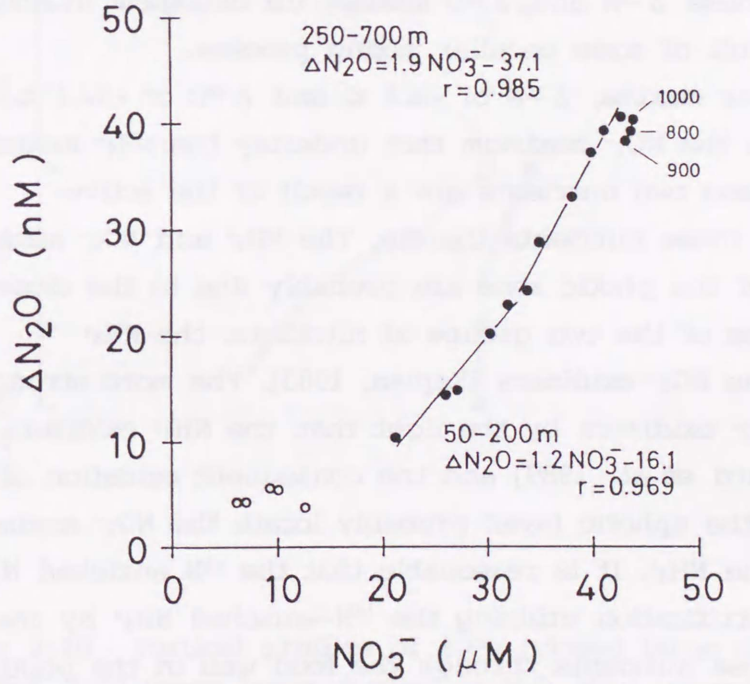

Figure V-9 Plots of apparent N_2O production (ΔN_2O) *vs.* NO_3^- concentration in the Monterey Bay water column. Open and closed circles present data from photic and aphotic zone, respectively.

Depth variations of $\delta^{15}N$ and $\delta^{18}O$ of N_2O, NH_4^+ and NO_2^- concentrations are shown in Fig. V-10. A possible reason for the irregular roughness of the vertical profiles of $\delta^{15}N$ and $\delta^{18}O$ in N_2O is an experimental error which results from the handling of small N_2O samples. As regarding $\delta^{18}O$ in particular, even tiny amount of H_2O contamination during chemical preparation causes profound interference of the accurate analysis of an oxygen isotopic ratio due to an oxygen isotopic exchange between CO_2 and H_2O, which would shift measurements lighter that the true value. Therefore, the slightly lower $\delta^{18}O$ of N_2O in this study than those in the Pacific Ocean water reported by Kim and Craig [1990] is not only due to the difference in depth between this study of surface-1000 m and their 200-5000 m, but may partly to such an artifact.

In full consideration of the above conditions, there can be seen two maxima of the $\delta^{15}N$ and $\delta^{18}O$ at the 50 m in depth, just beneath the bottom of the photic layer, and at 700 m depth with maximum N_2O concentration in the profiles. A rather linear T-S diagram (Fig V-11) suggests that these $\delta^{15}N$ and $\delta^{18}O$ maxima are biological in origin and not the result of some peculiar mixing process.

The shallower maxima, $\delta^{15}N$ of +8.3 ‰ and $\delta^{18}O$ of +42.4 ‰, corresponded to the NO_2^- maximum that underlay the NH_4^+ maximum. The elevations of these two nutrients are a result of the active regeneration of these nutrients therein. The NH_4^+ and NO_2^- maxima near the base of the photic zone are probably due to the closely coupled activities of the two groups of nitrifiers, the NH_4^+ oxidizers and the NO_2^- oxidizers [Kaplan, 1983]. The more strong inhibition of NO_2^- oxidizers by the light than the NH_4^+ oxidizers [Olson, 1981; Ward *et al.*, 1989] and the consequent oxidation of the NO_2^- to NO_3^- in the aphotic layer probably locate the NO_2^- maximum below that of the NH_4^+. It is reasonable that the ^{15}N enriched N_2O is produced via nitrification utilizing the ^{15}N-enriched NH_4^+ by the recycling of these nutrients through the food web in the photic layer. The higher $\delta^{18}O$ in N_2O, on the other hand, can be explained by considering the utilization of ^{18}O-enriched dissolved oxygen during the oxygen consumption for the regeneration of the nutrients [Kroopnik and Craig, 1976].

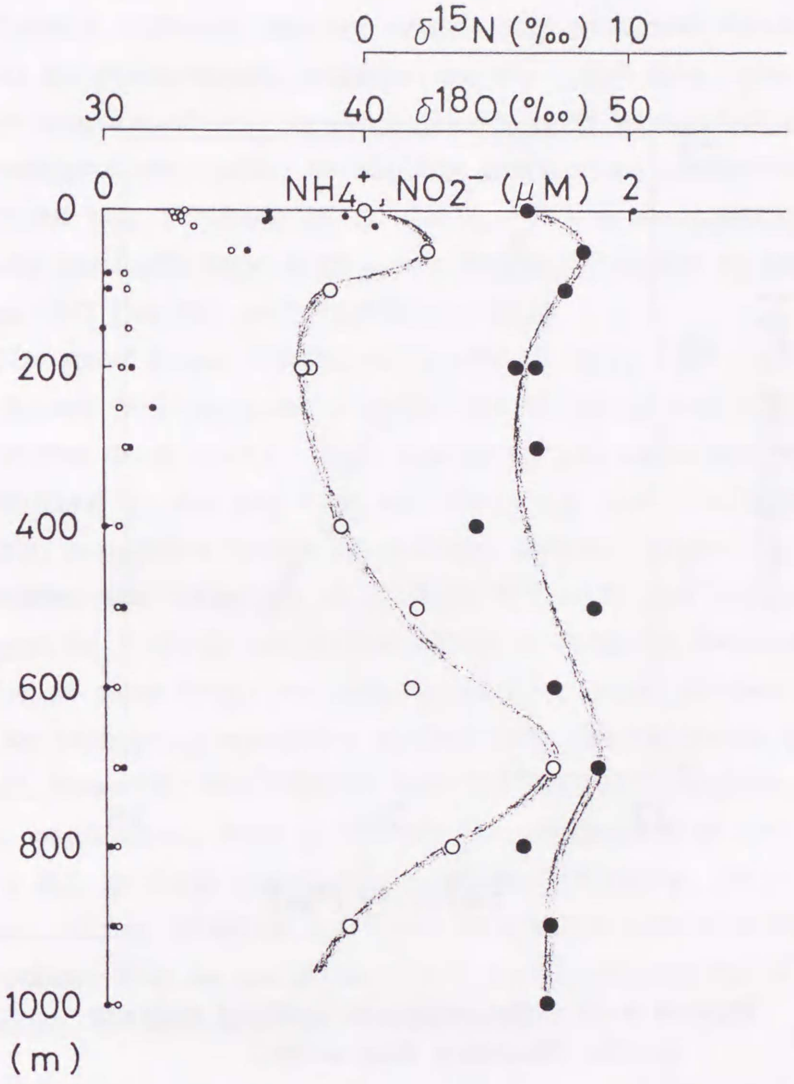

Figure V-10 Vertical profiles of $\delta^{15}N$ (closed large circles) and $\delta^{18}O$ (open large circles) in N_2O, NH_4^+ (closed small circles), NO_2^- (open small circles) concentrations from the Monterey Bay. Broad lines were drawn by hand.

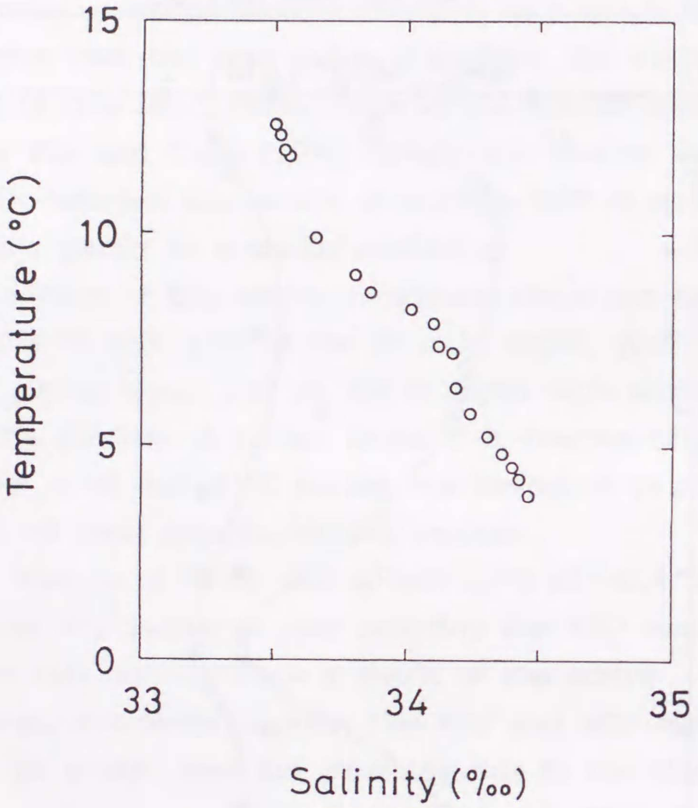

Figure V-11 Temperature-salinity diagram in the Monterey Bay water.

