THE BIOGEOCHEMISTRY OF NITROUS OXIDE

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CHAPTER I: INTRODUCTION

A) The Atmospheric N_2O Cycle

Nitrous oxide (N_2O) has a concentration of 300 parts per billion by volume (ppbv) in the atmosphere. N_2O has no known sinks in the troposphere and only minor variations (<1%) in concentration occur in the lower atmosphere (Hahn, 1979; McElroy, 1980). Recent data suggest that anthropogenic (industrial combustion, forest fires and agricultural) sources may be the cause of the observed secular increase in the concentration of atmospheric nitrous oxide (Weiss, 1981).

Figure I-1 shows the atmospheric nitrous oxide cycle. Concerns about anthropogenic nitrous oxide production have been twofold:

1) Nitrous oxide is the major source of nitric oxide in the stratosphere. NO enters a catalytic cycle that controls ozone levels in the stratosphere (Hudson and Reed, 1979, Figure I-2).

2) Absorption of infrared radiation by N_2O causes a greenhouse effect comparable to that of carbon dioxide, so that increases in the nitrous oxide content of the atmosphere should lead to global climate warming (Yung, Wang, and Lacis, 1976).

Anthropogenic nitrous oxide is not normally classified with nitrogenous pollution. Nitrogen pollutant gases (nitric oxide, nitrogen dioxide, and reaction products like peroxyacetylnitrate) undergo smog-producing photolytic reactions. They can cause respiratory distress in animals and humans, kill vegetation and limit crop yields (NAS, 1977), but they are oxidized on a short time scale (hours-days),

producing nitric acid aerosols, a major component of "acid rain". Nitric acid has been implicated in the low pH of rain, which appears to have killed off fish in lakes across the Canadian Shield (NAS, 1978) and in Norway. Contrary to the other nitrogen pollutant gases, nitrous oxide is unreactive in the lower atmosphere. Because of its long residence time in the troposphere, it is lost primarily by slow transport across the tropopause, followed by photolysis at wavelengths less than 2400 Angstroms, or by reaction with odd oxygen atoms in the stratosphere (McElroy, Wofsy and Yung 1977; Hudson and Reed, 1979, Figure I-2). N_2O transported to the stratosphere tends to deplete ozone, thus causing an increase in the intensity of biologically damaging ultraviolet radiation at the earth's surface (NAS, 1978).

B) N_2O in the Biological Nitrogen Cycle

It is impossible to predict or understand changes in atmospheric N_2O unless its role in the environmental nitrogen cycle is well understood. Nitrogen is the element which most widely limits biological productivity in terrestrial and marine ecosystems (Svensson and Soderlund, 1976; NAS, 1978; Nielsen and MacDonald, 1978). The global nitrogen cycle has been perturned by high levels of nitrogen fertilizer additions in modern agriculture (McElroy, Wofsy, and Yung, 1977), and by accelerated losses of soil nitrogen in Western commercial agricultural practices (Commoner, 1972).

The global nitrogen cycle is shown in Figure I-3 (after McElroy, 1976). Figure I-4 shows the currently accepted role of N_2O in the nitrogen cycle. Until recently denitrification was believed to be the major source of the gas in the environment, and considerable efforts

were made to measure nitrous oxide production in order to quantify denitrification. However, evidence has mounted in recent years that nitrous oxide plays a more complicated role in the nitrogen cycle than previously believed:

1) Aerobic production in the ocean

Nitrous oxide production occurs readily in aerobic habitats where denitritrification cannot proceed. In aerobic waters nitrous oxide bears a relation to nitrate and oxygen concentrations which suggests that a small but quite constant fraction (0.2 to 0.3%) of ammonia nitrogen released in biogenic decomposition and oxidized to nitrate is released as nitrous oxide. This pattern is observed in the North Atlantic (Yoshinari, 1976), the Eastern Pacific (Cohen and Gordon, 1978), and the Central Pacific (Elkins, 1978).

2) Aerobic production on land

Nitrous oxide production takes place in aerobic soil incubations (Freney, Denmead, and Simpson, 1979) and in well aerated agricultural soils (Bremner, Robbins, and Blackmer, 1980). Fields fertilized with nitrogen produce most nitrous oxide during the period when NH_4^+ is oxidized to nitrate (Breitenbeck, Blackmer and Bremner, 1980).

3) Anaerobic consumption

Anoxic waters with depleted nitrate and nitrite are undersaturated with nitrous oxide (Elkins, et al., 1978; Cohen and Gordon, 1978; Cohen, 1978). Denitrification thus seems to be a sink for nitrous oxide, rather than a source, in these systems. The large free energy change provided by using N_2O as a terminal electron acceptor provides the impetus for denitrifiers to consume the N_2O available to them.

D) Plan of the Thesis

In this thesis the bigeochemistry of nitrous oxide is examined from several viewpoints. In Chapter II, aerobic microbiological nitrous oxide production is studied using pure cultures of bacteria and algae. In Chapter III data on the spatial and temporal patterns of nitrous oxide production in a forest ecosystem are presented. Finally, the effects of human activity in increasing the N_2O burden to the atmosphere are discussed.

CHAPTER II

Synopsis

This chapter presents data on aerobic microbiological production of nitrous oxide. It is shown that N_2O is produced during normal growth of cultures of autotrophic nitrifiers as a constant fraction (0.3%) of the ammonium oxidized to nitrite. N_2O production yields (relative to NO_2^-) were similar in every species tested. While cell growth rate is virtually independent of oxygen, at low oxygen pressures, nitrite production declines, cell metabolic efficiency increases and N_2O production increases sharply. These results support inferences from field data that nitrification is a major global source of atmospheric N_2O, and that the oxygen concentration of the habitat is a critical controlling parameter.

Nitrite oxidizers are shown to produce only trivial amounts of N_2O. No N_2O is produced during the process reverse of nitrification, i.e., assimilatory reduction of nitrate and nitrite to ammonia by primary producers such as algae. Heterotrophic nitrifiers incapable of ammonium oxidation are found to produce N_2O as a major product during oxidation of hydroxylamine derivatives to nitrite .

Three possible biochemical processes for nitrous oxide production are assessed:

a) that N_2O is a true intermediate; b) that it is derived from a reductive pathway; and c) that it is produced from the oxidation of hydroxylamine derivatives. It is proposed that the last of these possible processes is most consistent with present results and with previous data on the biochemistry of nitrifiers.

Nitrous Oxide Production by Microorganisms

1) Introduction

Yoshida and Alexander (1970, 1971) first observed the production of N_2O by cultures of the nitrifying bacterium Nitrosomonas europea, a chemoautotroph which obtains its metabolic energy by oxidizing NH_4^+ to NO_2^-. There is a growing body of evidence from field studies to suggest that nitrification may be an important source of N_2O in both soils and aquatic systems (Breitenbeck, Blackmer, and Bremner, 1980; Bremner and Blackmer, 1978; Cohen and Gordon, 1978; Cohen and Gordon, 1979; Elkins, Wofsy, McElroy and Kaplan, 1980; Elkins, Wofsy, McElroy, Kolb and Kaplan, 1978; Hahn, 1974; Yoshinari, 1976).

In this chapter I present studies of N_2O production by cultures of nitrifying bacteria, with particular attention to the influence of the O_2 level maintained in the culture. A major fraction of the nitrifying activity in estuaries, streams, and lakes occurs in sediments and in biological films attached to detrital material (Curtis, Durrant, and Harman, 1975; Mortimer, 1941, 1942; Tuffy, Hunter and Matulewich, 1974). In these environments nitrification typically occurs at low concentrations of O_2 and high concentrations of NH_4^+, conditions similar to those maintained in the cultures. Field studies in aquatic systems (Elkins, et al., 1980; Elkins et al., 1978; Kaplan, Elkins, Kolb, McElroy, Wofsy and Duran, 1978; McElroy, Elkins, Wofsy, Kolb, Duran and Kaplan, 1978) suggest that low oxygen or high ammonia concentrations (or both) may enhance production of N_2O during nitrification. Similar behavior in agricultural soils may be inferred from recent work by Breitenback, Blackmer, and Bremner (1980), Bremner and Blackmer (1978) and Freney, Denmead and Simpson, (1978, 1979).

2) Materials and Methods

1) Analytical methods

A) N_2O

Gas samples to be analysed for nitrous oxide content are introduced into a 3 ml sample loop. An in-line trap at dry ice temperature removes water vapor. After the sample loop is flushed with more than 20 ml of sample gas at ambient pressure, the loop contents are injected into the column by means of a six-way Carle switching valve. The flow rate of the carrier gas (4% methane in argon) is 30 ml/min. The sample is separated on a column of Porapak Q maintained at 45°C in a thermostatted oven. The gases then flow through a Ni^{63} electron capture detector maintained at 350°C. The gas chromatogram permits determination of the volumetric proportions of nitrous oxide by comparison with standards of known concentration. Since the water vapor content of the gas samples varies with temperature and relative humidity, nitrous oxide is consistently expressed as parts per billion by volume in dry air (Elkins, 1978). Commercially prepared standard gases calibrated in our laboratory are injected from the same sample loop, and are run frequently during sample analysis. The standard deviation of replicate standards, and of replicate air samples, is between 0.5 and 1%. The apparatus used is illustrated in Figure II-1.

B) Nitrite

Nitrite was analyzed by the Griess-Illosvay method (Stainton, Capel and Anderson, 1977), with volumes scaled down by a factor of 10. The sample was stirred on a vortex mixer, 0.1 ml of a solution (containing 5 gm of sulphanilamide in 100 ml of concentrated hydrochloric acid and 800 ml of distilled water) were added and mixed. Within 5 minutes, nitrous acid (i.e. nitrite in solution) (HNO_2, pK=3.36) reacts with sulphanilamide to produce a diazonium salt. After 10 minutes 0.1 ml of a solution containing 0.5 gm of NNED (N-1-naphthylethylenediamine dihydrochloride) in 500 ml of distilled water was added and mixed. NNED reacts with the diazonium salt to produce an azo dye whose absorbance is read in spectrophotometer at a wavelength of 543 nanometers. Reagents were stored in a refrigerator. Fresh secondary standards were made every day, and no drift was seen in their absorbance. Replication of standards was within 1%. Blanks gave negligible readings. Both standards and blanks were run frequently.

C) Nitrate

Nitrate was analyzed by reduction to nitrite on a copper-coated cadmium column in a scaled down version of the method described in Stainton, Capel and Anderson (1977).

D) Cell Numbers

One ml of sample was preserved with 0.1 ml of formalin. The sample was filtered and stained with Acridine orange dye according to the method of Hobbie et al. (1977). Counting of cells was done in triplicate by Dr. Warren Kaplan in the haemocytometer of an epifluorescence

microscope. The purity of bacterial cultures was verified by streaking samples from each flask onto several sterile plates coated with Marine Agar 2216 (Difco) or its freshwater equivalent. Plates were examined for heterotrophic bacterial growth after four to seven days. The initial inoculum on a few occasions was contaminated and the experimental data discarded. In experiments with heterotrophic bacteria the optical density of the culture was measured at 543 nm.

II Equipment and Procedures

A) Flasks

Three-arm distillation flasks (550 ml) containing growth medium were incubated in a water bath maintained at 26±1°C by a thermostatted heater (Figure II-2). The flask contents were stirred magnetically to ensure rapid equilibration between gas and fluid phases. The central arm contained a fritted glass gas disk through which the medium was slowly and uniformly ventilated. The third arm contained the outflow tube leading to the gas chromatograph as well as a long syringe needle inserted into the medium. The needle was sealed off by a three-way stopcock. This arrangement allowed sampling of fluid for chemical or microbiological assays while keeping the growth medium isolated from the atmosphere. Sterile glass wool filters were installed upstream and downstream of the flask. The whole apparatus, including growth medium, was sterilized in an autoclave for 15 minutes. After cooling, the flask was hooked up to the flow lines and purged overnight with filtered, N_2O-free gas to eliminate all traces of atmospheric nitrous oxide. The system was leak tested and the outflow of each flask was analyzed for N_2O before the initiation of each experiment. N_2O was not detected prior to inoculation except when one commercially prepared gas tank was used. This tank was found to

contain 15 ppbv of N_2O and a background correction was therefore made.

B) Carrier Gas Flushing system

Gas from source tanks flowed to a three or six line manifold. Each line had its own flow controller, which regulated gas flow to a precision better than 1% over a two week period. Flow rates were regularly measured during each experiment using a bubble flowmeter attached to the outlet of each flask. Flow rates were adjusted to about 0.25 cm^3 sec^{-1} to give a gas residence time in the flask of about 20 minutes. The outflow could be connected to a dry ice trap which led into the sampling loop of the gas chromatograph.

Gas with mixtures of oxygen in argon, containing atmospheric levels of carbon dioxide (circa 330 ppm), were obtained from commercial suppliers or were prepared in the lab using a mixing manifold and tanks containing oxygen and nitrogen containing 330 ppm carbon dioxide. Accuracy of mixing was checked by analysis of oxygen, nitrogen, and carbon dioxide using a gas chromatograph equipped with a thermal conductivity detector. Gas standards consisted of mixtures prepared commercially and calibrated in our laboratory.

C) Sampling procedures

At each sampling interval the gas chromatograph column oven was cooled down from the temperature at which it was baked out between analyses (baking temperature 120°C, operating temperature 45°C). Repeated standards were run to check reproducibility. The gas outflow from each flask flushed the trap and sample loop for about 5 minutes before injection. Flow rates were measured in triplicate. Fluid samples were

obtained by opening the three way stopcock and withdrawing 0.3 ml of solution into a sterile syringe. This first sample was discarded through the outlet to flush out the syringe dead space. A 0.2 ml sample was subsequently withdrawn into the syringe and ejected into a small, clean pyrex flask. A 50 µl subsample was withdrawn from the flask with an automatic Eppendorf pipette, introduced into a clean pyrex test tube, diluted with 4.95 ml of distilled water, and analyzed for nitrite. Samples were periodically withdrawn and preserved with formaldehyde for Acridine orange cell counts. Water temperature and pH were measured. Sampling intervals ranged from three to eight hours around the clock. Glassware was washed in distilled water.

D) Growth media

Nitrifier growth medium was obtained from Stanley Watson and Frederica Valois in the Department of Biological Oceanography at the Woods Hole Oceanographic Institution, Woods Hole, Mass. This medium has been widely used for enrichment cultures of nitrifying bacteria in soils, sediments and water, and for experiments on growth, physiology, and biochemistry. It contains all of the essential minerals required by nitrifiers. The composition of the medium was as follows: (Watson, 1965):

$(NH_4)_2SO_4$	13.2 g
$MgSO_4.7H_2O$	200 mg
$CaCl_2$	20 mg
K_2HPO_4	114 mg
Chelated Iron (Sequestrene, 13%Fe)	130 µg Fe

$Na_2MoO_4.2H_2O$	1 µg
$MnCl_2 \cdot 4H_2O$	2 µg
$CoCl_2.6H_2O$	2 µg
$CuSO_4.5H_2O$	20 µg
$ZnSO_4.7H_2O$	100 µg

dissolved in one liter of either distilled water (for freshwater nitrifier cultures), aged, filtered Sargasso Sea water (for open ocean cultures), or an equal mixture of the two (for nitrifiers from estuarine and coastal waters).

A 5 liter batch of medium in a carboy was buffered prior to each experimental run by the addition of HEPES to bring it to 0.05 molar. HEPES (N-2 hydroxylethyl-piperazine-N'-2-ethane sulfonic acid) is a non-nitrogeneous buffer which does not markedly affect nitrifier growth (F. Valois and S. Watson, personal communication). The pH was titrated to 7.50 (the pK of Hepes at 25°C, and the pH of its maximal buffer capacity) with NaOH. 300 ml of buffered medium were used in each flask. Pure batch cultures of nitrifiers were maintained at 25°C, and at a pH of 7.5 by F. Valois in the laboratory of Dr. S. Watson. Prior to each experiment a sufficient volume of the culture was centrifuged in a sterile tube, resuspended in fresh medium and recentrifuged to yield a moderately dense culture which was transported to the lab at Harvard in an ice chest. An 0.5 ml inoculum was introduced into each flask via the sterile three way stopcock to initiate the experiment.