The deeper maxima, $\delta^{15}N$ of +8.7 ‰ and $\delta^{18}O$ of +47.1 ‰, in the layer with the minimum O_2 concentration and the maximum N_2O concentration would be owing to one or more of the following mechanisms those were previously supposed: (1) the production of isotopically heavier N_2O than the major product NO_3^- from nitrification utilizing heavier isotopically enriched NH_2OH *in vivo* due to its predominant oxidation to NO_2– [Kim and Craig, 1990], (2) the production of N_2O by denitrification, of which isotopic compositions are similar to slightly heavier as compared to the substrate NO_3^- [Yoshida *et al.,* 1989], (3) the consumption of N_2O pool by denitrification with preferential reduction of isotopically lighter N_2O [Wahlen and Yoshinari, 1985].

The good linear relationships among NO_3^-, PO_4^{3-} and AOU (Figs V-4, V-5, and V-6) suggest nitrification to be an important source of N_2O in the deep part (> 50 m depth) of the Monterey Bay water column. As referred to the fact that nitrifiers can use nitrite as terminal electron acceptors under low oxygen concentration by inducing nitrite reductase, the reduction of *in vivo* NO_2^- enriched with heavier isotopes as a result of isotope effect during its dominant oxidation to NO_3^- is more likely mechanism producing the deeper N_2O with heavier isotopic composition rather than the oxidation of heavy NH_2OH to N_2O. However, the present data set cannot elucidate which one of these mechanisms more governed the production of the isotopically heavy N_2O in deep ocean. For further discussion, we need isotopic determinations of other nitrogenous species participating in the N_2O metabolism, NO_3^- in particular, and direct measurements of microbial activities, nitrification and denitrification.

V-4. FRESHWATER

Sampling and Analysis

A. River and sewage waters

Riverine samples were collected in River Minami-Asakawa, a shallow urban stream in the western part of Tokyo (Fig. V-12). The lower reaches of the river is polluted by domestic waste waters brought through numbers of sewers to the river. The cumulative increase of N_2O concentration from the head stream to downstream in this river was observed in the preliminary study [Ueda, 1988]. Monthly observations by the author's laboratory over the past decade indicate that active nitrification occurs in-between MA4 and MA5. Water samples for stable isotopic analyses were taken at MA3, MA4 and MA5 on January 26, 1989. Sewages SW3 and SW4 which flow into the river between MA4 and MA5 were also collected.

Samples of secondary treated waste water were taken at JY0 in Kodaira, Tokyo on January 28, 1989. The treated water was pumped to this point from the Tamagawa Jouryu Sewage Treatment Facility, then discharged into River Tamagawa-Josui. By the preliminary observation, supersaturation of N_2O in the treated water was revealed and the N_2O concentration decreased as water flow due to emission to the atmosphere (data not shown).

Water samples were collected in 20 L polyetylene bottles used in groundwater sampling. Dissolved N_2O was extracted after the method that was described in Chapter III. Only nitrogen isotopic measurements were carried out after the Cu-reduction method using HITACHI RMU-6R ratio mass spectrometer. N_2O concentration was measured by the purge-trap GC method (see Chapter II). All the other chemical analyses were performed after the methods described in Chapter II.

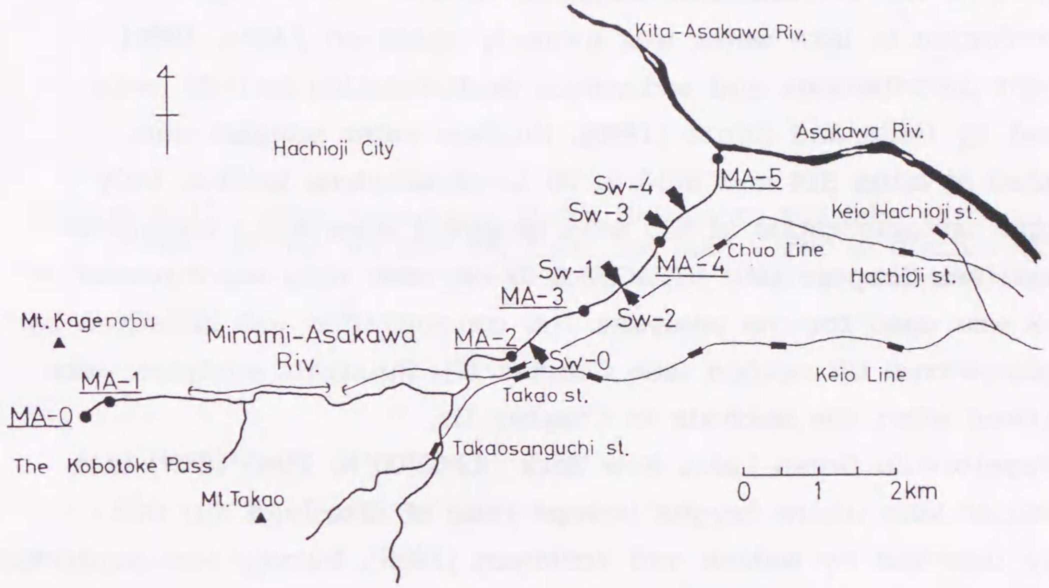

Figure V-12 River Minami-Asakawa. Site discriptions are as follows: MA0-one of the sources of the river; MA1-second-order stream of the river; MA2 to MA3-despite of sewage (SW0) discharge, in significant pollution of the river water is observed; MA4 to MA5-river water is eutrophied by externally loaded nutrients through sewage SW1, SW2, SW3 and SW4. Isotopic samples were collected at MA3, MA4, MA5, SW3 and SW4 on January 26, 1989.

B. Lake waters

Studies were conducted at Lake Teganuma, Chiba Prefecture, Japan (Fig. V-13) and Fayetteville Green Lake, New York, USA (Fig. V-14).

Lake Teganuma (36°00'N, 140°00'E) is highly eutrophied due to the huge amount of domestic waste water produced in its watershed. Because this lake was the former channel of the River Tonegawa, the mean water depth is about 1 m. Such an extremely shallow depth enhances sever eutrofication. Seasonal variation of the N_2O concentration in lake water was formerly observed [Ueda, 1988]. Nutrient distributions and sedimental denitrification activity were studied by Ueda and Ogura [1989]. Surface water samples were collected at sites St4 and St10 in 20 L polyethylene bottles. Only nitrogen isotopic ratios of N_2O were analyzed after the Cu-reduction method (see Chapter III). HITACHI RMU-6R ratio mass spectrometer at MKILS was used for the analyses. N_2O concentration was measured by the purge-trap GC method (see Chapter II). Nutrients analyses were performed after the methods in Chapter II.

Fayetteville Green Lake, New York (43°03'00"N, 75°47'48"W) is a meromictic lake where oxygen isotope ratio of dissolved N_2O was firstly reported by Wahlen and Yoshinari [1985]. Survey was conducted on May 23, 1990. Water temperature and dissolved O_2 concentration were measured at 1 m interval to 15 m depth by casting sensors (YELLOW SPRINGS INSTRUMENT Co.,Inc., Ohio). Water samples for chemical analyses (NO_3^-, NO_2^-, NH_4^+, DOC) were collected at 1, 4, 8, 12, 20, 22.5, 25, and 30 m in depth by a 1 L Van-Dorn bottle. Samples for isotopic analyses of N_2O were taken from 1 and 13 m depths by using an electric submersible pump. N_2O extraction was as same as for Teganuma samples. Only the nitrogen isotopic determinations of N_2O after the C/Au-reduction method (Chapter IV) were carried out with these samples. FINIGAN MAT delta-S at MBL was used for the analyses. N_2O and nutrients concentrations were determined after by the head-space technique (see Chapter II) and Technicon Autoanalyzer II at WCL&R.

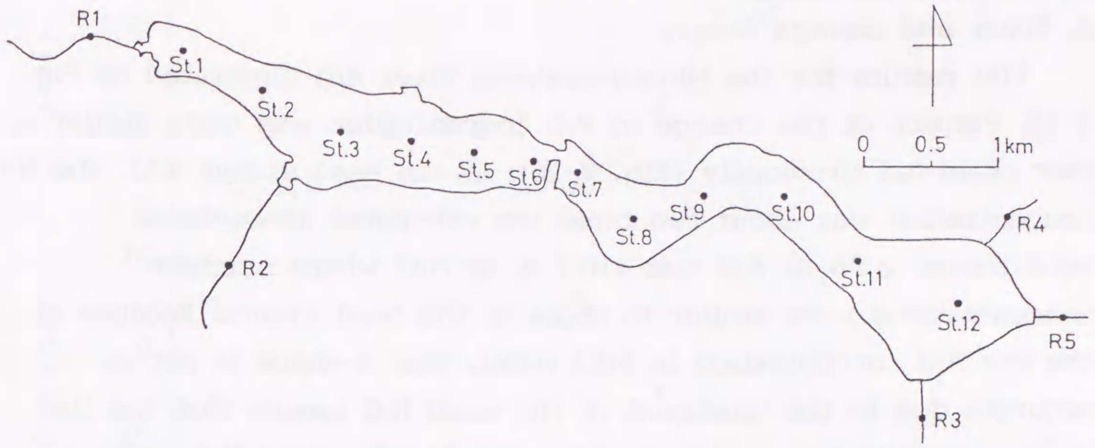

Figure V-13 Lake Teganuma. Water flows into the lake through rivers R1, R2, R3 and R4, then the lake water is discharged into a river R5. Isotopic samples were taken at St4 and St10 on May 29, 1989.