III. Results

A) N_2O and NO_2^- production are linked

Parallel production of nitrous oxide and nitrite was observed in all experiments with autotrophic nitrifiers. Control flasks in which growth was blocked with mercuric chloride (a decoupler of electron transport) produced no nitrite or nitrous oxide. Figure II-3 shows a triplicate set of experiments with the marine bacterium Nitrosoccus oceanus in which exponential curves have been least-square fitted to early portions of the cumulative production curves. Parallel production is indicated by similar slopes of the curves. As nitrite levels rise, the rate of production of both nitrite and nitrous oxide slows by about the same amount.

As production rates per cell decline, the nitrous oxide yield (defined as molves of nitrogen as N_2O produced divided by the moles of nitrite produced) remains quite constant (see Figure II-4). Figures II-5 to II-8 show representative N_2O head space concentrations, nitrite concentration in the media, and cell numbers for the yield curves of Figure II-4. The yield is found to be independent of cell density and independent of nitrite concentration from micromolar to millimolar levels (Figure II-6).

B) Nitrifiers grow well at low oxygen levels

Figure II-7 shows bacterial growth rates versus oxygen tension in the carrier gas. Growth did not vary more than 30% over the entire oxygen range between 0.5 and 20%. A weak maximum was found at 1% oxygen levels.

C) All genera of nitrifiers have comparable yields

Runs were made for species from every genus of autotrophic ammonium oxidizers listed in the standard taxonomic work (Watson, S.W., 1974). All showed N_2O yields between 0.1 and 0.5% (Figure II-8). The species tested at PO_2=0.2: Nitrosococcus oceanus (marine), Nitrosomonas marina (marine and estuarine), Nitrosomonas europea (freshwater and soil), Nitrosolobus multiformis (freshwater and soil), and Nitrosospira briensis (freshwater and soil), thus appear to have analogous product-yielding pathways. The only genus not tested was the recently discovered Nitrosovibrio (Harms, Koops, and Wehrmann, 1976). Representative runs of three soil and freshwater nitrifiers are shown in Figures II-9 to II-11.

D) Oxygen stimulates nitrite production

Nitrite production rate per cell increases non-linearly about seven times as oxygen increases from 0.5% to 20% (Figure II-12). The cellular growth yield, defined as the number of cells produced per mole of nitrite, varies inversely with oxygen, increasing at the lower oxygen levels (Figure II-13). Greater metabolic efficiency at low oxygen levels is not in accord with a simple thermodynamic model of growth in which metabolic efficiency is proportional to oxygen. This suggests that oxygen detoxification may be a significant energetic pathway in these organisms.

These results show that cell counts of nitrifying bacteria do not provide a meaningful measure of nitrification rates unless oxygen levels are specified. The ratio of nitrogen oxidized to carbon assimilated changes as a function of the oxygen concentration. Thus, assays such as the N-Serve inhibited dark uptake of HCO_3^- (Billen, 1976; Somerville, 1978)

(which assumes constant N to C ratios) may be inaccurate at low oxygen tensions.

E) Oxygen inhibits production of nitrous oxide.

Figure II-14 shows that at higher oxygen concentrations less nitrous oxide was produced per cell, the value decreasing as oxygen increases from 0.5 to 20%. Although N_2O/NO_2^- production yield rises sharply at low oxygen levels (Figure II-15), increases of N_2O production per cell with declining oxygen are smaller than the concommitant decline in nitrite production. The yield becomes quite constant (0.3%) for O_2>10% of the flushing gas.

F) Inhibitor results

Flasks containing 50 ml of sterile nitrifier medium were sealed with septum caps and autoclaved. The flasks received combinations of the following: a) inoculum: 10 ml of a moderately dense culture of Nitrosococcus oceanus; b) mercuric chloride: 1 ml of a saturated solution; c) N-Serve: 0.5 ml of commercial solution; d) hydrazine: 1 ml solution to bring its concentration to 10^{-3} molar; 3) thiourea: 1 ml solution to bring its concentration to 10^{-4} molar; f) acetylene: 1 ml solution to bring its concentration to 10^{-2} molar. All experiments were run concurrently.

Figure II-16 shows results from flasks inoculated with cells and medium alone, from flasks with nothing but medium, and from those with cells, medium and mercuric chloride. Initial N_2O values are somewhat less than atmospheric (300 ppbv) because of partial degassing of the medium during autoclaving. No N_2O was produced by the medium alone or by cells killed by mercuric chloride. The decline in N_2O

concentration in these cases results from replacement of the sampled headspace with N_2O-free gas during sampling.

Figure II-17 shows the effects of N-Serve and thiourea treatments. All cells inhibited with N-Serve behaved identically with uninoculated or killed controls. N-Serve did not generate N_2O by chemical reaction. It completely blocks nitrous oxide production. Acetylene behaved in identical fashion (W. Kaplan, personal communication). Addition of thiourea to growing cells did not inhibit nitrous oxide production, and there was no chemical production of nitrous oxide from thiourea. N-Serve, thiourea, and acetylene block nitrite production from ammonia (Billen, 1976; Mahli, Cook, and Nyborg, 1979; Hynes and Knowles, 1978). The results suggest that thiourea may act at a later point in the oxidation than do the other two inhibitors.

Figure II-17 also shows results of hydrazine treatments. Hydrazine reacted chemically with the medium, increasing nitrous oxide concentration by 150 ppbv in 36 hours. Much larger amounts of nitrous oxide were produced when hydrazine was added to either live or mercury-killed cells. Hydrazine appears to react chemically with some component of the cell to produce nitrous oxide. It has been suggested that hydrazine blocks nitrite formation but not hydroxylamine decomposition, by channeling nitrogen into nitrous oxide (Yoshida and Alexander, 1971). This suggestion is refuted by our data, since hydrazine exerts its effect on dead cells.

G) Assimilatory nitrate-nitrite reduction does not release N_2O

A pure culture of the open ocean dinoflagellate Exuviaella procentrum (No. A312, from $42^{o}6'N$, $68^{o}4'W$) was grown in Guillard's f/2 medium containing nitrate as the only nitrogen source. Medium and culture were provided by Dr. Larry Brand in Woods Hole. The experiment was performed in a closed flask sealed with a silicone rubber stopper, containing 150 ml of medium and 150 ml of air. The culture was sampled periodically, via a port, for nitrate and nitrous oxide. Visual observations showed increasing chlorophyll content, and microscopic examination of the culture two days after termination of the experiment showed that the alga had grown to maximal densities.

Nitrate levels declined during a one week period from 598 μM/L to 454 M/L as algae took up nitrate for reduction to amino compounds. During this time nitrous oxide concentrations in the flask showed no change (Figure II-18). Assimilatory nitrate reduction by Exuviaella does not appear to generate N_2O.

H) Nitrite oxidation is not a significant source of N_2O

Pure cultures of the freshwater nitrifier Nitrobacter Winogradskii and a marine species of the same genus were provided by S. Watson and F. Valois and incubated in sealed flasks. Samples were withdrawn periodically by syringe for analysis of nitrous oxide. The concentrations of nitrate and nitrite were analyzed at the end of the experiment. Only small amounts of nitrous oxide were produced (see table). Most of the nitrite in the medium was oxidized to nitrate, and the nitrous oxide yield was about $2x10^{-7}$, a thousand fold less than that of ammonium oxidizers. Most of the N_2O was produced toward the end of the experiment, when

oxygen concentrations may have approached zero.

Concentration of N_2O in Headspace (ppb)

Time (days)	Nitrobacter Winogradskii (freshwater)	Nitrobacter marina (seawater)
0	300	300
14	396.9	429.9
40	452.1	1179.0
60	808.8	1036.2
after 60 days NO_2^- (mM/L)	20.27	18.34
NO_3^- (mM/L)	90.82	90.77

I) Heterotrophic nitrifiers produce large amounts of N_2O

Bacillus cereus, a heterotrophic bacterium isolated from the human upper gut, was found by Reinaldo Gomez and Barbara Richardson in the Nutrition and Food Sciences department at M.I.T. to be capable of oxidizing acetohydroxamate to nitrite.

Cultures of these bacteria, provided by the M.I.T. group, were grown at 37°C in 1% yeast extract with 1% M-9 Buffer (Na_2HPO_4, KH_2PO_4, NaCl, $MgSO_4.7H_2O$). 0.2% NH_4HCO_3 and/or 0.2% acetohydroxamic acid were added to some flasks. Optical densities of the cultures are shown in Figure II-19. The cultures growing in the flask with no additions was the first to reach peak optical density, followed by those in flasks to which ammonia had been added, and then by those to

acetohydroxamate had been added. The slowest growing culture was in a flask to which both ammonia and acetohydroxamate had been added.

Flasks in which cells grew in anamended media and flasks in which cells grew with only ammonia added showed no nitrous oxide or nitrite production. The bacteria appear to be incapable of ammonia oxidation.

Figure II-20a and b show results of flasks with added acetohydroxamate. The following sequence of events is observed: a) before inoculation the medium contained 30 mM/L of nitrite, and small amounts of nitrous oxide were generated (first point); as no nitrous oxide production was observed in flasks to which acetohydroxamate was not added, a chemical reaction of the latter compound appears to generate N_2O; a possible reaction is:

$$R\text{-}C(=O)NHOH + HNO_2 \rightarrow R\text{-}C(=O)OH + N_2O + H_2O$$

b) during the lag phase no nitrite was produced or consumed, but nitrous oxide production increased steadily; c) at the initiation of the logarithmic growth phase the initial nitrite in the culture was suddenly and completely consumed and nitrous oxide production rates fell to zero; throughout the log phase nitrite concentrations and nitrous oxide production rates remained at zero; d) after maximal optical densities were reached, both nitrite and nitrous oxide suddenly began to be produced; nitrite levels rose rapidly at first, slowed, and came to a halt at a constant concentration as cell optical densities declined and a stationary phase was reached; e) as nitrite production slowed, nitrous oxide production declined, and when nitrite concentrations levelled off, no more nitrous oxide was produced; production of the two compounds thus appears to be closely linked.

The flask treated with both acetohydroxamate and ammonia produced similar results as those treated with acetohydroxamate alone (Figure II-20c). Once again, nitrous oxide was made by a chemical process in sterile media (first point), but its rate of production was greater in the presence of ammonia. As soon as nitrite was taken up by the cells the reaction ceased.

In these experiments heterotrophic nitrifiers that are incapable of ammonia oxidation but are capable of oxidizing to NO_2^- organic derivatives of hydroxylamine produce nitrous oxide as a major product (yields 25% to 50%).

Discussion: O_2, N_2O, and Nitrification

Earlier studies (Carlucci and McNally, 1960; Carlucci and Strickland, 1968; Gunderson, Carlucci and Bostrom, 1966) indicated that Nitrosomonas europea and Nitrosococcus oceanus could grow at oxygen tensions as low as 1% of atmospheric. These studies utilized solid media or liquid media containing particulate $CaCO_3$, and it was difficult to control oxygen tension and pH. Total uptake rates of ^{14}C were consistently faster at low oxygen (Carlucci and McNally, 1960; Gunderson, Carlucci, and Bostrom, 1966), whereas production of nitrite was most rapid at oxygen levels near atmospheric (Gunderson, Carlucci, and Bostrom, 1966). In the present work, cell growth rates were slightly larger at reduced concentrations of oxygen, but cultures generated much less nitrite (per cell) during growth. This finding is consistent with earlier results in both carbon-14 uptake and nitrite production and with the original observations of ZoBell (1935).

Loveless and Painter (1968) studied the growth of Nitrosomonas europea at low oxygen tension. Painter (1970) used these data to estimate a Michaelis-Menten half saturation constant of 0.3 mg/liter for oxygen (the concentration at which the growth rate is reduced to half of its high oxygen value). Our data would appear to imply a slightly lower value (ca. 0.15 mg liter). However, the growth rates do not appear to fit a simple model with Michaelis-Menten kinetics, since a decline in the rate appears to occur at higher levels of oxygen.

Our values for the number of cells produced per mole of ammonium oxidized to nitrite (0.65×10^6 cells per micromole) may be compared with earlier results for Nitrosomonas europea (Belser and Schmidt, 1980; Engel and Alexander, 1968; Painter, 1970) and Nitrosococcus oceanus (Watson, 1965), about 1.0×10^6 and 0.5×10^6 cells per micromole, respectively. These cell yields are somewhat lower than those recently reported by Belser and Schmidt (1978) for soil organisms (ca. 5×10^6).

Yoshida and Alexander (1970) observed yields of nitrous oxide between 2 and 25% relative to nitrite during 3 hour incubations of N. europea (10^9/ml) in sealed Warburg flasks. Most of the nitrous oxide had appeared by the time of the first observation, one hour after inoculation. The present studies indicate much lower yields of N_2O from nitrifying organisms including N. europea, except at very low concentrations of oxygen. (Cultures contaminated with Fusarium sp. were observed to produce large quantities of N_2O). At the start of each experiment I observed significant N_2O generation by the inoculum during preparation. This N_2O appeared in flasks with both live and killed cultures and was flushed out by the gas stream in about 0.5 hr. Subsequent production of N_2O paralleled production of nitrite with the ratios shown in the

figures. It is possible that some of the N_2O observed by Yoshida and Alexander may have been produced during preparation of the cells, and that the dense cultures which they employed might have consumed the oxygen in the culture medium faster than it could infuse from the head space. The high yields they observed would in this case reflect the enhanced yield of N_2O at reduced levels of oxygen.

The present results support the view that nitrification is a major source of N_2O in natural systems. Nitrification can proceed in environments where oxygen concentrations are very low, for example in organic rich sediments. Under micro-aerophilic condition copious amounts of nitrous oxide may be produced.

Human activities create high concentrations of oxygen demanding substances in a variety of environments, such as polluted waters, heavily fertilized soils, or areas where live-stock are maintained. Oxidation of reduced nitrogen from the decomposition of organic matter or from fertilizers is usually accomplished by nitrifying bacteria. Thus, human perturbations to the environment may increase the global rate of nitrification at low oxygen tension. The result could be the release of globally significant quantities of nitrous oxide, the rate of N_2O release may increase nonlinearly should the accumulation of oxygen demanding wastes also increase.

Mechanisms of Nitrous Oxide Production

Kluyver and Doncker (1926) and Corbet (1934, 1935) proposed that the oxidation of NH_4^+ to NO_2^- proceeds via two-electron steps. An intermediate at the redox state of hydroxylamine (-1) and one at the redox state (+1) of hyponitrous acid ($H_2N_2O_2$), nitroxyl (NOH), or nitrous oxide (N_2O)

would be expected. Two separate enzymes are now known to be involved: a membrane-attached ammonia oxidase which releases hydroxylamine, and a soluble hydroxylamine oxidase which releases nitrite and which contains the entire electron transport chain (Hooper, 1978). N_2O (as well as hyponitrous acid) cannot be taken up and oxidized to nitrite (Falcone, Shug and Nicholas, 1962, 1963; Anderson, 1963, 1964a,b, 1965a,b,c). Consequently N_2O does not appear to be a true intermediate. N_2O and NO are produced under anoxic conditions by the purified NH_2OH oxidase (Falcone, Shug, and Nicholas, 1963). N-15 labelling shows that these gases are largely derived from nitrite (Ritchie and Nicholas, 1972; Hooper and Terry, 1979). In the absence of oxygen, nitrite may substitute as a terminal electron acceptor, being reduced to NO, N_2O, or N_2. This "denitrifying" pathway does not appear to provide energy to the cell since nitrifiers cannot grow under such conditions. If nitrite were a competitor with oxygen as an electron acceptor then the production of N_2O should depend on the ratio of nitrite to oxygen. The fact that nitrous oxide production is independent of nitrite concentrations from levels as low as a few micromolar to eight millimolar (Figures II-9) argues against this possibility. The latter levels vastly exceed nitrite levels in most nitrifying environments. Several lines of evidence suggest an alternative pathway of N_2O production:

1) Ammonia oxidizers are the only organisms prominent in nitrogen redox transformations in which hydroxylamine is a free intermediate. It is known not to be an intermediate in assimilatory and dissimilatory nitrite reduction (Hewitt, 1975; Cole and Brown, 1980; Yoshinari, 1980).

2) While nitrifiers can oxidize NH_2OH to NO_2^-, N_2O and NO the amount of NH_2OH consumed exceeds that of the known products (Nicholas and Jones, 1960; Anderson, 1965; Hooper and Nason, 1965; Rees, 1968a, b; Yoshida and Alexander, 1970; Hooper and Terry, 1979).

3) NH_2OH reacts rapidly with amides, ketones, and ketoacids to make substituted hydroxylamines (Roberts and Caserio, 1965; Csaky, 1948).

4) NH_2OH substitutes readily for ammonia in enzymatic aminations (Wallace and Nicholas, 1969; Elliott, 1948; Waelsch, Borek, Grossowicz and Schou, 1950; Stumpf and Loomis, 1950; Grossowicz, Wainfan, Borek and Waelsch, 1950; Virtanen and Jarvinen, 1951).

5) The products of hydroxylamine reactions (enzymatic and non-enzymatic) with major metabolites are hydroxamic acids and oximes (Figure II-21). These compounds are known to produce N_2O by a variety of reactions including spontaneous oxidations (Smith, 1965).

6) The microbial oxidation of hydroxamates has been observed to produce large amounts of N_2O (previous section).

We propose here that N_2O (and perhaps NO) are derived from the oxidation of hydroxylamine derivatives. This view appears more consonant with the evidence than the competing hypotheses.

CHAPTER III

SYNOPSIS

The bulk of atmospheric N_2O is derived from land (McElroy, 1981). While previous studies have examined N_2O production from agricultural soils, few data are available from undisturbed ecosystems. This chapter presents a study of the annual cycle of nitrous oxide emissions from an unperturbed ecosystem, the watershed 6 of the Hubbard Brook Experimental Forest in New Hampshire. The area studied is the site of a 16 year biogeochemical monitoring program. Nitrogen has been previously shown by input-output analysis to be tightly cycled in the ecosystem, but the balance is readily disturbed by perturbations such as timber harvesting, which greatly enhance nitrate loss from the soil. In the course of this program, nitrous oxide evolution from the forest floor occurred almost entirely during spring and early summer and declined to low values during the rest of the growing season. The flux of N_2O was highly variable spatially and temporally, and was sensitive to the moisture content of the soil. Production occurred in well aerated soils, and minor consumption occurred in waterlogged, anoxic sites. The mean N_2O production rate per unit area from the ecosystem was slightly less than what is believed to be the global average production rate. Although N_2O production at Hubbard Brook is less intense than in more productive or heavily fertilized soils, undisturbed forests appear to be a significant source of atmospheric N_2O.

Carbon dioxide evolution, a measure of total soil respiration, was found to be primarily a function of soil temperature. Emission rates for CO_2 were not closely correlated with N_2O production.

Nitrification and ammonification thus seem to be only weakly coupled in this ecosystem.

CHAPTER III

NITROUS OXIDE PRODUCTION FROM FOREST SOIL

1) Introduction

At least 11 MT of N_2O-N must be applied annually to the atmosphere to balance the stratosphere photolysis of this gas. About 90% of the N_2O supply is supplied by land sources (McElroy, 1981). A global budget for N_2O sources must distinguish between agricultural and non-agricultural ("unperturbed") habitats, in order to permit assessment of anthropogenic impacts on the global cycle of the gas. Although many measurements of nitrous oxide production in fertilized agricultural land have been made, no studies of N_2O production in unperturbed terrestrial ecosystems are available. It was assumed until recently that all nitrous oxide production in soils was due to denitrification, and indeed the desire to quantify denitrification has been a major stimulus for study of the gas in soils (Nielsen and McDonald, eds., 1978). However, denitrification takes place under anoxic conditions where it is extremely favorable energetically to use nitrous oxide as a terminal electron acceptor. Hence, much of the N_2O produced during denitrification may be consumed without being released to the atmosphere.

Aerobic oxidation of ammonia by autotrophic nitrifiers has been shown recently to produce nitrous oxide at rates which increase at low oxygen levels; this process could therefore be a major source of N_2O (Goreau et al., 1980, and Chapter II). The results of nitrogen fertilizer additions to experimental plots and of laboratory soil incubations also indicate that nitrification could be a source of N_2O under normal aerobic field conditions (Freney, Denmead, and Simpson, 1978; 1979; Breitenbeck, Blackmer and Bremner, 1980). Thus measurement

of nitrous oxide fluxes to and from soils may allow estimates of nitrification and denitrification to be made when one of the two processes predominates. In poorly aerated soils with strong redox gradients, both processes may operate in spatial proximity; nitrous oxide fluxes in such settings are a complex function of the rates of both processes.

In this chapter, I present a year-long study of temperature, moisture, nitrous oxide production, and carbon dioxide production in undisturbed Northern Mixed-Hardwood forest soils.

2) Methods

a) Sample Area

Most of the measurements reported here were carried out at Watershed 6 of the Hubbard Brook Experimental Forest in the southern White Mountains, New Hampshire. This ecosystem has been extensively studied for 16 years (Likens et al., 1977). The watershed is underlain by impermeable bedrock of the Littleton Formation, a sillimanite grade metasedimentary gneiss. Input-output mass balances have been measured for elements without major gaseous exchange by monitoring the chemistry and quantity of incoming precipitation and stream outflow (Bormann, Likens, Fisher, and Pierce, 1968; Likens, Bormann and Johnson, 1969; Likens, Bormann, Johnson, Fisher and Pierce, 1970; Bormann, Likens, Siccama, Pierce and Eaton, 1974; Likens and Bormann, Pierce, Eaton, and Johnson, 1977; Likens, Bormann, Pierce and Reiners, 1978; Bormann and Likens, 1979).

Nitrogen entering the ecosystem in rainfall slightly exceeds that lost in river outflow (6.5 versus 4.0 kg N/Ha/yr), and both are considerably less than the estimated plant uptake (79.6 kg N/Ha/yr) or litter contributions to the forest floor (63.2 kg N/Ha/yr).

Mineralization is estimated to be 69.7 kg N/Ha/yr (Bormann, Likens and Melillo, 1977). While cycling of nitrogen is very conservative in undisturbed conditions, extensive leaching of soil nitrate occurs when the forest is cut (Likens, Bormann, Johnson, Fisher and Pierce, 1980; Likens, Bormann, Pierce and Reiners, 1978). After an experimental clearcut, nitrate losses rose and then declined to a new conservative steady state; 11.6 times more nitrate was lost from a cutover watershed than from the unperturbed one over a ten year period (Likens, Bormann, Pierce, and Reiners, 1978). Nitrate losses during this period were 28 percent of total ecosystem nitrogen. 3.17×10^{4} ammonium oxidizers/gram of dry soil were found in the control forest soils, while 5.65×10^{5} gm^{-1} were found in soils from the cutover forest. The difference, a factor of eighteen, exceeds the one-sided 95% confidence limit of roughly three times the estimate of mean cell densities (Smith, Bormann, and Likens, 1968).

Six sites at an elevation of 580 m were chosen to represent the forest floor of the lower section of watershed 6. This Watershed is the unperturbed watershed with which the perturbed ones have been compared (Likens, Bormann, Pierce, Eaton and Johnson, 1977; Bormann and Likens, 1979). The forest is dominated by Acer saccharoides (Sugar Maple), Fagus grandifolia (Beech), and Betula alleghaniensis (Yellow Birch) (Bormann, Siccama, Likens and Whittaker, 1970). All sites were within 100 meters of each other. At each site gas fluxes were simultaneously measured at two locations on the forest floor within a meter of each other, and the same locations were reoccupied for each measurement. The forest floor was minimally disturbed: fresh leaves and the litter layer were left intact and only an occasional surface twig was removed where it

intersected the edge of the dome and would have disturbed the soil during dome emplacement.

b) Equipment and methods

Aluminum chambers (14x28x57 cm) were emplaced 2 cm into the ground. Gas samples were slowly withdrawn from the chamber via a three-way stopcock into a gas-tight syringe following flushing of the small stopcock dead volume. Ambient air was allowed to enter the chamber via a three way stopcock located at the opposite corner of the chamber, which was opened only while the syringe was being filled. This procedure prevented pressure gradients between the chamber and soil air. The chamber volume was mixed gently by a toy aeroplane propeller driven at a few cps through a sealed bushing by an external battery powered motor. The inner edge of the perimeter of the chamber was milled to a sharp edge to minimize soil disturbance during insertion.

Vertical profiles of nitrous oxide in soil were determined by withdrawing 20 cc of soil air from fritted-glass ports mounted at various depths in a 3/4 inch steel soil probe. Each port was connected via fine tubes to a three way stopcock through which gas samples were withdrawn into syringes following flushing of the dead volume of the sample lines.

Gas samples were stored in ground-glass syringes and analyzed within a day of sampling. A water seal was maintained around the barrel of the syringe. Storage tests showed no change in gas concentrations during two week periods. The gas samples were introduced into two sample loops connected in series. The content of one loop was injected via a six way Carle valve into a Porapak Q column at 60^{o}C with an electron capture (Ni-63 source) detector (ECD) at 350^{o}C. The other

sample loop was injected into parallel Porapak Q and Molecular Sieve columns at room temperature; these columns were in series with a thermal conductivity detector (TCD) maintained at room temperature. Nitrous oxide was detectable on the ECD with a precision of about two parts per billion. The detection limit for carbon dioxide on the TCD was 200 parts per million with a precision of about 10 ppm. Instrument response was found to be linear down to the limit of detection. The electron capture detector also gave a linear response to carbon dioxide but with a detection limit (roughly 600 ppm) three times higher than the TCD.

Typical sets of nitrous oxide data are shown in Figure III-1. The fit to straight lines was usually well within the one sigma standard error bars of nitrous oxide measurements. About 10% of measurements showed some curvature. A few percent of the measurement series were rejected because the variance from the regression line exceeded twice the analytical precision. The time series allows measurement of fluxes down to about 10^8 molecules of N_2O cm^{-2} second $^{-1}$.

Carbon dioxide evolution curves were usually smooth and concave (Figure III-2). The shape of these curves are similar to solutions of the diffusion equation from soil into a closed volume for differing surface flux boundary conditions. Curvature may be produced by back diffusion as the head space equilibrates with the upper layers of soil. Curvature of CO_2 concentration functions in the dome appeared frequently at sites where simultaneous observations of nitrous oxide showed linearity with time. Atmospheric CO_2 concentrations are 10^3 times N_2O concentrates but CO_2 fluxes were about 10^5 times N_2O fluxes. Consequently headspace CO_2 levels equilibrate with soil

air much faster than N_2O levels. It was possible to estimate the initial CO_2 flux quite accurately from the initial slope of the curve.

Soil temperatures were measured by a mercury thermometer inserted two centimeters into the forest floor. No temperature difference was found to occur under chambers: a result of the high thermal conductivity and reflectivity of aluminum.

Soil matric potential (soil suction) was measured with a tensiometer. The matric potential, in centibars, is the pressure established across a water-permeable, gas-impermeable ceramic element when pure water equilibrates with soil moisture. The matric potential is zero in pure water, and increases as soils get drier. Because of the size of the ceramic element used, the measurement is representative of the uppermost six to eight centimeters of the forest floor. Temperature and matric potential were measured at a location between the two flux chambers.

c) Equipment Tests

Equipment tests were performed on forest soils in Middlesex Fells, Massachusetts, and on Cape Cod recessional moraine soils.

Flux measurements were made in a mixed oak-maple-white birch stand and in a red pine stand at Middlesex Fells. Chambers were removed and replaced with chambers of different height, and a second set of flux measurements made. This was followed by removal, replacement, and a third flux determination. Each site gave the same flux on each repetition, indicating that the repeated emplacement of domes did not perturb the flux of nitrous oxide from the forest floor. In a more violent test a box was emplaced for a long period of time, samples were

withdrawn every five minutes, and the box was vigorously disturbed after 50 minutes by my jumping heavily on top of the chamber. After a further 25 minutes, the chamber was jumped on ("stomped") for five full minutes as vigorously as possible without actually dislodging it, and further measurements made. The results (Figure III-3) indicate that nitrous oxide concentrations in the chamber increased linearly throughout, with a small (1%) perturbation following "stomping". This variation is only slightly larger than the standard deviation of repeated standard air measurements. A third test was made (by M. Keller and S. Wofsy) to see if gas fluxes were enhanced by the cut made in litter material during insertion of the dome. In June 1981, flux measurements at five sites were made in the usual fashion, then repeated after making two slits (to 3 cm depth) in the forest floor material inside the dome. Fluxes were indistinguishable before and after making the additional slits. It is concluded that the gentle insertion used in the field does not measurably perturb the flux of gases from the soil.

The Cape Cod site was in the middle of a 2x2 m area of bare soil kept clear of weeds on the edge of a vegetable garden. The well drained sandy loam had good sun exposure. It had been mulched with dried seaweed (largely Codium fragile and Fucus species) over a year before. A series of measurements was taken as the soil dried after heavy rains that followed a long dry period. The results (Figure III-4) showed that the flux of nitrous oxide declined from a high of 203×10^9 molecules/cm^2/sec to one tenth this amount as the soil dried over a nine day period.

3) Results and Discussion

a) Physiochemical parameters: soil temperature and moisture

The temperature and moisture variations throughout the 1980 growing season at the six forest floor sites are shown in Figure III-5. The first measurements were made about three days after ground thaw. The ground had frozen during cold conditions and low snowfall in the winter of 1979-1980. This condition is exceptional at Hubbard Brook: "a significant microclimatologic feature of this area is that even the uppermost layer of the forest soils usually remains unfrozen during the coldest months because of the thick humus layer and a deep snow cover" (Likens, Bormann, Pierce, Eaton, and Johnson, 1977). Since the initiation of the Hubbard Brook Ecosystem Study, lack of insulating snow cover had permitted soil frost only during the dry winters of 1969-1970 and 1973-1974. On both occasions the nitrate content of streamwaters during spring thaw was elevated above the norm (Likens, Bormann, Pierce, Eaton and Johnson, 1977).

Following ground thaw all sites were very moist, but only one site (site 2) approached water saturation. Those sites that were initially driest had the highest initial soil temperatures, and their temperature and matric potential increased most rapidly. Soil temperatures reached maximum values of about 20^{o}C in early August. All sites showed drying trends until August. Sites 2 and 6 remained the wettest throughout the year. The former site was the wettest during the spring, but later dried out to a greater extent than site 6. Site 2 lay in a topographic low about 4 meters across, while site 6 was located 3 meters from and 1.5 meters above the principal stream of the watershed. Site 3 was the driest throughout the year, and reached the

highest soil temperatures. It was located on the shoulder of a knoll having up to two meters of relief above areas 5 meters distant. Sites 1, 4, and 5 were located in fairly flat, featureless portions of forest floor with an inclination close to that of the average slope (12-13^{o}, Bormann, Siccama, Likens, and Whittaker, 1970). These sites showed intermediate levels of temperature and matric potential.

During the early part of the summer of 1980 rainfall was lower than average. (Figure III-6). Following intense thundershowers in early August, most sites showed a drop in matric potential, followed by drying later in the month. Fall rain and declining soil temperature were concurrent with declining matric potential late in the growing season. Soils remained slightly damp to the touch at all but the driest sites. The thick humus layer appears to have protected the soil from excessive temperatures and moisture loss by evaporation, and the driest soils had matric potentials (about 60 centibars) well below the typical wilting potential for temperate forest trees (about 150 centibars, Larcher, 1980). Small hygrophytic cryptograms and phanerograms appeared to be partially affected by soil dryness at some sites: The fern Dryopteris spinulosa and the clubmoss Lycopodium lucidulum remained green at most sites throughout the summer, but the latter appeared to have suspended foliage production.