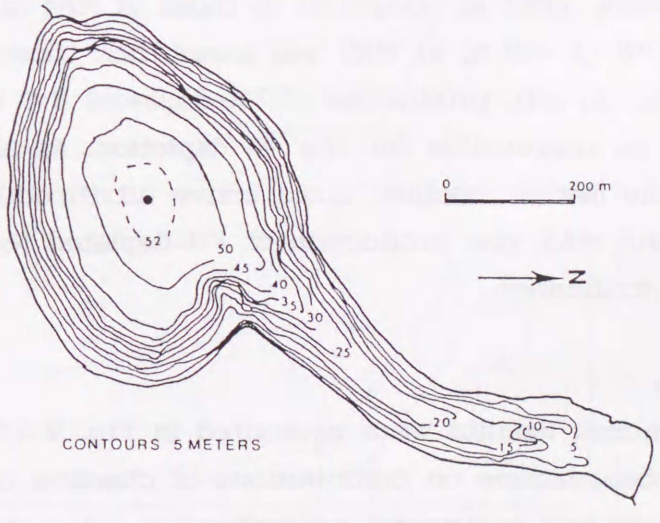

Figure V-14 Fayetteville Green Lake. Sampling was carried out at the point with maximum depth on May 23, 1990.

Results and Discussion

A. River and sewage waters

The results for the Minami-asakawa River are illustrated as Fig. V-15. Pattern of the change in N_2O concentration was quite similar to that observed previously (Fig. V-16). At the head stream MA1, the N_2O concentration was about two times the calculated atmospheric equilibrium. $\delta^{15}N$ of N_2O was +10.7 ‰ at MA3 where nutrient concentrations were similar to those in the head waters. Because of the low N_2O concentration in MA3 water, this δ-value is not so accurate due to the treatment of the small N_2O sample that the the measurement is comparable to the value for dissolved N_2O under equilibrium condition with atmosphere as +7.3 ‰ within the experimental error. The $\delta^{15}N$ of the N_2O in the equilibrium is calculated with the mean $\delta^{15}N$ of +6.5 ‰ (Table V-1) and the enrichment factor in dissolution of ε =0.8 ‰ [Yoshida, 1984].

The ^{15}N contents of N_2O decreased dramatically from MA3 to MA5 via MA4 as the N_2O concentration increased. The $\delta^{15}N$ of N_2O in the sewages of SW3 and SW4 are -9.2 ‰ and -0.5 ‰, respectively, which are comparable to -2.9 ‰ obtained with N_2O in the secondary treated sewage effluent collected at JY0. Since the water discharge rates of SW3 and SW4 were small as compared to those of MA4 and MA5, and because the $\delta^{15}N$ of -38 ‰ at MA5 was lower than those in these in-flowing sewages, *in situ* production of ^{15}N-depleted N_2O between MA4 and MA5 must be responsible for the ^{15}N depletion. As already mentioned in the method section, since active nitrification occurs between MA4 and MA5, the production of ^{15}N-depleted N_2O was probably produced by nitrification.

B. Lake waters

Lake Teganuma: Results were presented in Fig. V-17 together with the previous observations on distributions of chemical constituents in the lake water and sedimental denitrification rates [Ueda, 1988; Ueda and Ogura, 1989]. Dissolved N_2O is characterized by the negative $\delta^{15}N$ in the upper basin and the positive value of +14.7 ‰ in the down basin of the lake. The later is the highest $\delta^{15}N$ in this study.

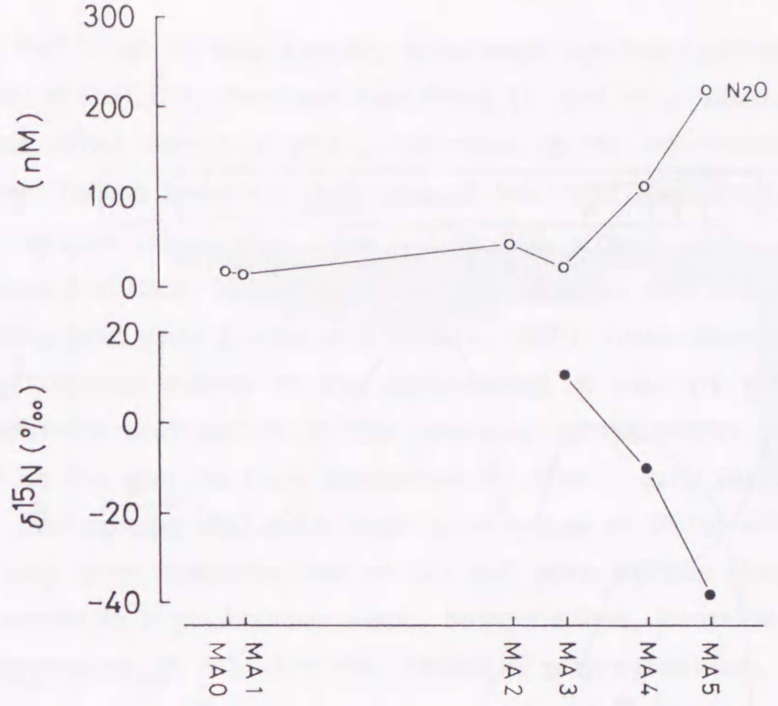

Figure V-15 Concentrations and $\delta^{15}N$ measurements of N_2O in River Minami-Asakawa on January 26, 1989.

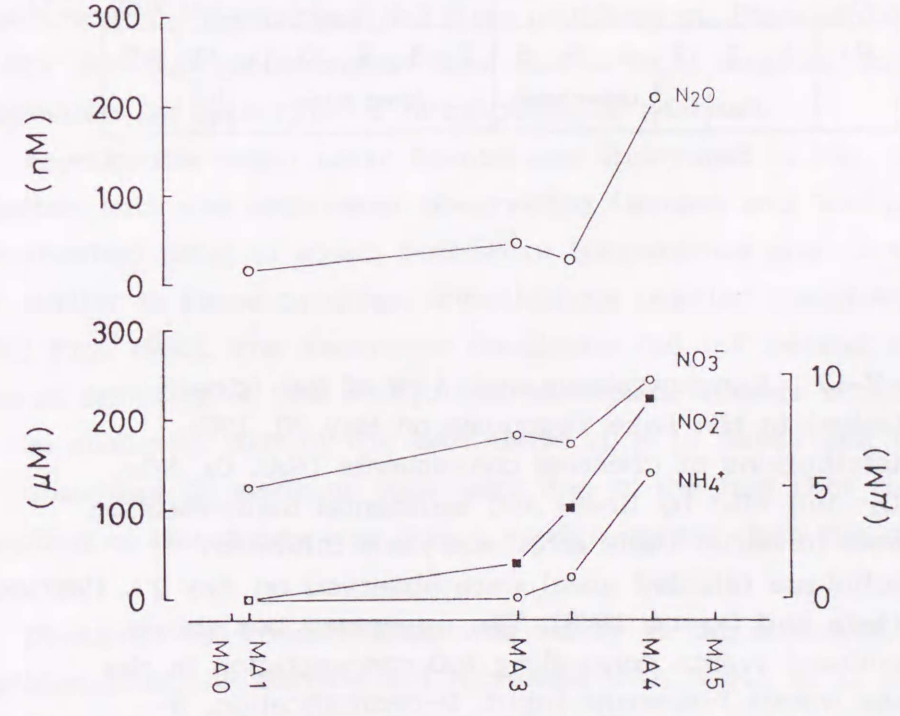

Figure V-16 Concentrations of N_2O, NO_3^-, NO_2^- and NH_4^+ in River Minami-Asakawa on October 26, 1986 [*Ueda*, 1988]. NO_2^- is in nM scale.