B) Nitrous Oxide Fluxes: Relations with Temperature and Moisture

Figure III-7 shows the time history of nitrous oxide production at each site. Some sites show considerable variation between fluxes in adjacent chambers whereas others are very similar. The variance between sites exceeds that within sites, and clear differences in seasonal

patterns of N_2O production were observed at different sites:

a) Site 3, the warmest and driest site gave the largest fluxes in early spring, a few weeks after ground thaw. The flux rapidly declined after the matric potential rose to around 18 centibars, and remained low thereafter.

b) Most sites had maximal fluxes in early spring as the soil warmed and dried, and again in late July following thunderstorms. Largest fluxes at this time occurred under drier conditions than earlier maxima (which had occurred at lower soil temperatures). Very low fluxes were found from August on.

c) Each site followed a different trajectory in temperature-moisture space and had a different pattern of nitrous oxide evolution. A plot of nitrous oxide fluxes as a function of both temperature and matric potential is presented in Figure III-8. The different symbols represent different flux magnitudes. Highest fluxes are found in a crudely parabolic band. Nitrous oxide fluxes were low under very wet (<2 cb), very dry (>30 cb), very warm (T>18^{o}C), and cooling Fall conditions. The frequency of fluxes measured in each flux class as a function of temperature is shown in the table below, along with the percentage contribution fluxes in that class make of all measured fluxes in that temperature class:

Temperature (^{o}C)	Low	Intermediate	High
	$<1.10^{9}$	$1 - 3x10^{9}$	$>3x10^{9}$ cm^{-2} sec^{-1}
0 - 5	0 (0)	1 (25)	3 (75)
5 - 10	10 (45.4)	8 (36.3)	4 (18.2)
10 - 15	7 (43.8)	6 (37.5)	3 (18.7)
15 - 20	7 (41.2)	8 (47.2)	2 (11.8)

High fluxes are characteristic of low temperatures following thaw, but make only a minor and decreasing proportion of measured fluxes at temperatures warmer than $5^{o}C$. No low values were found at low temperature. Low fluxes were the plurality between 5 and $15^{o}C$ but intermediate fluxes were the largest category under conditions warmer than $15^{o}C$.

Figure III-9 (top) shows the frequency of matric potential observed during the course of the year. Figure III-9 (bottom) is a plot of nitrous oxide flux as a function of matric potential. Fluxes at the wettest sites were very small. Occasionally, small negative fluxes were measured in waterlogged soils.

C) Nitrous Oxide Fluxes in a Global Context

Figure III-10 shows the nitrous oxide flux from Hubbard Brook soils through the 1980 growing season. Fluxes were low throughout the second half of the growing season, but the overall temporal pattern is complex. Spatial and temporal variability are related to moisture-temperature patterns, microtopography, and (probably) the local distribution of plant roots and detritus. The mean flux required over the Earth's surface to balance the rate at which nitrous oxide is lost by photolysis in the stratosphere (McElroy, Wofsy and Yung, 1977; Hudson and Reed, 1979) is shown by the arrow. If the ocean contributes 1 MT/yr (McElroy, 1981) then the ocean flux should be 0.3×10^{9} and the land flux 9.26×10^{9} molecules/cm^{2}/sec. The average flux at Hubbard Brook is approximately half of the global average and one seventh the land average. Productivity is not high in northern mixed hardwood forest or podzolic soils (Whittaker and Likens, 1973; Ajtay, Ketner, and Duvigneaud, 1979). Nevertheless nitrous oxide fluxes from this ecosystem are comparable (Figure II-32)

to those observed in Iowa soybean fields (Bremner, Robbins, and Blackmer, 1980), unfertilized Ontario corn and tobacco fields (McKenney, Wade and Findlay, 1978), and Colorado cornfields (Hutchinson and Mosier, 1979). On the other hand, Hubbard Brook fluxes are considerably less than those of fields which have been heavily fertilized (McKenney, Wade and Findlay, 1978) and those of garden soils that have received organic mulch (Figure III-15).

The average N_2O flux at Hubbard Brook is about 0.2 kg N ha^{-1} yr^{-1} ($1.5x10^9$ molecules/cm^2/sec) and net primary productivity is 5740 kg C ha^{-1} yr^{-1} (Whittaker, Bormann, Likens and Siccama, 1974). Global terrestrial primary production is estimated at $60x10^{12}$ kg C/yr (Ajtay, Ketner, and Duvigneaud, 1979). If we assume that fixation of C and incorporation of N are approximately balanced by decay processes, and if we adopt the view that N_2O production is a by-product of nitrification, then we might expect N_2O eflux to be roughly proportional to primary production. With these assumptions global production of N_2O would be estimated at $2.3x10^6$ tons yr^{-1}, which suggests that forest biomes could be major global sources of N_2O. Production of N_2O by forests would have been underestimated if a larger proportion of plant nitrogen at Hubbard Brook is provided by ammonium rather than by nitrate or nitrite, as compared to the global average. We note in this context that at Hubbard Brook soil ammonium levels are ten times those of nitrate (Melillo, 1977), whereas the opposite is typical of most soils (Russell, 1973; Nye and Greenland, 1960), in which nitrate has been shown to be the major plant nitrogen source (Haynes and Goh, 1978; Stalfelt, 1972; Mariakulandai and Manickam, 1975; Russell, 1973; Jones, 1979, National Research Council, 1979). Consequently the above estimate

of global "natural" N_2O production may be low. A more accurate estimate must await further work in more productive forests under a wider range of conditions.

D) N_2O and the nitrogen cycle at Hubbard Brook

The average N_2O loss from the ecosystem (about 0.1 kg N/Ha/yr) is very small compared to the input of fixed N in precipitation (6.5 kg N/Ha/yr, 90% NO_3^-, 10% NH_4^+), loss of N in streamwater (4 kg N/Ha/yr as NO_3^-) plant uptake (79.6 kg N/Ha/yr) or litter fall (60.4 kg N/Ha/yr) (Bormann, Likens and Melillo, 1977; Gosz, Likens, and Bormann, 1972). Ammonia levels in streamwater are very small during the entire year and are lower in concentration than rainfall. On the other hand, stream nitrate levels are low during the growing season, but reach 3 mg/L in the spring (Figure III-11). The ecosystem loses nitrate in the spring and gains it in summer (Likens et al., 1977). During the growing season, plants appear to be capable of virtually suppressing ecosystem nitrogen loss. Ammonia nitrogen levels in soil are about 10 times those of nitrate, and both nutrients have soil levels about three times higher in fall and winter than during the growing season (Melillo, 1977). Rates of mineralization and nitrification in the forest floor have been studied by following nitrate and ammonia concentrations of bags containing leaf letter (Melillo, 1977). Ammonification was estimated at 69.7 kg N/Ha/yr with nitrification about one sixth this value. Such measurements may not be representative of the forest floor for three reasons.

a) Roots were excluded from the litter bags. Microbial densities are much higher in the "rhizosphere" (the diffusion boundary layer around root surfaces) except those of nitrifying bacteria, which are lower (Nye and Tinker, 1977; Alexander, 1977).

b) The nitrogen content of decomposing litter increases by 50% in a year while dry weight decreases by 40% (Gosz, Likens and Bormann, 1973). Litter has a high C/N ratio so that decomposers must withdraw nitrogen from the soil in order to use carbon from decomposing litter (Gosz, Likens, and Bormann, 1976). This demand is thus about 27.1 kg N/Ha/yr. In the litter-bag experiments a high C/N ratio may suppress nitrification rates, particularly in acid soils like Hubbard Brook (Russell, 1973).

c) Vertical profiles of N_2O measured using a soil probe with fritted glass sampling ports showed maximal N_2O concentrations in the upper layer of the mineral soil (Figure III-14). If a significant part of N_2O production by nitrification occurs in mineral soil, the litter bag studies of Melillo would underestimate nitrification rates in the ecosystem.

Several mechanisms could lead to nitrous oxide production in soils. Chemical sources are regarded as insignificant in normal soil (Focht and Verstraete, 1977). Heterotrophic nitrification is believed to be insignificant in most soils (Russell, 1973; Focht and Verstraete, 1977; Alexander, 1977), but little work on soil heterotrophic nitrifers has been done. A heterotrophic nitrifier oxidizing hydroxamate has been observed to emit large amounts of N_2O (Chapter II). Large N_2O production rates by the fungus Fusarium sp. were observed in contaminated flasks containing cultures of the freshwater nitrifier Nitrosomonas europaea.

These fungi are known to be heterotrophic nitrifers (Doxtader and Alexander, 1966). Thus, it is possible that heterotrophic nitrifiers play a major role in generating N_2O even if such organisms are only minor sources of NO_2^-.

Nitrous oxide is not produced during assimilatory nitrate reduction by plants, fungi and bacteria (Hewitt and Smith, 1974; Beevers, 1976) or algae (Chapter II; Goreau et al., 1980). The two major condidates for N_2O sources in forest soils are denitrification and autotrophic nitrification; heterotrophic nitrification is also a possible source of N_2O.

Denitrification requires exclusively anoxic habitats whereas autotrophic nitrification is exclusively oxic. My data are consistent with autotrophic nitrification being the major source of N_2O. Waterlogged conditions which promote anoxia were found only at site 2 in the early spring following thaw. Only small N_2O fluxes were found under these (wet) conditions (Figure III-9) and it appears that aerobic soils are the dominant source of N_2O in the ecosystem, and that net anaerobic sources or sinks of N_2O are small. At several sites with differing moisture levels diffusion coefficients were calculated from measured fluxes and vertical concentration gradients of nitrous oxide (Figure III-14) in the forest floor. Diffusion coefficients were larger in drier soils and were found to fit the relationship $D=0.000025\ \ S^{1.74}$, where D is the effective soil gas diffusion coefficient in cm^2/sec and S is the matric potential in centibars. This relationship indicates an expected increase in effective diffusion as pore space becomes more air filled. The forest floor was sufficiently dry at all sites (except site 2 in early spring) so that diffusion could keep soils well aerated in the face of respiratory demands for oxygen (equal in magnitude but opposite in sign to CO_2 fluxes; next

section, see Brady, 1974). Further work on vertical concentration profiles and flux measurements for N_2O, CO_2, CH_4 and O_2 can be done to quantify gas production as a function of oxygen tension.

Nitrification is inhibited by soil dryness to a much greater degree than ammonification (Seifert, 1970, 1972; Domergues, Belser and Schmidt, 1978; Belser, 1979) but is relatively less inhibited by low soil temperature (Russell, 1973). The observations reveal depression of N_2O production and rise in CO_2 production in warm dry soils, as compared to cool moist soil. This result could be interpreted as support for the view that N_2O production is linked to nitrification in soils.

If it is assumed that a) soils at Hubbard Brook were well aerated through the year and that b) nitrification is the major source of N_2O, with average N_2O production yields of 0.2-0.3% (Chapter II, Goreau et al., 1980) nitrification rates of 31 to 47 kg N/Ha/yr may be inferred from the measured N_2O fluxes. This estimate exceeds that of litter bag studies (ca.11 kg N/Ha/yr). If this interpretation is confirmed, it would imply that a significant portion of total nitrogen demand may be provided by nitrate. Acid soils like Hubbard Brook are often regarded as inconducive to nitrification (Belser, 1979), and so the inference that nitrate and nitrite may be rapidly cycled is unexpected. However, plants generally grow better on nitrate than on ammonia (Hewitt and Smith, 1974; Haynes and Goh, 1978; Nye and Tinker, 1977; Miller, 1980). The free energy required to reduce nitrate to ammonia is about equal to that needed to pump protons actively out of cells when ammonium is taken up; hence, metabolic costs are fairly similar with either source of nitrogen (Middleton and Smith, 1979). Nitrate or nitrite uptake can be coupled to bicarbonate ion loss, aiding removal of a metabolic

waste product (CO_2) from the roots.

E) Carbon Dioxide Fluxes from the Forest Floor

Carbon dioxide is produced by microbiological oxidation of soil organic matter and by plant root respiration. The CO_2 flux is a direct measure of metabolism in the shallow, well-aerated forest floor, and provides a measure against which production of other gases may be campared.

The average carbon dioxide flux from the ecosystem (Figure III-12) showed a pattern similar to that of soil temperature (Figure III-5). The occurrence of peak soil temperature, coincided with maximal carbon dioxide fluxes at each site. Maximum CO_2 fluxes were fairly similar in value at all sites (Figure III-13) despite marked differences in matric potential. As soil temperatures dropped in the fall, carbon dioxide fluxes declined most rapidly at dry sites. Seasonal patterns of carbon dioxide evolution are similar to those of three Minnesota forests (Reiners, 1968), mixed deciduous forests at Oak Ridge, Tennessee (Edwards, 1975), and an oak-pine forest at Brookhaven, New York (Woodwell and Botkin, 1973). Mean annual CO_2 fluxes at Hubbard Brook (.67 kg $C/m^2/yr$) are comparable to reported values in other forests (Figure III-16).

Soil respiration rates have been previously estimated by two techniques:

1) Trapping of CO_2 exhaled into a dome, using a strong alkali solution. Long dome emplacement is required, which perturbs the upper layers of the soil system much more than the brief emplacements used in this work. The KOH often fails to trap a considerable portion of the carbon dioxide, and large corrections are necessary (Schulze, 1967).

2) Infrared gas analysis of air pumped past the forest floor at fairly high speeds. Fluxes can be overestimated, because of suction of soil air into the flow by the pressure gradient in the chamber. No other study of which we are aware has used the short term initial flux measurement technique described here.

Net primary production on watershed 6 has been estimated to have been 1.32 kg dry weight/m^2/yr between 1956 and 1960, and 1.07 kg dry weight/m^2/yr between 1961 and 1965; the non-tree contribution was under 1% (Whittaker, Bormann, Likens and Siccama, 1974). These estimates were derived from regression equations between trunk diameter at chest height, tree height, branch weight, leaf weight, stem weight, and root weight. In constructing the carbon mass-balance it was assumed that 55% of Gross Primary Production was consumed in autotrophic respiration: 1.40 kg dry weight/m^2/yr, of which 0.23 kg/m^2/yr was below ground (Whittaker et al., 1974). Net ecosystem production was estimated to be 0.435 kg/m^2/yr between 1956-1960, and 0.29 kg/m^2/yr between 1961 and 1965 (Whittaker et al., 1974). Forest floor organic carbon was estimated to have increased by 0.033 kg/m^2/yr (Bormann and Likens, 1979). Litter fall on watershed 6 is 0.57 kg/m^2/yr in the lower watershed where these experiments took place (Gosz, Likens and Bormann, 1972). These figures are expressed in grams of dry weight, about half of which is carbon.

Heterotrophic respiration of carbon should equal net primary production (0.57 kg C/M^2/yr) plus soil autotroph respiration (0.23 Kg C/M^2/yr) minus the net increase in forest biomass (about 0.3 kg C/m^2/yr). The predicted soil CO_2 evolution rate (circa 0.5 km C/m^2/yr) is 75% of that measured (0.67 kg C/m^2/yr). The difference could result from larger amounts of root respiration than

assumed or in annual variations in biomass and litter accretion.

The N_2O cycle and man

The atmosphere contains 1500 MT of N_2O-N (McElroy, 1980). About 11 MT/yr are removed by stratospheric photolysis. The measured rate of increase of atmospheric N_2O, 0.5 ppbv/yr (Weiss, 1981), corresponds to an annual addition of 2.5 MT/N_2). Oceanic production now appears to be less than 4 MT/yr (Elkins et al., 1978; Cohen and Gordon, 1979; Weiss, 1981). Thus 8-12 MT/yr must come from land. If Hubbard Brook is representative of global conditions, about 2.3 MT/yr of N_2O-N are produced in forest soils (Chapter III). This estimate is rather uncertain because: a) estimates of global terrestrial primary production are uncertain; b) the turnover rates for nitrogen are not well-determined, c) the fraction of the global average of plant nitrogen derived from nitrate may be different from the fraction at Hubbard Brook and d) sources or sinks of N_2O may not scale directly with primary productivity. More data are needed to derive a more accurate estimate of the strength of this source.