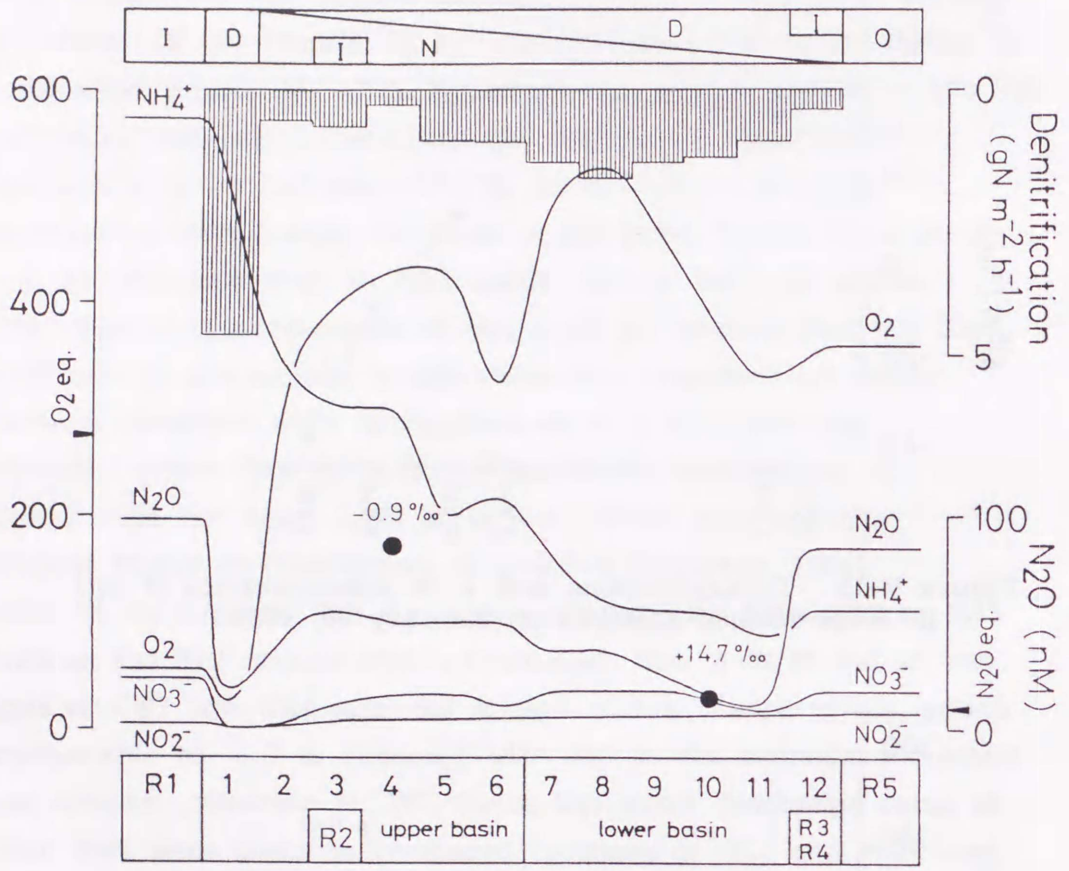

Figure V-17 Concentrations and $\delta^{15}N$ of N_2O (closed circles) in the Lake Teganuma on May 20, 1989. Distributions of chemical constituents (N_2O, O_2, NO_3^-, NO_2^- and NH_4^+ by lines) and sedimental denitrification rates in aerial basis after acetylene inhibition technique (shaded area) were observed on May 27, 1987 [*Ueda and Ogura,* 1989]. The uppermost box shows dominant ivents controlling N_2O concentration in the lake water: I-riverine input, D-denitrification, N-nitrification, O-output to the reiver. Numbers in the bottom box correspond those in Figure V-13.

N_2O from R1 was rapidly consumed by denitrification near the river mouth St1, because dissolved O_2 and NO_3^- were exhausted in the water. This means *in situ* production of the ^{15}N-depleted N_2O in the upper basin. Dramatic decrease of NH_4^+ and significant increase of NO_3^- in the lake water from St2 to St6, where major part of the bottom sediment is composed of sandy loam, are the general trend throughout year [Ueda and Ogura, 1989]. Such nitrogen transformation is attributed mainly to the assimilation of NH_4^+ by phytoplankton community and partly to the microbial nitrification. The ^{15}N-depleted N_2O at St4 can be thus explained by the *in situ* nitrification.

Concerning N_2O with high $\delta^{15}N$ value at St10, where the sediment is silty clay, consumption of the N_2O pool by the denitrification in sediment is a probable reason. Nevertheless, because of the requirement of NO_3^- for the dominant phytoplankton, *Microcystis sp.*, remaining NO_3^- fraction to be used by denitrifiers would be enriched with ^{15}N, which may result in the production of ^{15}N-enriched N_2O. The NH_4^+ in the down basin water might also be enriched in ^{15}N through the assimilation and the nitrification. This may result in production of ^{15}N-enriched N_2O from nitrification. However, decreases of NO_3^- and N_2O concentration from St.8 to St.11 suggest the N_2O production by such type of nitrification to be small.

Fayetteville Green Lake: Results are illustrated in Fig. V-18 together with the midsummer observation [Wahlen and Yoshinari, unpublished data] in which profiles of temperature and dissolved O_2 are similar to those by other investigators [Wahlen and Yoshinari, 1985; Fry, 1986]. The meromictic conditions did not persist at the time of sampling of this study. The incomplete summer stratification in the shallower part of the lake above 10 m in depth and the absence of subsurface O_2 maximum zone were due to the fact that the time of sampling of this study was 3 to 4 months earlier than the other reports.

Dissolved N_2O concentration was nearly to the atmospheric equilibrium at the surface and increased with depth to 12 m, but the extent of the accumulation is lower than the results of Wahlen and Yoshinari [1985]. The NO_3^- concentration is higher in the surface but significantly lower in the deeper part than those in 1982. The high

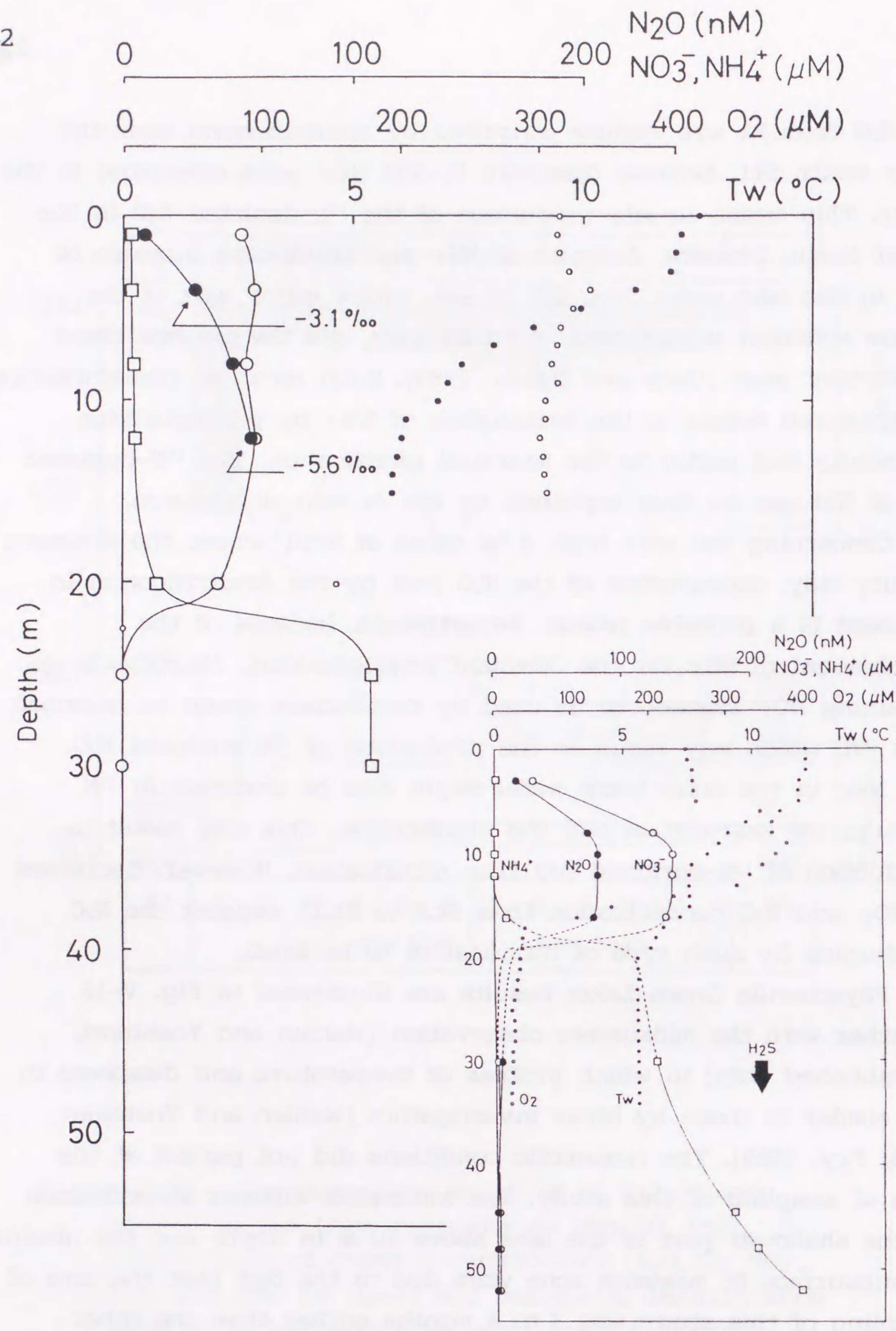

Figure V-18 $\delta^{15}N$ of N_2O and vertical profiles of temperature (small closed circle), O_2 (small open circle), N_2O (large closed circle), NO_3^- (large open circle), and NH_4^+ (large open square) at the Fayetteville Green Lake on May 22, 1990. A previous observation at the same location on August 24, 1982 [*Wahlen and Yoshinari,* unpublished data] is presented as a reduced illustration.

NO_3^- concentration in the surface reflects low assimilation by the primary producers in the sampling period. Lack of the deeper accumulation of NO_3^- is due to insufficient nitrification activity. Both are a result of the incomplete stratification of the upper part of the lake.

The $\delta^{15}N$ of N_2O was lower at 12 m depth than at 4 m depth (Fig. V-19). These $\delta^{15}N$ measurements are comparable to and -6.0 ‰ which was measured at 21 m depth in the Lake Kizaki, a Japanese alpine mesotrophic lake, where N_2O is considered to be produced by nitrification [Wada *et al.,* 1991]. Although stable isotopic compositions of other nitrogenous species were not measured, it may not be unreasonable to suppose that nitrification is mainly responsible for these N_2O with negative $\delta^{15}N$ values.

V-5. ISOTOPIC CHARACTERIZATION OF ENVIRONMENTAL NITROUS OXIDE

Nitrogen Isotopic Composition of N_2O from Different Environments and their Emission Potential

Fig. V-19 illustrates relationship between excess N_2O ($\triangle N_2O$) and its $\delta^{15}N$ calculation in various aquatic environments. Note that $\triangle N_2O$ is presented logarithmically. As a general trend, nitrogen isotopic compositions of aquatic N_2O is likely characterized as a function of the degree of N_2O supersaturation. Namely, the lower $\triangle N_2O$ the higher $\delta^{15}N$ and vise versa can be seen in the figure.