If all fertilizer nitrogen (4×10^7 T/yr) were applied as ammonia and nitrified, only ca 0.12 MT/yr of N_2O-N would be obtained (based on a yield of 0.3%). Hence, direct stimulation of nitrification by fertilizer addition may be less significant than indirect effects due to man's activities on natural N_2O production in soils. Other anthropogenic inputs may be provided by plant biomass burning (Crutzen, Heidt, Krasnec, Pollock, and Seiler, 1979) and by fossil fuel combustion (Weiss and Craig, 1976). The magnitude of each of these sources is poorly known. The discussion below focusses on soil sources and man's potential effects on the magnitude of the N_2O eflux.

The spatial and temporal variability of N_2O fluxes from soils are large, and many measurements are needed to characterize "average" fluxes from a given habitat adequately (Chapter III). Production of N_2O appears to respond in a complex fashion to environmental conditions. Moisture and temperature conditions are much more variable in agricultural soils than in the forest soils studied here. For example, diurnal variations are larger and wetting/drying cycles are shorter in agricultural soils. Nitrous oxide fluxes are expected to show variability on shorter time scales in agricultural soils, as compared to forest soils. Agricultural soils are known to nitrify during short bursts, particularly in the tropics (Russell, 1973), and irrigated agricultural soils are particularly likely to contain lowered oxygen concentrations which enhance N_2O production.

The major part of global primary production occurs in the tropics. Nitrate leaching is extensive in tropical soils, and nitrification often takes place in week-long, but very intense, periods of soil wetting following the first heavy rains of the rainy season (Nye and Greenland, 1960). These habitats should show intense seasonal enhancements of N_2O fluxes into the atmosphere. While the tropics are likely to be a significant global N_2O sources, some tropical habitats may be surprisingly small contributors. Grasslands are well known to have very low nitrification rates (Nye and Greenland, 1960; Russell, 1973); tropical rain forests on laterite soils have low nitrifier counts and low soil nitrate contents (Jordan, Todd & Escalante, 1979). In these soils nutrients appear to move directly from rapidly decomposing litter to plant roots via symbiotic saprophytic fungi, bypassing the soil micro-flora (Stark and Jordan, 1978). The

great variability of tropical soils (NAS, 1972) and flora make estimates of tropical N_2O sources impossible until data can be collected in different tropical habitats during the entire year.

There is a strong impetus for examining nitrification and N_2O release in tropical agricultural and forest soils. High nitrification rates, nitrifier counts, and nitrate levels occur in Ghanaian evergreen rain forests and semi-deciduous forests (Nye and Greenland, 1960). Forests or fertile volcanic soils may have intrinsically rapid nitrate cycling even before conversion to agriculture (Jordan, 1981). In many tropical soils nitrification proceeds at matric potentials as high as 15 bars (Russell, 1973). Low nitrifier counts occur in acid tropical forest soils and in tropical savannahs, but the pH rises and the abundance of nitrifers increases rapidly after the forests are burned and the land is cleared for agriculture (Nye and Greenland, 1960). These results suggest that human perturbation of the tropical nitrogen cycle, by conversion of forests to agricultural lands may result in sharp increases of nitrification and associated N_2O production as the soil humus of a nutrient-poor forest is rapidly oxidized. Tropical forests in Asia, South America, and Africa are being cleared at an accelerating rate (NAS, 1980). This phenomenon, along with the massive historical deforestation of Europe, Asia, and North America (Thomas, 1956) may have altered the global N_2O cycle to an unknown extent. As 98% of soil nitrogen is organic (Russell, 1973), relatively small decreases in humus content may have significant effects on ammonification and nitrification rates. Studies of N_2O fluxes from soils of varying ages since clearance, and comparison with those of unperturbed habitats, are needed to evaluate this possibility.

Decomposition of wastes presents another possible perturbation to the nitrogen and nitrous oxide cycles. Human and agricultural wastes are increasingly concentrated for disposal. Figure IV-1 shows an example of high N_2O concentrations produced in a pile of composting vegetation. Composting is much more efficient if it is done aerobically rather than anaerobically, and it is frequently managed in a manner that promotes nitrification. For example, feedlot cattle wastes are often decomposed in lagoons kept aerated by bubbling and mixing (Jones, 1979). Intense nitrification of organic wastes was exploited in ancient Egypt and India to produce saltpeter (KNO_3). Because gunpowder was a key to military success, aerobic decomposition of horse manure was made compulsory for farmers by Charles Vasa of Sweden in the seventeenth century and by Napoleon in the early nineteenth century. Nitrification is intense under these conditions; when wood ash is added, saltpeter (KNO_3) crystalizes in white crusts. The extreme sensitivity of nitrous oxide production to low oxygen levels makes waste disposal of particular interest to studies of the N_2O cycle. High oxygen demand and high nitrogen mineralization rates are generated in rapidly decomposing organic wastes, so that anthropogenic production of nitrous oxide could rise rapidly and non-linearly with increasing decomposition (Goreau, et al., 1980), fueled by increasing population and increasing per capita production of wastes. This potential problem could be alleviated to some degree if decomposition of human and agricultural wastes proceeded under dispersed and well-aerated conditions, or if these wastes were fermented anaerobically. The latter process conserves about 99% of the nitrogen (NAS, 1977), and the anaerobic conditions allow denitrification to consume any N_2O produced early in decomposition.

Nitrous oxide "pollution" thus may be an environmental hazard whose severity could rapidly increase. If the biological nitrogen cycle is a major source of atmospheric N_2O, prediction of future atmospheric N_2O burdens require knowledge of the frequency with which different oxygen gradients occur in nitrifying habitats and the associated N_2O eflux from nitrification and from denitrification. Such knowledge requires further study of the coupled nitrogen, oxygen, and carbon biogeochemical cycles in natural and perturbed habitats. The gas flux measurements described in this chapter may provide a useful approach to such problems.

FIGURE CAPTIONS

Figure I-1 THE NITROUS OXIDE CYCLE IN THE ATMOSPHERE

Nitrous oxide is produced by biological processes in the oceans and in soils and freshwaters. It is also released from combustion of fossil fuels and from forest fires and fires used to clear land for agriculture. The gas has no known reactions in the troposphere, and has a constant concentration of 300±1 ppbv. Above the tropopause N_2O levels decline. Stratospheric ultraviolet photolysis of nitrous oxide is believed to be the major atmospheric sink of the gas. Photolysis of N_2O is the major stratospheric source of NO.

Figure I-2 NITROUS OXIDE - OXYGEN REACTIONS IN THE STRATOSPHERE

Reactions 1 through 5 represent the reactions by which ozone is produced and consumed. Reaction 6 shows the ultraviolet photolysis of nitrous oxide. Reactions 7 and 8 are reactions of nitrous oxide with metastable oxygen atoms (produced by reaction 5) that consume nitrous oxide. Reactions 9 and 11 show the cyclic series of reactions by which NO produced by reaction 3 can catalytically act to affect ozone levels. The sum of these three reactions is given in reaction 12.

Figure I-3 THE GLOBAL NITROGEN CYCLE ACCORDING TO MCELROY (1976)

In this scheme emphasis is placed upon the rapid biological reactions by which the biosphere produces and consumes atmospheric nitrogen gases. The symmetry of the scheme shown is disrupted by the large amount of human industrial nitrogen fixation for fertilizers. While almost exclusively applied to soils, much anthropogenically fixed nitrogen may find its way to water bodies, causing eutrophication. Most of pool sizes and fluxes should be regarded as approximate. Units are in metric tons or metric tons per year.

Figure I-4 THE NITROGEN CYCLE IN TERMS OF NITROGEN REDOX STATES

The various nitrogen biogeochemiocal transformations are presented as oxidation-reduction reactions. Reaction 1 takes place only anaerobically or in anaerobic sites in plant and algal tissues protected by oxygen-binding pigments. Reaction 2, 3, and 6 occur both aerobically and anaerobically. Reaction 4 occurs only in the presence of molecular oxygen, while reactions 5 takes place only in its absence. Of all the compounds shown only those at zero oxidation state and at the most oxidized and reduced states are common in the environment. N_2O is a trace constituent of the atmosphere, and nitrite is found in detectable amounts only in unusual ("unbalanced") situations of intense pollution or of high or low pH.

Figure II-1 GAS CHROMATOGRAPH SYSTEM

The plumbing arrangement used for this thesis research allowed measurement of several gases in single gas samples simultaneously by two gas chromatographs. The electron capture detector was used for routine high precision measurements of nitrous oxide, and occasionally for high levels of carbon dioxide and acetylene. The

thermal conductivity detector was used for measurements of carbon dioxide, oxygen, and nitrogen.

Figure II-2 EXPERIMENTAL FLASKS USED FOR MICROBIOLOGICAL NITROUS OXIDE PRODUCTION MEASUREMENTS

Flasks incubated at a fixed temperature were used to measure production of gases from microorganisms during growth. Samples for cell counts and for nutrient product determinations were withdrawn through the three way valve in order to prevent exposure of the medium to air. A continuous flow of experimental gas bubbled through the medium and the outflow gas from the flask was allowed to flow directly into the gas chromatograph sampling loop.

Figure II-3 REPLICATES SHOWING PARALLEL NITRITE AND NITROUS OXIDE PRODUCTION

Typical results from three experimental flasks innoculated with Nitrosococcus oceanus and incubated under 20% oxygen. The origin of each set of results is displaced by one decade for clarity. Parallel production of nitrite and nitrous oxide was observed without exception in over a hundred experiments using autotrophic nitrifiers. Cumulative production of NO_2^- and N_2O are plotted.

Figure II-4 CONSTANCY OF N_2O PRODUCTION YIELD DURING EXPERIMENTAL RUNS

Results from typical runs of Nitrosomonas marina at different oxygen levels showing constancy of the yield during the course of experimental runs. In deriving average yields from 3 to 6 such runs were averaged together. Variability observed in the yield exceeds the precision of the measurements and appears to be due to minor variability in physiological rates in the various flasks. The yield was not found to depend on inoculum size, bacterial growth rate, or source of the inoculum. The N_2O production yield is calculated as the ratio of instantaneous nitrous oxide concentrations divided by the difference of nitrite concentrations over some time interval. When nitrite production is of the order of the sensitivity of the measurement, early in the experiment, the yield estimate has a large standard error. For this reason detailed measurements usually began after 20 hours.

Figure II-5

N_2O head space concentration, NO_2^- concentration in medium, and cell density for representative runs at 20%, 5%, 2% and 0.5% O_2 in Nitrosomonas sp. (marine).

Figure II-6 shows data from a representative flask in which nitrification was allowed to proceed until it became self limiting. Nitrite levels rose from 30 to nearly 12,000 micromoles per liter. N_2O production yields were quite constant at 0.4+0.1%, declining appreciably only after nitrite levels exceeded 8,000 micromolar.

Figure II-7 CELL GROWTH RATE AS A FUNCTION OF OXYGEN

The growth rate of Nitrosomonas marina at various oxygen tensions was determined by cell counts using the acridine orange epifluorescence technique. Bacteria was found to increase their

numbers at the same rate to within 35% over the entire range from 0.5 to 21% oxygen. A maximal value was noted at 1% oxygen. As ammonium oxidizers are known to be incapable of growth without oxygen the last point on the left can be connected to the origin.

Figure II-8 NITROUS OXIDE YIELD OF AUTOTROPHIC NITRIFYING BACTERIA

All species were run at 21% oxygen, and exhibited similar production of nitrite and nitrous oxide. The species listed include species from every known genus of ammonium oxidizer except one. The yield (in percent) is given as the average value plus or minus the standard error.

Figure II-9 Nitrite and nitrous oxide concentrations from a representative run of Nitrosolobus multiforma. Yield was 0.10+.02%.

Figure II-10. Nitrite and nitrous oxide concentrations from a representative run of Nitrosomonas europea. Nitrite production was linear. N_2O production constant, and the yield was 0.70+.05%.

Figure II-11. Nitrite and nitrous oxide concentrations in a representative run of Nitrosospira briensis. The yield was 0.12+.03%.

Figure II-12 NITRITE PRODUCTION PER CELL AS A FUNCTION OF OXYGEN

Marine Nitrosomonas sp. produced more nitrite per cell per day as levels of oxygen increased. Because of the stoichiometry of the oxidation it follows that cells growing at low oxygen levels have markedly lowered oxygen consumption rates, which may be adaptive in situations where excessive oxygen consumption by nitrifiers acts to limit their growth. Units are mg-at (N)/cell/day.

Figure II-13 CELLULAR GROWTH YIELD AS A FUNCTION OF OXYGEN

The cell growth yield is defined here as the number of cells of marine Nitrosomonas produced per mole of ammonium oxidized to nitrite. Cells are able to produce about five times as many cells per mole of nitrite at oxygen levels of 0.5% than they can at 21%. Metabolic efficiency in converting the free energy released from ammonium oxidation into cell biomass is thus greater at low oxygen levels. This strongly suggests oxygen detoxification and the presence of catalase, peroxidase, or superoxide dismutase.

Figure II-14 NITROUS OXIDE PRODUCTION AS A FUNCTION OF OXYGEN

Marine Nitrosomonas produces more nitrous oxide per cell at low oxygen levels. The fractional increase of nitrous oxide production at low oxygen levels is considerably less than the decline in nitrite production per cell. Units are mg-at (N)/cell/day.

Figure II-15 NITROUS OXIDE YIELDS AS A FUNCTION OF OXYGEN

The yield of nitrous oxide (defined as the mole fraction of nitrous oxide-nitrogen produced relative to nitrite-nitrogen produced) rises sharply at low oxygen levels in marine Nitrosomonas. Preliminary results show similar patterns in other species of ammonium oxidizers (Fred Lipschultz, personal communication).

Figure II-16 NITROUS OXIDE PRODUCTION IS DEPENDENT ON ENZYMATIC ELECTRON TRANSPORT

Nitrifier growth medium was autoclaved in a flask. To some flasks an inoculum of Nitrosococcus oceanus was added and to some mercuric chloride was added. $HgCl_2$ kills cells by irreversibly inhibiting electron transport pathways from which cells derive their energy. Flasks were sealed with a septum-cap stopper, and samples of head space removed via a syringe following 10 minutes of shaking. Volume was kept constant by addition of an equal volume of N_2O-free gas. Controls showed that minor amounts of nitrous oxide diffused across the septum cap stopper. Small amounts of nitrous oxide contained in the innoculum account for the difference in the bottom two curves. No nitrous oxide was produced by the medium or by cells to which mercuric chloride was added.

Figure II-17 EFFECTS OF N-SERVE, THIOUREA AND HYDRAZINE ON NITROUS OXIDE PRODUCTION

Procedures identical to those of the preceding figure were followed. No nitrous oxide was produced by cells to which N-Serve was added. Thiourea, which blocks ammonium oxidation to nitrite, failed to prevent nitrous oxide production.

In the absence of cells, nitrous oxide was formed by a chemical reaction between hydrazine and the medium. Amounts produced were comparable to those generated by cell inocula in the absence of inhibitor. Much larger amounts of nitrous oxide were produced when hydrazine was added to inoculated cultures whether or not mercuric chloride was added (note change in scale). A chemical reaction between hydrazine and some constituent of the cell appears to be responsible.

Figure II-18 LACK OF NITROUS OXIDE PRODUCTION BY AN ALGA

A pure culture of the tropical open-ocean dinoflagellate Exuviaella procentrum (Clone #A-312) was grown in a medium in which nitrate was the only nitrogen source. Nitrogen required for cell growth was thus exclusively provided by nitrate reduction to ammonia. The culture grew in a 300 ml B.O.D. bottle with 150 ml air head space which was sampled by syringe needle through a silicone rubber stopper. Nitrate declined smoothly during growth without any change in nitrous oxide concentration.