The right arrows for the Monterey Bay (from 50 m to 700 m excepting dotted line from the photic layer), Green Lake (4 m to 21 m depth) and River Minami-Asakawa (upper leach MA3 to lowest leach MA5 via MA4) correspond production of ^{15}N-depleted N_2O by nitrification. Whereas the left arrows for the Lake Teganuma (upper basin to down basin) and the Lake Kizaki [Wada *et al.*, 1991] (21 m to 23 m depth) show enrichment of ^{15}N in N_2O through the denitrification. The large arrow from the plots for the deep N_2O in the Monterey Bay water (800-1000 m) to the plots of the oxygen deficient layer at the eastern tropical North Pacific [Yoshida, 1984] also suggests the ^{15}N-enrichment in N_2O as denitrification proceed. Groundwater N_2O presented as open circles and squares are characterized by higher concentrations and lower $\delta^{15}N$ than the surface water N_2O.

One can thus predict a potential source strength of N_2O in a given aquatic environment from the nitrogen isotopic analysis as well as elucidate major mechanisms controlling a size of N_2O pool therein by tracing the plots in the $\triangle N_2O$-$\delta^{15}N$ diagram.

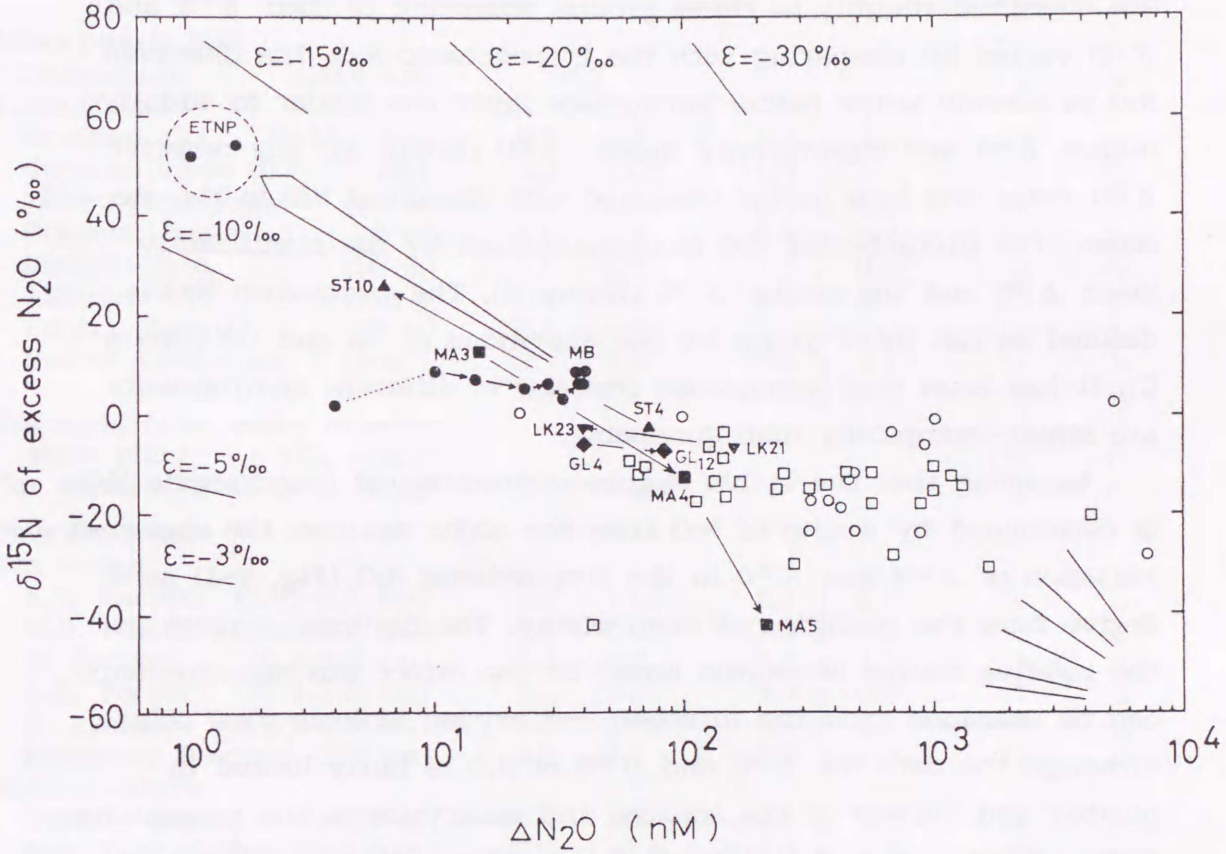

Figure V-19 Relationship between concentration of excess N_2O ($\triangle N_2O$) and its $\delta^{15}N$ in aquatic systems. Open circles squares present data for groundwaters. Abbreviations are as follows: MB-Monterey Bay, GL-Fayettiville Green Lake, MA-River Minami-Asakawa, ST-Lake Teganuma, LK-Lake Kizaki [*Wada et al.*, 1991]. The $\delta^{15}N$ was calculated with the mean $\delta^{15}N$ of +6.5 ‰ for the tropospheric N_2O (see text) and ε=-0.8 ‰ during dissolution [*Yoshida*, 1984]. Arrows show the direction of the net reaction. Encircled plots are data for the eastern tropical North Pacific [*Yoshida*, 1984]. A large arrow means proceeding of denitrification in open ocean.

Two-Isotope Map of N_2O

Tables V-3 and V-4 summarize all the available information on nitrogen and oxygen stable isotopic compositions of environmental N_2O, from which a $\delta^{15}N$-$\delta^{18}O$ map for N_2O is compiled (Fig. V-20).

The map strongly suggests that N_2O from different environments are classified roughly in three groups according to their $\delta^{15}N$ and $\delta^{18}O$ values by comparing with the tropospheric N_2O. The dissolved N_2O in oceanic water bellow subsurface layer has similar to slightly higher $\delta^{15}N$ and significantly higher $\delta^{18}O$ (Group A). Any negative $\delta^{15}N$ value has been never observed with dissolved N_2O in the sea and ocean. The groundwater N_2O is characterized by the significantly lower $\delta^{15}N$ and the similar $\delta^{18}O$ (Group B). The combustion N_2O is defined as the third group by the depletions of ^{15}N and ^{18}O (Group C). It has been thus recognized that N_2O in different environments are stable-isotopically distinguishable.

Assuming that the stable isotope composition of tropospheric N_2O is determined by mixing of N_2O from the major sources, the observed variation of $\delta^{15}N$ and $\delta^{18}O$ in the tropospheric N_2O (Fig. V-1) must derive from the condition of such mixing. The findings suggest that the relative source strengths based on the direct flux measurements can be examined from the nitrogen and oxygen isotopic view point. Although the data for $\delta^{15}N$ and $\delta^{18}O$ of N_2O is fairly limited in number and variety of the sources and reservoirs at the present time, future development of this kind of two-isotope diagram for N_2O as prepared in this study is believed to help in both the source identification and the budget discussion on N_2O.

TABLE V-3 Summary for Nitrogen Stable Isotope Ratios of N_2O from Different Environments

	$\delta^{15}N$ (‰)		n	Ref.
	Range	mean ± SD		
Atmospheric N_2O				
Stratosphere (20.8 km)	19.2	—	1	a
Troposhere				
Boulder, Col. (0-13.1 km)	0.0 – 9.3	5.2 ± 2.0	26	a
Central Japan (0.7-8.3 km)	8.3 – 15.5	11.3 ± 2.0	12	b
Tokyo Metlopolis (10 m)	6.6 – 10.1	8.1 ± 1.0	12	b
Otsuchi, Iwate (8 m)	6.9 – 9.1	7.9 ± 0.7	12	b
Hachijo-jima (50 m)	6.6 – 8.0	7.2 ± 0.5	8	b
Albany, N.Y. (sur.)	5.5 – 7.7	6.6 ± 0.7	15	c
Fuchu, Tokyo (5 m)	5.5 – 5.6	—	2	c
Pacific Ocean Air (sur.)	3.7 – 8.6	7.2 ± 1.1	16	b
	6.9 – 7.1	7.0 ± 0.1	5	d
Stackgas from waste incineration plant (-NO_x, -SO_2)^1	1.6	—	1	c
Car exhaust gas (-NO_x, -SO_2)^1	-11.6 – -22.6	—	2	c
Dissolved N_2O				
Sea waters				
E.T. Pacific (0.05-0.5 km)	3.3 – 14.2	8.1 ± 4.0	10	b
W.N. Pacific (sur.-4.8 km)	3.4 – 15.0	12.3 ± 8.5	9	e
N. Pacific (0.2-5.0 km)	6.3 – 9.7	8.2 ± 1.3	6	d
N.E. Pacific (0.3-2.8 km)	8.5 – 9.8	9.0 ± 0.5	7	d
S. Pacific (0.3-4.9 km)	6.4 – 8.9	7.6 ± 1.1	7	d
Monterey Bay (sur.-1.0 km)	4.2 – 8.7	6.8 ± 1.3	13	c
Groundwaters				
Central Japan (0-250 m)	-28.4 – 2.7	-10.2 ± 9.3	22	c
New York States (0-216 m)	-32.0 – -2.4	-14.7 ± 7.0	30	c
Fresh surface waters				
Minamiasakawa River				
upper stream	10.7	—	1	c
middle courses	-10.6	—	1	c
downstream	-39.8	—	1	c
inflowing sewage	-0.5 – -9.2	—	1	c
Treated sewage (JYO)	-2.9	—	2	c
Lake Teganuma (upper basin)	-0.9	—	1	c
(down basin)	14.7	—	1	c
Green Lake (4 m depth)	-3.1	—	1	c
(12 m depth)	-5.6	—	1	c
Lake Kizaki (21 m depth)	-6.0	—	1	f
(23 m depth)	-1.1	—	1	f

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[2] NO_x and SO_2 were eliminated by conc. NaOH solution at the sampling.