Figure II-19 GROWTH OF HETEROTROPHIC NITRIFIERS

Growth of heterotrophic nitrifiers as measured by the optical density of cultures diluted by a factor of 100, at 543 nm. Bacteria without added nitrogen reached maximal optical densities most rapidly (Flask 1), followed by those to which ammonia had been added (Flasks 3 and 4), and those to which acetohydroxamate had been added (Flasks 5 and 6). The flask to which both acetohydroxamate and ammonia was added took longest to reach peak optical density. Replicate flasks showed considerable diversity in the optical density as a function of time.

Figure II-20 PRODUCTION OF NITROUS OXIDE AND NITRITE BY ACETOHYDROXAMATE OXIDATION

Nitrous oxide concentrations in flask outflow and nitrite concentrations in fluid as a function of time are shown for a pure culture of Bacillus cereus supplied with acetohydroxamate. It should be noted that N_2O concentrations measure instantaneous production rate, while NO_2^- concentrations measure cumulative production or consumption. Small amounts of nitrous oxide were produced during lag phase (The first point shows that some N_2O was produced prior to inoculation, due to chemical oxidation of acetohydroxamate). No nitrous oxide was produced during cell growth phase, and small amounts of nitrite in the medium were completely consumed. Nitrous oxide was produced at rates proportional to the rate of nitrite production during the decline to stationary phase, and production of both N_2O and NO_2^- started and ceased simultaneously. In figure (c) ammonia was also present in the medium: the lag phase duration was increased, and chemical production of N_2O prior to inoculation (first point) was stimulated.

Figure II-21 Chemical and enzymatic pathways leading to oximes and hydroxamates.

Figure III-1 TYPICAL NITROUS OXIDE EVOLUTION CURVES USED FOR FLUX DETERMINATIONS

The nitrous oxide concentration in the flux chamber virtually always increased linearly but occasional measurements in areas of high flux where the soil surface was wet showed some curvature. The two top curves show examples of the most marked curvature found at Hubbard Brook. Only rarely (5%) could a straight line or simple curve not be fitted to the data with a fit equal to or better than the precision for replicate air samples (0.5 to 1%). The initial portion of the fitted line was used to estimate nitrous oxide flux from the ground in cases where back diffusion from the head space to the ground caused curvature. This procedure introduced negligible error in the flux estimate.

Figure III-2 TYPICAL CARBON DIOXIDE EVOLUTION CURVES USED FOR FLUX DETERMINATION

While about 20% of carbon dioxide concentration increases in the flux chamber were linear, the rest showed distinct curvature even when nitrous oxide increase was linear. This difference results from the fact that carbon dioxide fluxes were very much larger, and apparently more effective in saturating the head space by equilibration with the top layers of soil. The smoothness of the curves allowed precise measurement of initial CO_2 flux even when curvature was most marked. The error bar in the lower right hand corner represents the precision of individual CO_2 measurements.

Figure III-3 TEST OF SENSITIVITY OF FLUX MEASUREMENTS TO MECHANICAL DISTURBANCE

At the time indicated by the first arrow the flux chamber was jumped upon twice. At the time marked by the second arrow I jumped up and down on the flux chamber for five full minutes, jumping about two feet into the air and landing as heavily as possible. Care was taken to land squarely and simultaneously with both feet so as not to dislodge the chamber and allow air to enter. The standard deviation of replicate standards or air samples was 0.5 to 1%. At this level of precision the data is an excellent fit to a straight line, indicating that pressure fluctuations and mechanical disturbance of the box do not measurably perturb the flux of gas from the ground.

Figure III-4 NITROUS OXIDE FLUXES FROM A DRYING GARDEN PLOT FREE OF VEGETATION

The nitrous oxide flux (crosses) scale is at left, while that of the soil matric potential (dots) is at right. Note the marked decrease in flux as the soil dries out and the matric potential rises. While the soil (a well-aerated, sandy loam) at this site was free of vegetation, mulch had been turned into the ground to a depth of about 10-15 centimeters the previous year. Fluxes found were considerably above the mean global value needed to balance stratospheric photolysis of nitrous oxide (about 3×10^9 molecules/cm^2/sec).

Figure III-5 ANNUAL CYCLES OF SOIL TEMPERATURE AND MATRIC POTENTIAL AT 6 HUBBARD BROOK SITES

The scale at left indicates the temperature of the soil at 2 cm depth in degrees centigrade and the soil matric suction between 2 cm and 8 cm depth in the soil, in units of centibars of pressure. All sites show general warming-drying and cooling-wetting patterns over the year, but there are marked differences in amplitude and phase from site to site. Temperatures are indicated by crosses, and matric potential by squares.

Figure III-6 ANNUAL PRECIPITATION AT WATERSHED 6 DURING 1980 COMPARED TO 1956-1974 AVERAGES

Precipitation was measured by rain gauges. Data were kindly provided by Dr. Eaton of Cornell. The long term averages were taken from Likens, Bormann, Pierce, Eaton and Johnson, 1977. It is noted that precipitation at Hubbard Brook was above average in the spring and fall, but markedly lower than normal during January, February, May, June and July.

Figure III-7 ANNUAL PATTERNS OF NITROUS OXIDE EVOLUTION AT HUBBARD BROOK FOREST FLOOR SITES

Nitrous oxide flux histories over the 1980 growing season at each of six sites are shown. Fluxes are in units of 10^9 molecules/cm^2/sec; the sensitivity of the analytical method is about 10^8 molecules/cm^2sec. The bars show the range of values measured fluxes at adjacent plots at each site. At each site measurements were made simultaneously at two adjacent areas of forest floor. The lines show the mean value at each time, and the bars indicate the fluxes at each of the two areas. It is noted that at some sites adjacent areas have very similar fluxes, while at other sites they differ considerably. Nevertheless patterns of variability between sites exceeds that within them

Figure III-8 NITROUS OXIDE FLUXES IN THE TEMPERATURE-MATRIC POTENTIAL PLANE

Fluxes of nitrous oxide in 10^9 molecules/cm^2/sec are plotted in the temperature (in degrees centigrade) - matric suction (in centibars) plane. Very wet, very dry, and very warm conditions inhibit nitrous oxide generation. Optimal matric potential for nitrous oxide production varies roughly as the square of temperature. Each site followed a trajectory starting at the lower left hand corner of the graph following ground thaw, moved upwards to the right during the summer, and returned towards the lower left hand corner in late fall. Each site mapped out a unique portion of the plane. Symbols used are: dot = F $1x10^9$; triangle = $1x10^9$ F $3x10^9$; square = F $3x10^9$ molecules/cm^2/sec.

Figure III-9 FLUXES OF NITROUS OXIDE AS A FUNCTION OF MATRIC POTENTIAL

The lower figure shows the maximum, mean, and minimum nitrous oxide fluxes in each matric potential interval. The symbol at right indicates the precision with which each measurement was done, showing that variability vastly exceeds the precision of the measurements. Fluxes decline sharply at values of matric potential

below 10 centibars. The histogram at top shows the frequency with which the various matric potential conditions were encountered. Because of the range frequency of very dry conditions, this portion of the flux-suction curve has the lowest statistical reliability.

Figure III-10 AVERAGE NITROUS OXIDE FLUX FROM HUBBARD BROOK SOILS DURING 1980

The average flux of all 12 sites through the 1980 growing season is shown by the line. Bars representing the 90% confidence limits derived from the variability of the measured fluxes at all sites at each point in time. The confidence interval greatly exceeds the precision of the measurements, and represents real temporal and spatial variability from site to site. Many measurements are needed to characterize the mean flux, as variance is approximately equal to the mean. The mean global flux required to balance the stratospheric photolysis of nitrous oxide is indicated by the arrows. High mean fluxes were found from April to July, but only low values were observed later in the year.

Figure III-11 NITRATE AND AMMONIA CONCENTRATIONS IN WATERSHED 6 RUNOFF

Nitrate and ammonia concentrations in mg/L are shown. Data were kindly provided by Dr. John Eaton of Cornell University. Ammonia is plotted on a scale expanded by a factor of ten. All river NH_4^+ values are close to the limit of detection. Nitrate and ammonia levels are similar to those measured in previous years. Also shown are the ranges of nitrate and ammonia in rainfall. When trees are not in leaf, nitrate outputs exceed precipitation inputs because of soil nitrification and leaching but during the growing season plant nitrate uptake allows little loss of the ion in streamwater.

Figure III-12 AVERAGE CARBON DIOXIDE FLUXES FROM HUBBARD BROOK SOILS DURING 1980

Carbon dioxide fluxes (in 10^{14} molecules/cm^2/sec, $gC/cm^2/yr$, and $Kg\ C/m^2/yr$) averaged over all 12 sites are shown by the line. Bars indicate the 90% confidence intervals. Prior to July carbon dioxide measurements by thermal conductivity detector were not possible due to lack of appropriate instruments and fluxes were too small to be measurable with the electron capture detector. The upper limit for these is shown by the arrow and the dashed line is a suggested upper limit. Note the correlation between temperature and carbon dioxide flux, and the fact that the mean values considerably exceed the variance. Variance is greater than the precision of the flux measurements, and reflects real differences from site to site. The dashed line has been used in estimating the average annual flux.

Figures III-13 CARBON DIOXIDE FLUXES FROM HUBBARD BROOK FOREST FLOOR SITES IN 1980

The carbon dioxide flux (in 10^{14} molecules/cm^2/second) at each site through the 1980 growing season are shown in these figures. The bars show the range of values found at adjacent plots at each site.

Figure III-14 VERTICAL N_2O CONCENTRATION GRADIENTS IN HUBBARD BROOK SOIL

N_2O concentrations in soil air samples from various depths taken from a multi-port soil probe in October 1, 1979. The three lower profiles were in fairly dry soils (matric potential 40-50 cv), the upper at a damp site (15 cb). Note the difference in vertical scale. Fluxes of N_2O were $2.1x10^9$ molecules/cm^2/sec (top figure) and from 0.9 to $1.5x10^9$ molecules/cm^2/sec (bottom figures). Balancing these concentration gradients against the flux measured just before at the same spot, apparent diffusion coefficients between $2.46x10^{-3}$ and $2.8x10^{-2}$ cm^2/sec were estimated. The smaller diffusion coefficients were found in moist soil. For this range of values, diffusion to a depth of 5 cm takes from a quarter to three hours. N_2O concentration maxima were noted only in the mineral soil.

Figure III-15

Nitrous Oxide fluxes in various habitats compared to Hubbard Brook.

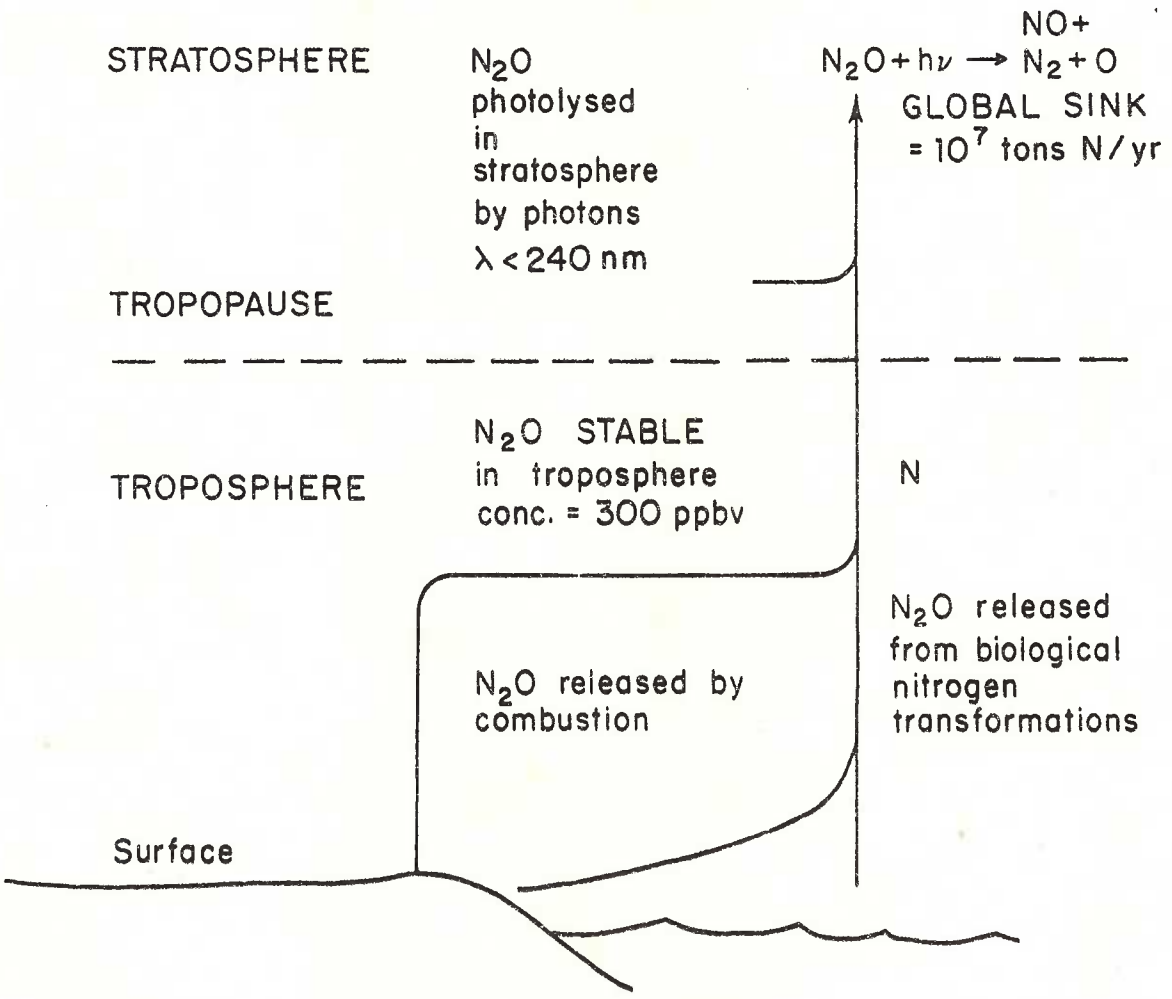
Figure III-16

Carbon dioxide fluxes in various habitats compared to Hubbard Brook.

Figure IV-1 VERTICAL N_2O CONCENTRATION GRADIENT IN DECOMPOSING MULCH

A 1 m deep pit, 1 m^2 in area, was dug on Cape Code recessional moraine soils at Quisset, Mass. The pit was filled with green freshly picked weeds from a 40 m^2 garden. About 10% of dry seaweed (Codium fragile + Fucus species) and about 20% soil (brown loam) were mixed in. The vertical profile of nitrous oxide was measured using a multi-port soil probe. The very large concentration gradients near the surface indicate that such common composting procedures could stimulate N_2O production.