Literature cited: a. Moore [1973], b. Yoshida [1984], c. This study, d. Kim and Craig [1990], e. Yoshida *et al.* [1990], f. Wada *et al.* [1991]

TABLE V-4 Summary for Oxygen Stable Isotope Ratios of N_2O from Different Environments

	$\delta^{18}O$ (‰)		n	Ref
	Range	mean ± SD		
Atsospheric N_2O				
Troposphere				
Albany, N.Y. (sur.)	42.7 – 48.3	45.4 ± 2.1	6	a
(sur.)	32.3 – 41.2	37.0 ± 2.7	8	b
Fuchu, Tokyo (5 m)	31.6 – 35.2	—	2	b
Pacific Ocean (sur.)	43.6 – 45.4	44.7 ± 0.7	5	c
Enclosed housing air in the nitrification facility	21.7 – 23.1	22.4 ± 1.0	2	d
Soil gas	35.9 – 45.0	39.8 ± 4.7	3	a,e
Stackgas from coal-burning power plant (+NO_x, +SO_2)[1]	58.9 – 59.9	59.4 ± 0.7	2	a
Stackgas from waste incineration plant (-NO_x, -SO_2)[2]	26.9	—	1	b
Car exhaust (+NO_x, +SO_2)[1]	46.9	—	1	a
(-NO_x, -SO_2)[2]	15.2 – 19.4	17.3 ± 3.0	2	b
By-product in the manufacture of monomers for 6,6- and 6,12-nylon	16 – 26	—	NA	f
Dissolved N_2O				
Sea waters				
N. Pacific (0.2-5.0 km)	45.6 – 56.6	52.7 ± 4.4	5	c
N.E. Pacific (0.3-2.8 km)	45.3 – 57.3	53.0 ± 3.9	7	c
S. Pacific (0.3-4.9 km)	41.9 – 50.7	45.9 ± 3.6	7	c
Monterey Bay(sur.-1.0 km)	37.6 – 47.1	40.2 ± 2.0	11	b
Groundwaters				
Central Japan (0-250 m)	31.6 – 50.4	36.3 ± 5.1	13	b
New York State (0-216 m)	30.8 – 41.6	34.6 ± 3.0	29	b
Fresh surface waters				
Green Lake, New York	93.0	—	1	a

NA: non available

[1] NO_x and SO_2 were not removed at the sampling.

[2] NO_x and SO_2 were eliminated by conc. NaOH solution at the sampling.

Literature cited: a. Wahlen and Yoshinari [1985], b. This study,
c. Kim and Craig [1990], d. Yoshinari and Wahlen [1985],
e. Yoshinari [1991], f. Thiemens and Trogler [1991]

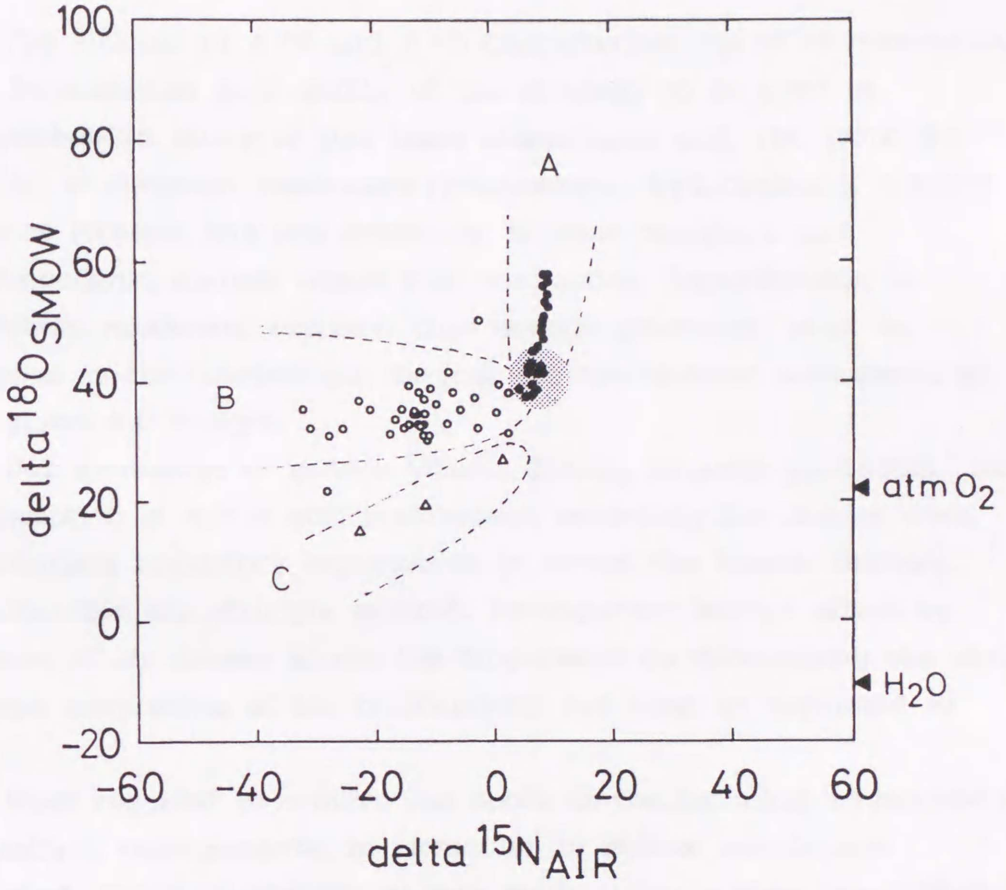

Figure V-20 $\delta^{15}N$-$\delta^{18}O$ map of N_2O from different environments. All data are listed in TABLE V-3 and V-4. Legend is as follows: Open circles-groundwater N_2O, Closed circles-oceanic N_2O, Open triangle-combustion N_2O, Shaded large circle-tropospheric N_2O. $\delta^{18}O$ in the atmospheric O_2 and the mid-latitude meteoric water are together shown.

CHAPTER VI

FUTURE PROBLEM

The success in $\delta^{15}N$ and $\delta^{18}O$ characterizations of environmental N_2O demonstrates good ability of the strategy to be used in biogeochemical study of this trace atmospheric gas. Yet, more data for N_2O in dominant reservoirs (troposphere, hydrosphere), natural sources (oceanic and soil emissions, biomass burning), and anthropogenic sources (fossil fuel combustion, byproduction in industrial processes, emission from sewage treatment) must be compiled in the two-isotopic diagram for the isotopic assessment of the global N_2O budget.

Our knowledge of isotope effects during biogenic production and consumption of N_2O is still insufficient, especially for oxygen ones. Appropriate laboratory experiments to reveal the kinetic isotopic fractionation are strongly awaited. An apparent isotope effect by mixture of air masses across the tropopause on determining the stable isotope composition of the tropospheric N_2O must be evaluated as well.

What required to achieve the above is the technical advancements in isotopic measurements, in particular to reduce sample size required. The best analysis in this study using a mass spectrometer accompanied with the micro-cold fingers still needs at least 1 μmole of N_2O, which means 100 liter of the tropospheric air and much more of the stratospheric air must be collected for the analyses. A direct measurement of without any conversion of N_2O to other compounds [Yoshida, personal communication] is probably the next stage to fill the requirement. That will enable us not only to simplify the handling of samples but also to be free from a risk of fractionation derived from the chemiccal preparations.

Provision of an internationally accepted N_2O standard for the isotopic measurements and intercalibration among different analytical techniques are the responsibility of our participants to establish the stable isotopic strategy [Yoshinari, 1991b].

APPENDIX 1 Data for Groundwaters in Japan Page 1/3

Station Code:	Date	Tw (℃)	SC (μS · cm^{-1})	pH	O_2 (μM)	$-\Delta O_2$ (μM)	N_2O (nM)	ΔN_2O (nM)	NO_3^- (μM)	Cl^- (μM)
J01:	8/30/88	16.3	ND	ND	ND	–	441	431	442	ND
	1/30/89	14.8	ND	ND	ND	–	475	465	561	552
	2/23/89	16.0	ND	ND	181	118	423	413	580	ND
	4/24/89	16.0	ND	ND	180	119	495	485	442	ND
	6/20/89	16.0	ND	ND	ND	–	393	383	ND	ND
	8/21/89	16.1	223	7.5	173	126	441	431	ND	ND
	10/23/89	16.0	ND	8.3	195	104	749	739	ND	ND
	8/ 2/91	17.0	242	8.6	183	110	355	345	375	496
J02:	8/30/88	17.5	ND	ND	ND	–	1080	1070	210	530
	1/30/89	15.0	ND	ND	ND	–	554	544	224	493
	2/23/89	16.3	ND	ND	75	222	1030	1020	234	ND
	4/24/89	16.3	ND	ND	157	140	836	826	218	ND
	6/20/89	16.3	ND	ND	ND	–	676	666	ND	ND
	8/21/89	16.7	178	7.8	27	270	945	935	ND	ND
	10/23/89	16.3	ND	8.2	77	220	1430	1420	ND	ND
	8/ 2/91	16.5	252	8.5	91	205	433	423	283	510
J03:	6/26/91	16.6	198	6.3	221	74	1240	1230	373	456
J04:	6/26/91	15.7	164	6.2	230	71	256	502	436	362
J05:	6/26/91	16.2	193	6.0	246	52	287	277	701	423
J06:	6/26/91	16.1	194	6.2	249	49	885	875	730	443
J07:	8/17/91	17.2	163	6.0	259	33	434	424	493	407
J08:	8/17/91	18.7	201	6.2	203	80	280	271	624	387
J09:	9/16/89	17.8	ND	6.5	165	123	69	60	ND	ND
J10:	10/10/89	16.2	188	7.6	220	78	119	109	ND	ND
J11:	10/08/89	16.6	810	ND	25	270	81	71	ND	ND
J12:	10/20/89	16.5	965	6.1	42	254	690	680	213	ND
J13:	10/20/89	14.3	402	6.1	24	286	1030	1022	274	ND
J14:	10/20/89	14.9	442	6.1	81	225	5280	5270	1079	ND
J15:	5/17/89	16.3	288	6.4	293	4	800	790	615	930
J16:	5/17/89	15.5	227	6.1	273	29	169	159	601	549

ND: Not determined.

APPENDIX 1 Data for Groundwaters in Japan Page 2/3

Station Code	Tw	SC	pH	O_2	$-\Delta O_2$	N_2O	ΔN_2O	NO_3^-	Cl^-
Date	(℃)	(μS · cm^{-1})		(μM)	(μM)	(nM)	(nM)	(μM)	(μM)
J17: 5/17/89	15.5	358	6.5	186	116	361	351	814	896
J18: 5/17/89	16.0	257	6.5	144	155	135	125	327	690
J19: see Apendix 2									
J20: 12/26/86	15.5	208	5.9	282	20	571	561	797	569
6/ 5/87	16.2	246	6.0	261	37	332	322	733	465
7/21/87	15.7	189	5.8	268	33	554	544	701	400
12/29/87	15.4	212	6.1	258	45	426	426	777	549
8/28/88	15.7	214	6.0	267	34	466	466	764	428
1/13/89	15.6	ND	6.0	248	53	565	565	783	ND
4/17/89	15.8	ND	ND	263	37	308	308	712	ND
7/ 1/89	15.6	ND	6.0	258	43	326	326	561	437
8/30/91	15.7	186	6.0	248	53	306	306	553	442
J21: 12/26/86	15.8	210	5.9	242	58	454	444	854	563
12/29/87	16.0	208	6.1	284	15	346	336	725	656
8/28/88	15.9	209	6.0	263	36	501	491	714	675
1/13/89	16.0	ND	5.9	258	41	595	585	815	ND
4/17/89	15.3	ND	ND	258	45	115	105	663	ND
7/ 1/89	15.8	ND	5.9	240	60	364	354	682	531
8/30/91	16.1	188	6.0	229	70	336	326	533	443
J22: 6/ 5/87	15.7	286	6.4	261	40	464	454	437	450
7/21/87	16.3	238	6.2	229	68	593	583	482	613
12/29/87	16.8	242	6.2	224	70	414	404	519	531
8/28/88	16.5	252	6.3	238	58	420	410	757	466
1/13/89	16.8	ND	6.4	204	90	699	689	465	ND
2/23/89	16.4	ND	ND	256	41	360	350	ND	ND
7/ 1/89	15.2	252	6.3	236	68	471	461	622	550
9/ 3/91	16.6	259	6.3	215	80	436	426	498	404
J23: 12/26/86	17.1	243	6.3	252	40	304	294	515	507
2/23/89	16.2	ND	ND	275	23	301	291	559	456
7/ 1/89	15.3	189	5.8	259	44	399	389	622	496
J24: 12/26/86	15.6	217	6.2	199	102	796	786	20	462
6/ 5/87	16.7	270	6.2	214	81	1410	1400	307	507
7/21/87	16.6	226	6.2	199	96	1210	1200	304	423
12/29/87	16.7	253	6.1	197	98	736	726	404	563
8/28/88	16.4	242	6.2	203	93	505	495	458	524
1/13/89	16.7	ND	6.2	259	36	1490	1480	414	ND
7/ 1/89	16.7	242	6.1	213	82	560	550	363	411
9/ 3/91	17.6	246	6.2	198	94	593	584	293	545

ND: Not determined.

APPENDIX 1 Data for Groundwaters in Japan Page 3/3

Station Code	Tw	SC	pH	O_2	$-\Delta O_2$	N_2O	ΔN_2O	NO_3^-	Cl^-
Date	(℃)	(μS · cm^{-1})		(μM)	(μM)	(nM)	(nM)	(μM)	(μM)
J25: 12/26/86	15.2	247	6.7	300	4	54	44	503	383
6/ 5/87	17.5	318	6.7	286	4	39	30	460	389
7/21/87	17.5	265	6.5	271	19	293	284	503	389
12/29/87	14.8	235	ND	302	5	30	20	465	456
8/28/88	16.3	266	6.8	290	7	180	170	568	546
J26: 10/ 8/89	14.7	325	ND	273	34	108	97	ND	ND
J27: 10/10/89	14.5	152	7.4	285	24	32	22	ND	ND
J28: 6/30/89	14.3	59	5.8	216	94	76	66	181	ND
J29: 10/20/89	11.4	225	7.4	252	78	490	478	1200	ND
J30: 10/10/89	12.7	970	6.5	288	33	7060	7049	2143	ND
J31: 5/17/89	19.9	342	7.3	253	24	153	144	261	620

ND: Not determined.

APPENDIX 2 Data of Periodical Observations at J19 Page 1/3

Date	Tw (℃)	SC (μS · cm^{-1})	pH	O_2 (μM)	ΔO_2 (μM)	N_2O (nM)	ΔN_2O (nM)	NO_3^- (μM)	Cl^- (μM)	Discharge (L · sec^{-1})
10/27/86	15.6	182	5.9	255	46	454	444	599	ND	ND
12/26/86	15.5	160	6.0	280	22	289	279	607	456	8.7
1/27/87	15.3	178	ND	282	21	250	240	624	434	5.3
2/ 3/87	15.3	175	ND	295	8	271	261	552	372	4.8
2/10/87	15.3	165	ND	280	23	282	272	623	445	7.6
2/17/87	15.3	167	ND	279	24	216	206	604	428	4.8
2/25/87	15.5	198	ND	283	19	422	412	596	445	5.1
3/ 6/87	15.5	172	ND	276	26	429	419	585	394	5.1
3/13/87	15.5	168	ND	287	15	424	414	590	439	6.5
3/24/87	15.2	177	ND	283	21	345	335	584	439	6.4
4/ 2/87	15.2	167	ND	285	18	455	445	587	468	6.4
4/15/87	15.2	179	ND	ND	–	428	418	620	485	5.7
4/22/87	15.5	179	6.1	281	21	305	295	597	518	5.5
5/ 1/87	15.3	163	ND	279	24	330	320	577	465	3.4
5/ 8/87	15.5	164	ND	288	13	309	299	565	462	.8
6/ 5/87	15.7	203	ND	60	40	261	251	544	470	4.9
6/10/87	15.7	177	6.0	276	25	273	263	557	423	5.4
6/17/87	15.6	173	ND	260	41	226	216	581	524	3.3
6/25/87	15.6	192	ND	249	52	200	190	580	490	4.5
7/ 8/87	15.5	172	ND	246	56	243	233	537	400	5.6
7/13/87	15.6	169	ND	272	30	247	237	549	456	6.7
7/21/87	15.8	169	6.0	255	45	128	118	482	389	7.0
8/ 5/87	15.5	172	ND	263	7	260	250	533	366	11.2
8/14/87	15.7	172	6.2	263	38	303	293	493	468	10.9
8/22/87	15.5	167	ND	265	37	232	222	481	417	10.1
8/31/87	15.6	178	ND	ND	–	254	244	467	456	11.0
9/ 8/87	15.7	185	ND	248	53	275	265	512	462	14.8
9/14/87	15.6	182	ND	274	27	293	283	542	451	12.5
9/22/87	15.5	189	ND	255	47	307	297	543	445	13.3
10/19/87	15.7	171	5.9	237	64	316	306	515	476	19.6
10/26/87	15.4	186	ND	249	53	313	303	470	372	20.1
11/ 2/87	15.7	187	ND	248	53	344	334	553	428	16.3
11/12/87	15.8	180	ND	252	48	329	319	538	428	17.2
11/16/87	15.6	163	ND	277	24	321	311	ND	ND	16.6
11/18/87	15.8	184	ND	254	46	289	279	567	468	14.0
11/25/87	15.6	179	ND	251	50	296	286	611	479	15.3
12/ 9/87	15.3	170	ND	ND	–	292	282	601	473	11.9
12/28/87	15.6	185	ND	257	45	301	291	605	479	7.3
1/ 6/88	15.5	165	ND	282	20	260	250	619	ND	7.1
1/12/88	15.4	179	ND	273	29	234	224	587	462	6.5

ND: Not determined.