N_2O CYCLE IN THE ATMOSPHERE



NITROUS OXIDE - OXYGEN REACTIONS

$O_2 + h\nu \ (\lambda < 242\,nm) \rightarrow O + O$

$O + O_2 + M \rightarrow O_3 + M$

$O_3 + h\nu \rightarrow O_2 + O$

$O_3 + O \rightarrow 2O_2$

$O_3 + h\nu \ (\lambda < 310\,nm) \rightarrow O_2 + O(^1D)$

$N_2O + h\nu \ (\lambda < 240\,nm) \rightarrow N_2 + O$

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

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

NO_x - OXYGEN REACTIONS

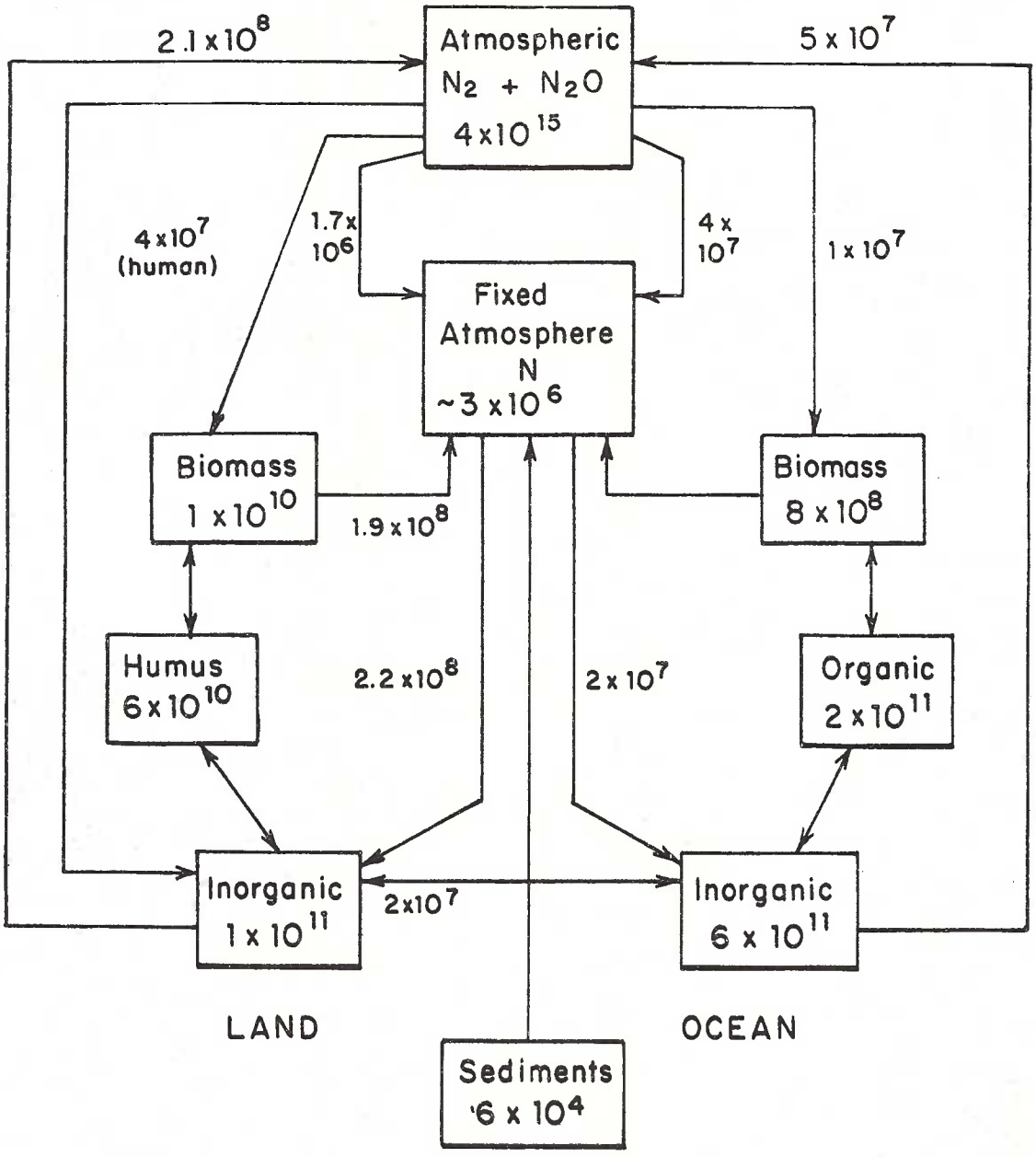
$NO + O_3 \rightarrow NO_2 + O_2$

$O_3 + h\nu \rightarrow O_2 + O$

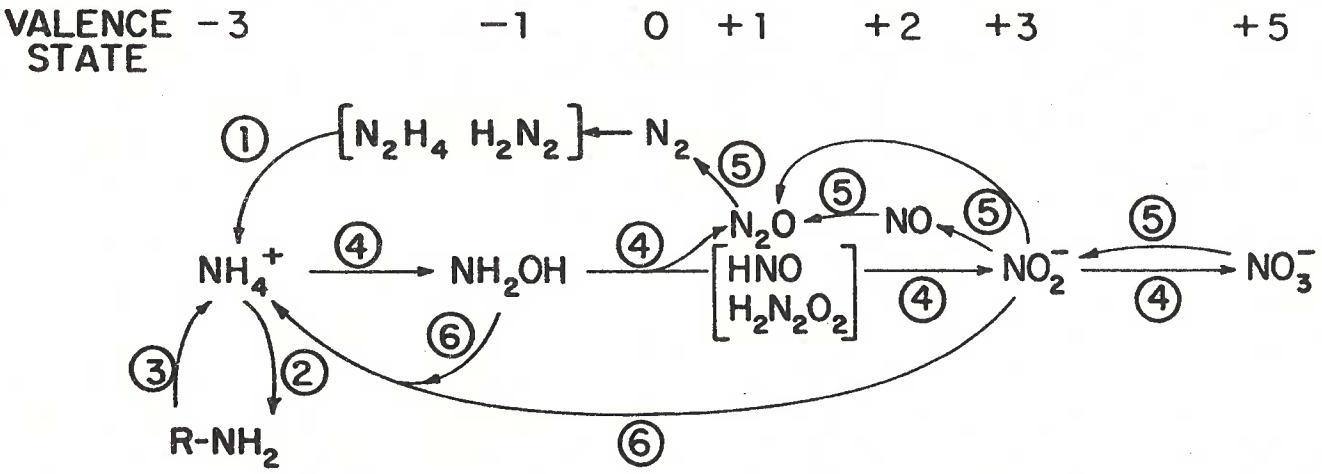
$NO_2 + O \rightarrow NO + O_2$

---

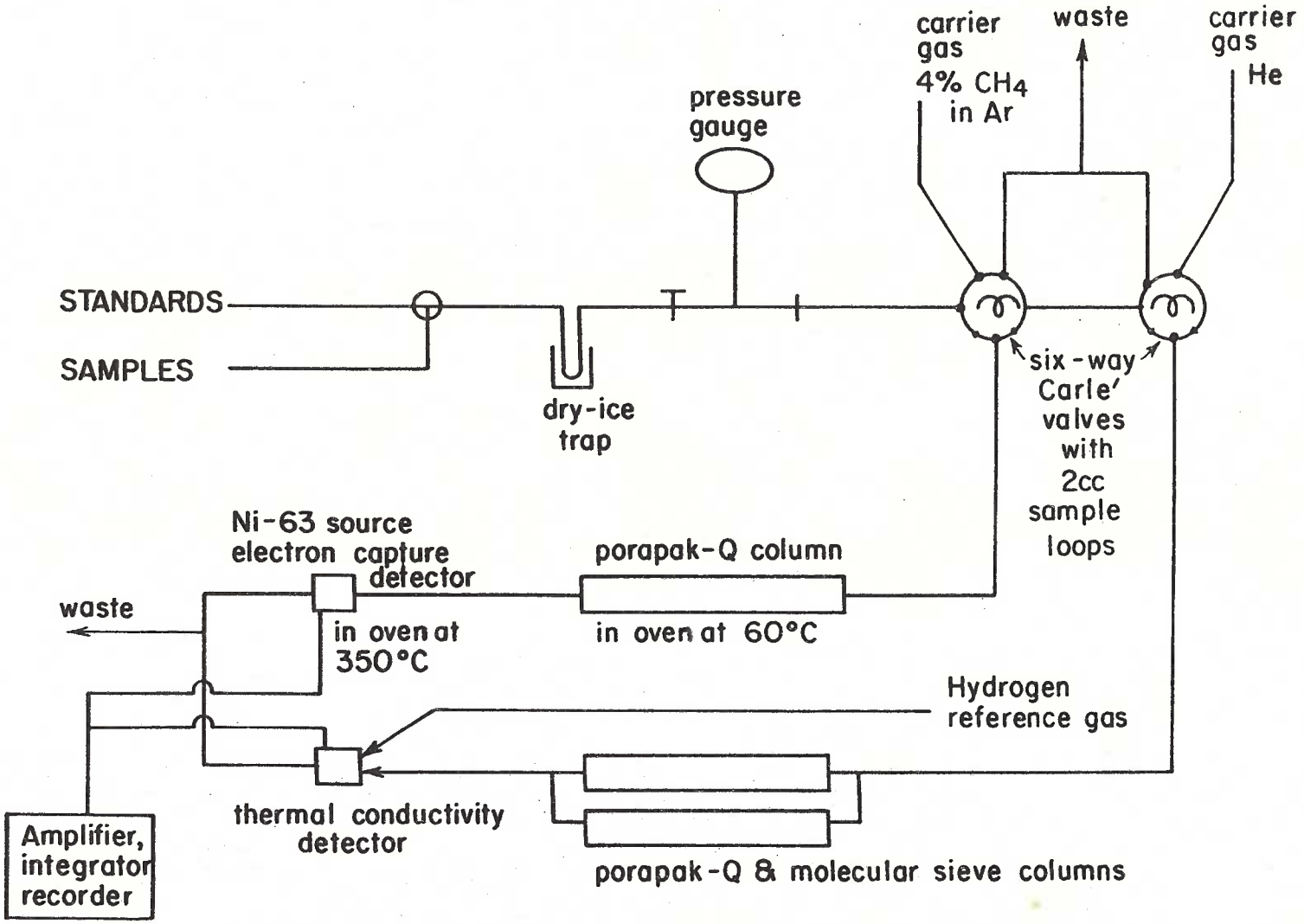
net $2O_3 + h\nu \rightarrow 3O_2$

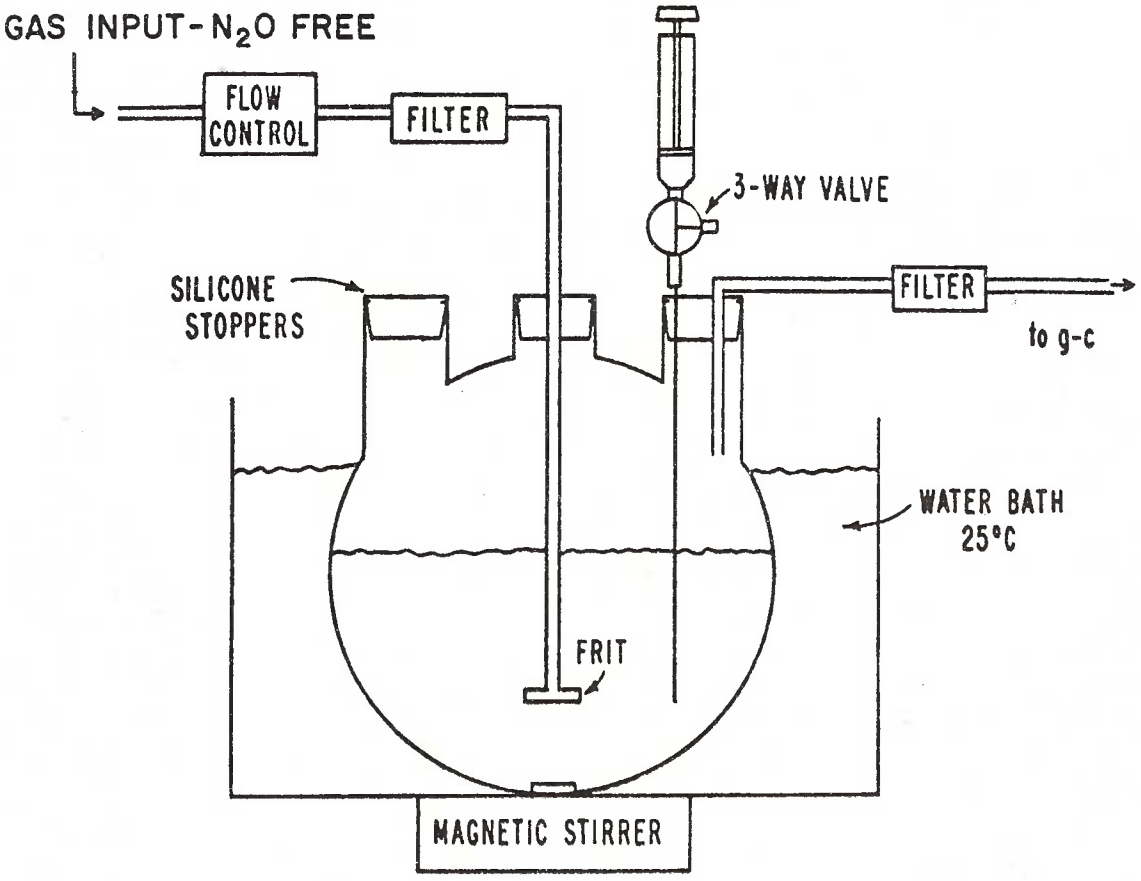

2.1 x 10^8
Atmospheric
N2 + N2O
4x10^15
5 x 10^7
4x10^7
(human)
1.7x
10^6
4 x
10^7
1 x 10^7
Fixed
Atmosphere
N
~3 x 10^6
Biomass
1 x 10^10
1.9 x 10^8
Biomass
8 x 10^8
Humus
6x10^10
2.2 x10^8
2 x 10^7
Organic
2 x10^11
Inorganic
1 x 10^11
2x10^7
Inorganic
6 x 10^11
LAND
OCEAN
Sediments
6 x 10^4