APPENDIX 2 Data of Periodical Observations at J19 Page 2/3

Date	Tw (℃)	SC (μS · cm^{-1})	pH	O_2 (μM)	ΔO_2 (μM)	N_2O (nM)	ΔN_2O (nM)	NO_3^- (μM)	Cl^- (μM)	Discharge (L · sec^{-1})
1/19/88	15.3	179	ND	ND	–	245	235	600	468	6.6
1/27/88	15.1	173	ND	277	28	243	233	-	ND	6.2
2/ 5/88	15.5	166	ND	300	2	237	227	560	485	5.0
2/13/88	15.5	174	ND	283	18	227	217	509	425	4.3
2/18/88	15.6	171	ND	276	25	203	193	591	462	3.7
3/15/88	15.6	179	ND	280	22	214	204	586	465	3.1
3/22/88	15.2	171	ND	257	47	212	202	402	355	5.0
3/29/88	15.2	178	ND	287	17	226	216	555	428	6.7
4/ 6/88	15.3	181	ND	283	21	222	212	537	468	8.3
4/19/88	15.4	159	ND	280	22	236	226	410	423	7.9
4/26/88	15.7	176	ND	274	29	256	246	504	423	8.7
5/ 2/88	15.7	167	ND	274	27	274	264	398	423	10.3
5/10/88	15.7	168	ND	267	34	294	284	513	406	9.9
5/17/88	15.7	158	ND	263	38	301	291	494	439	9.3
5/24/88	15.7	178	ND	260	40	287	277	458	456	11.1
5/30/88	-	196	ND	258	43	293	283	540	377	11.1
6/ 7/88	15.7	161	ND	265	36	282	272	524	428	10.4
6/14/88	15.9	162	ND	241	59	293	283	513	465	11.3
6/21/88	15.9	183	ND	252	47	306	296	523	425	9.9
6/28/88	15.9	188	ND	261	38	303	293	523	448	12.0
7/ 5/88	5.7	172	ND	253	48	307	297	543	456	15.2
7/12/88	5.9	182	ND	236	64	299	289	525	428	13.3
7/19/88	5.7	178	ND	249	51	300	290	527	428	22.4
7/26/88	5.8	174	ND	246	54	275	265	511	439	19.4
8/ 3/88	5.8	-	ND	244	56	316	306	522	ND	20.2
8/12/88	5.9	172	ND	218	81	299	289	476	439	38.7
8/17/88	5.8	152	ND	248	52	293	283	489	394	53.0
8/26/88	6.0	158	ND	244	55	300	290	494	ND	37.1
8/30/88	5.8	157	ND	210	90	352	342	535	428	38.2
9/ 7/88	5.8	178	ND	232	68	312	302	583	468	32.7
9/13/88	5.8	182	ND	-	-	314	304	575	ND	27.6
9/20/88	5.4	166	ND	238	65	330	320	611	465	27.4
9/29/88	5.8	173	ND	243	57	324	314	511	ND	44.9
10/ 4/88	5.9	168	ND	-	-	325	315	531	434	43.8
10/13/88	5.8	176	ND	245	55	336	326	539	431	33.8
10/18/88	5.8	176	ND	236	64	361	351	565	ND	30.4
10/25/88	5.8	180	ND	233	67	511	501	565	ND	26.5
11/ 1/88	5.8	186	ND	248	52	518	508	563	451	19.4
11/ 9/88	5.7	173	ND	239	62	467	457	575	434	19.4
11/15/88	5.4	68	ND	243	60	473	463	616	445	21.1

ND: Not determined.

APPENDIX 2 Data of Periodical Observations at J19 Page 3/3

Date	Tw (℃)	SC (μS · cm^{-1})	pH	O_2 (μM)	ΔO_2 (μM)	N_2O (nM)	ΔN_2O (nM)	NO_3^- (μM)	Cl^- (μM)	Discharge (L · sec^{-1})
11/22/88	15.3	172	ND	240	63	550	540	576	448	18.2
11/29/88	15.2	162	ND	257	47	492	482	562	439	14.8
12/ 8/88	15.6	166	ND	258	43	508	498	616	437	10.2
12/14/88	15.8	172	ND	257	43	496	486	595	434	10.9
12/21/88	15.2	168	ND	256	48	504	494	573	434	8.6
1/12/89	15.6	ND	ND	264	37	394	384	593	ND	5.0
2/13/89	15.6	ND	ND	273	28	286	276	587	ND	ND
4/17/89	15.7	173	ND	239	62	249	239	534	ND	ND
5/15/89	15.7	ND	ND	263	38	340	330	538	ND	ND
6/12/89	15.7	ND	ND	257	44	297	287	531	ND	ND
6/20/89	15.6	ND	ND	ND	–	297	287	ND	ND	ND
7/18/89	15.7	ND	ND	244	56	318	308	484	ND	ND
8/21/89	15.6	ND	5.9	235	56				ND	ND
9/21/89	15.6	ND	ND	ND	–	397	387	537	ND	ND
10/17/89	15.6	ND	ND	223	78	492	482	529	ND	ND
7/29/91	15.7	ND	ND	238	63	206	196	554	283	4.3
8/30/91	15.8	159	6.0	238	62	257	247	470	347	4.8

ND: Not determined.

APPENDIX 3 Data for Groundwaters in New York State

Station Code: Date	Tw (℃)	SC (μS · cm^{-1})	pH	O_2 (μM)	$-\Delta O_2$ (μM)	N_2O (nM)	ΔN_2O (nM)	NO_3^- (μM)	Org.C (μM)
U01: 3/ 3/90						160			
U02: 5/ 2/90	11.5	735	6.5	190	140	572	560	227	32
U03: 5/ 2/90	11.5	1476	6.8	150	180	155	143	886	43
U04: 5/ 2/90	12.0	734	7.0	194	132	78	67	222	31
U05: 5/ 2/90	11.7	797	6.3	36	292	4320	4310	474	83
U06: 5/ 2/90	11.4	1253	6.6	147	183	1660	1650	412	64
U07: 5/ 2/90	13.5	1186	6.8	243	72	182	171	1643	58
U08: 5/ 2/90	10.8	938	6.4	230	105	701	689	1229	64
U09: 3/14/90						570		542	
U10: 3/14/90						908		907	
U11: 5/10/90	12.6	208	6.1	308	14	781	770	292	<16
U12: 5/10/90	11.8	229	5.9	245	83	385	374	366	<16
U13: 5/10/90	12.4	166	6.2	289	34	243	232	186	<16
U14: 5/10/90	12.0	456	6.0	228	98	1294	1283	561	18
U15: 5/10/90	12.2	460	6.2	248	76	1194	1183	519	16
U16: 5/10/90	12.8	198	6.4	193	127	1013	1002	299	<16
U17: 5/23/90	11.7	1360	7.1	294	34	155	144	121	51
U18: 5/23/90	10.6	857	7.2	73	264	125	113	321	67
U19: 5/23/90	9.0	699	7.7	153	197	315	302	165	92
U20: 5/23/90	8.5	596	7.1	198	156	149	136	96	150
U21: 3/29/90	12.2	261	6.2	281	43	337	326	397	ND
U22: 3/29/90	12.5	225	6.0	234	88	254	243	217	ND
U23: 3/29/90	11.3	96	6.3	247	84	145	133	91	ND
U24: 3/29/90	10.8	39	6.2	125	210	16	4	4	ND
U25: 3/29/90	11.5	91	6.1	272	58	265	253	258	ND
U26: 3/29/90	11.6	104	5.6	253	76	450	439	392	ND
U27: 3/29/90	11.3	56	5.8	266	65	137	125	152	ND
U28: 3/29/90	12.7	59	6.1	125	196	81	70	60	ND
U29: 3/29/90	12.0	159	5.5	219	107	308	297	294	ND
U30: 3/29/90	11.8	33	6.0	31	328	55	44	nd	ND

ND: Not determined.
nd: not detected.

APPENDIX 4 Data for Monterey Bay Water

Depth (m)	Temp. (℃)	Sal. (‰)	O$_2$ (μM)	AOU (μM)	N$_2$O (nM)	NO$_3^-$ (μM)	NO$_2^-$ (μM)	NH$_4^+$ (μM)	PO$_4^{3-}$ (μM)
0	–	33.500	287	-26	13.4	12.5	0.31	2.02	1.72
2	12.520	33.500	280	-20	13.7	6.1	0.27	0.63	0.91
5	12.510	33.500	281	-21	13.7	6.1	0.27	0.63	1.04
9	12.510	33.500	281	-21	13.9	6.9	0.30	0.93	1.05
11	12.340	33.510	276	-14	14.6	6.9	0.27	0.62	0.96
18	11.990	33.530	261	10	15.0	9.2	0.30	0.93	1.08
21	11.790	33.550	255	16	14.9	9.9	0.35	1.04	1.16
50	9.774	33.658	171	104	19.9	21.2	0.49	0.55	1.81
80	9.023	33.804	132	141	23.7	26.1	0.08	0.02	2.05
100	8.642	33.858	126	157	24.2	27.1	0.09	0.01	2.15
150	8.259	34.065	93	189	29.8	30.3	0.10	0.00	2.47
200	7.968	34.108	80	207	32.4	31.6	0.11	0.09	2.53
250	7.386	34.112	74	217	33.6	33.8	0.06	0.19	2.67
300	7.267	34.177	51	240	38.3	34.8	0.08	0.09	2.88
400	6.461	34.178	38	260	42.5	37.7	0.06	0.00	3.13
500	5.783	34.240	20	281	46.8	39.6	0.05	0.06	3.16
600	5.333	34.302	14	292	48.9	41.0	0.04	0.00	3.35
700	4.860	34.341	13	295	50.2	42.5	0.04	0.05	3.38
800	4.521	34.395	14	297	48.6	43.7	0.04	0.01	3.43
900	4.252	34.423	17	296	47.9	43.2	0.04	0.03	3.56
1000	3.840	34.450	22	283	49.8	43.6	0.04	0.00	3.50

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