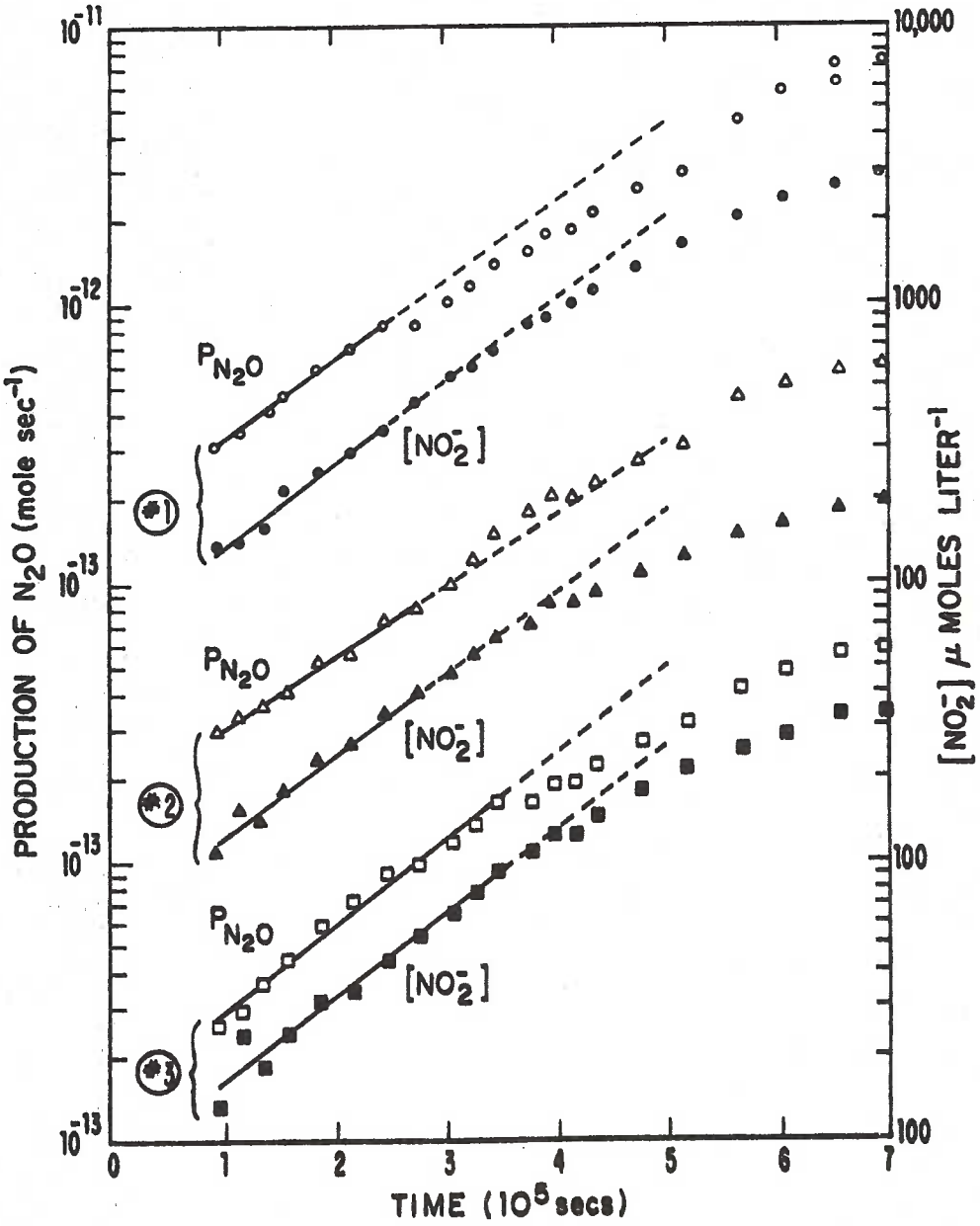
NITROGEN CYCLE: CURRENT VIEW

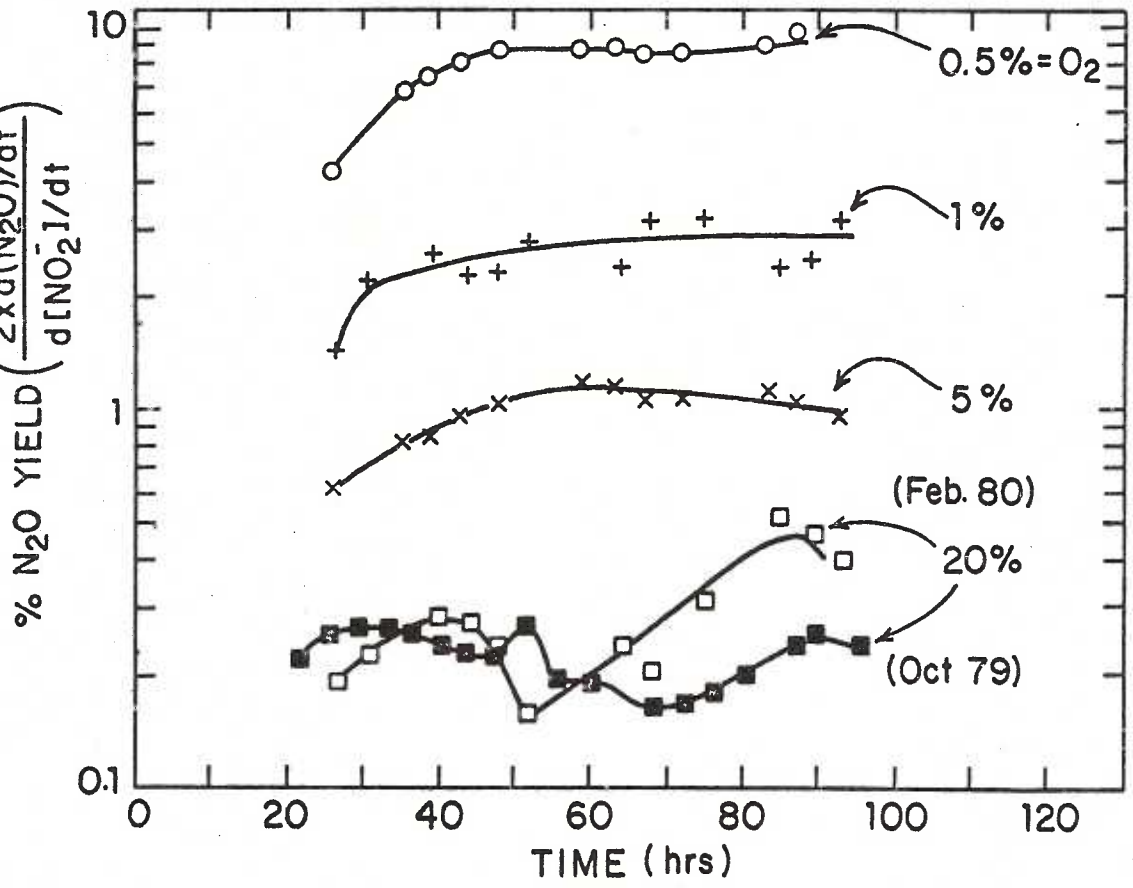


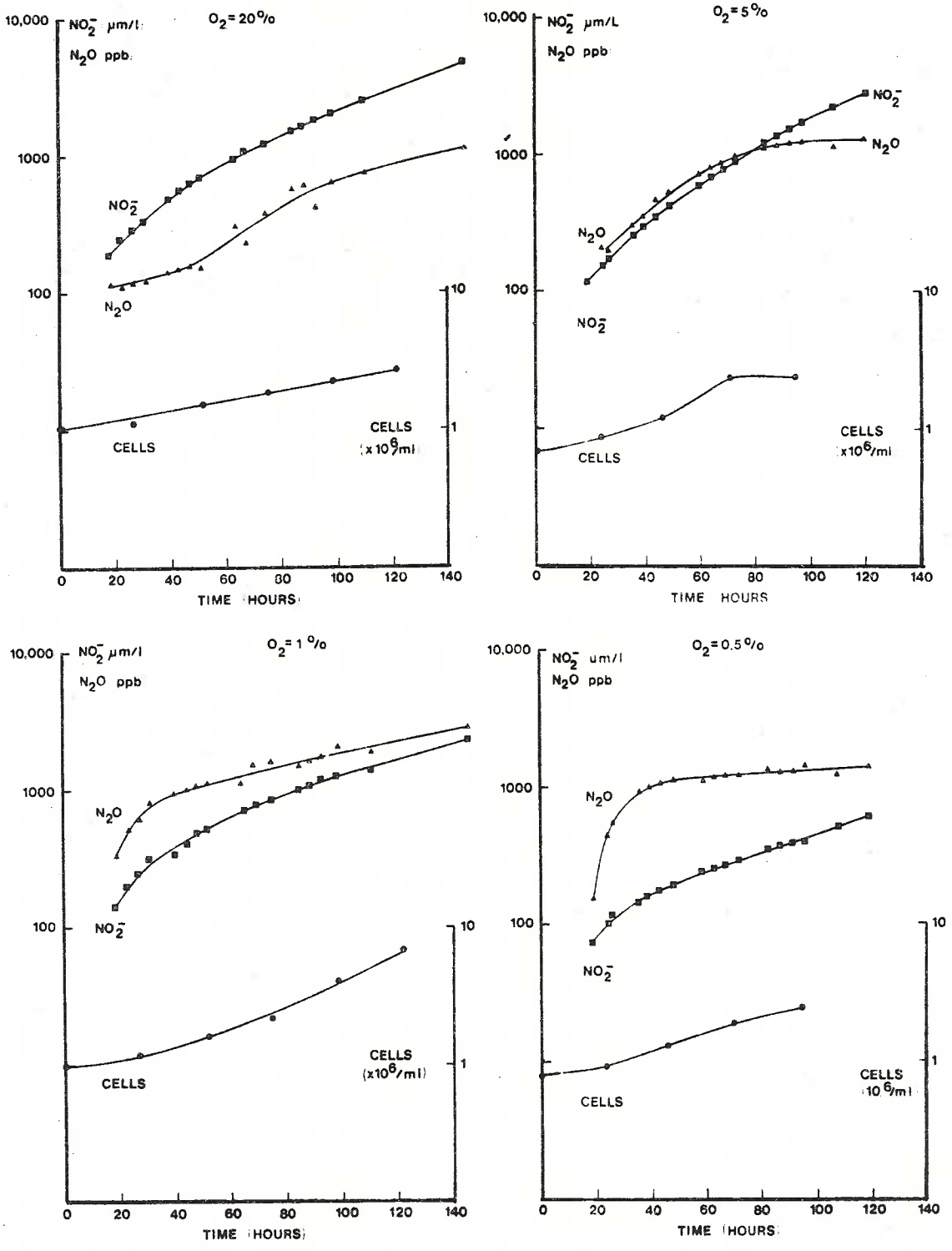
KEY TO NITROGEN TRANSFORMATIONS	
	① Nitrogen fixation
	② Amination
	③ Deamination
	④ Nitrification
	⑤ Denitrification
	⑥ Assimilatory and dissimilatory reduction

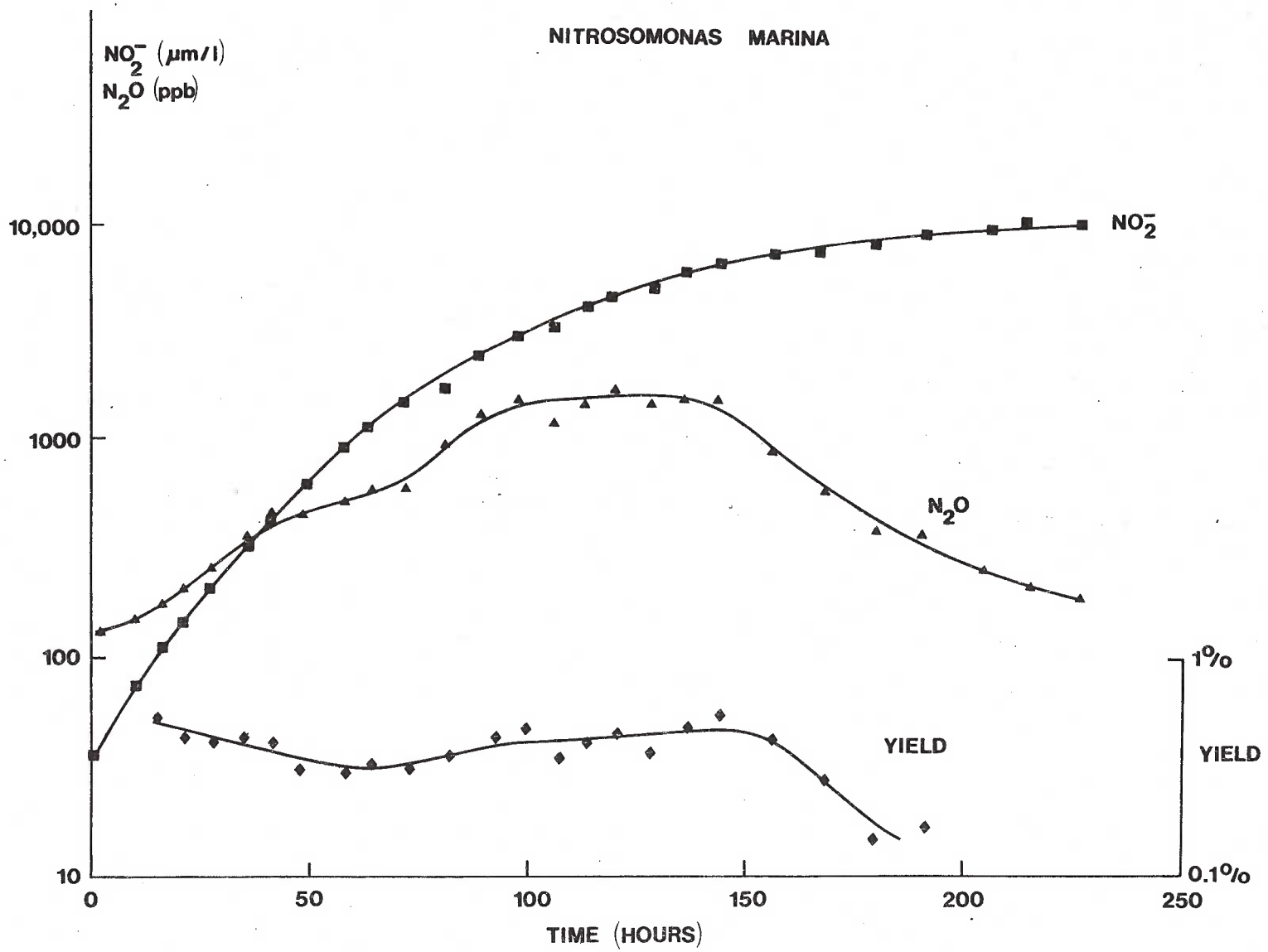

carrier gas 4% CH_4 in Ar
waste
carrier gas He
pressure gauge
STANDARDS
SAMPLES
dry-ice trap
six-way Carle' valves with 2cc sample loops
Ni-63 source electron capture detector
in oven at 350°C
porapak-Q column
in oven at 60°C
waste
Hydrogen reference gas
thermal conductivity detector
Amplifier, integrator recorder
porapak-Q & molecular sieve columns

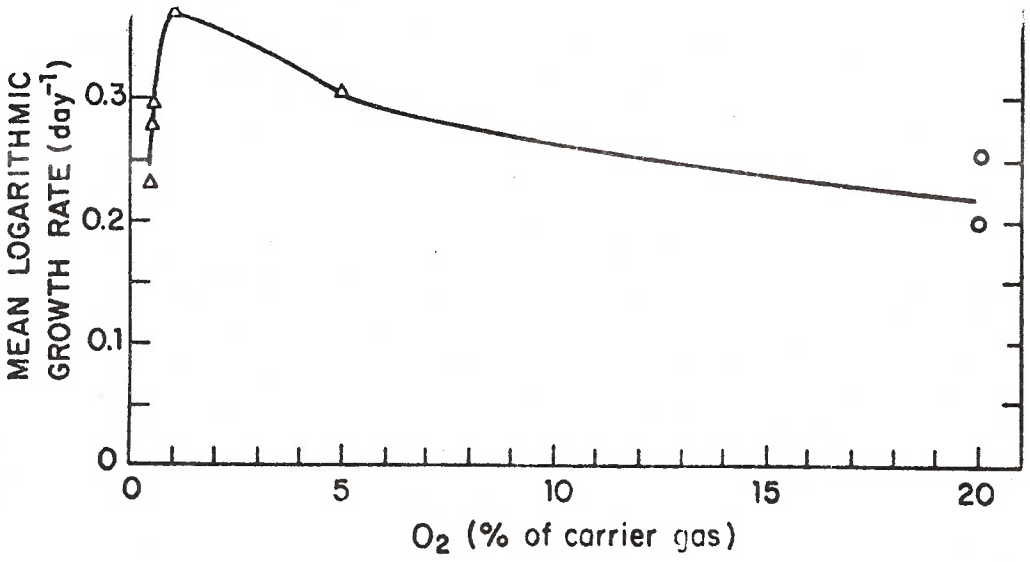

GAS INPUT-N_2O FREE
FLOW CONTROL
FILTER
3-WAY VALVE
SILICONE STOPPERS
FILTER
to g-c
WATER BATH 25°C
FRIT
MAGNETIC STIRRER


PRODUCTION OF N_2O (mole sec^{-1})
10^{-11}
10^{-12}
10^{-13}
10^{-13}
10^{-13}
P_{N_2O}
$[NO_2^-]$
#1
P_{N_2O}
$[NO_2^-]$
#2
P_{N_2O}
$[NO_2^-]$
#3
0
1
2
3
4
5
6
7
TIME (10^5 secs)
10,000
1000
100
100
100
$[NO_2^-]$ μ MOLES $LITER^{-1}$


10
1
0.1
0
20
40
60
80
100
120
TIME (hrs)
% N2O YIELD (2xd(N2O)/dt / d[NO2-]/dt)
0.5%=O2
1%
5%
(Feb. 80)
20%
(Oct 79)


O_2 = 20%
NO_2^- µm/l
N_2O ppb
10,000
1000
100
NO_2^-
N_2O
CELLS
CELLS (x10^6/ml)
10
1
0 20 40 60 80 100 120 140
TIME (HOURS)
O_2 = 5%
NO_2^- µm/L
N_2O ppb
O_2 = 1%
NO_2^- µm/l
N_2O ppb
O_2 = 0.5%
NO_2^- um/l
N_2O ppb

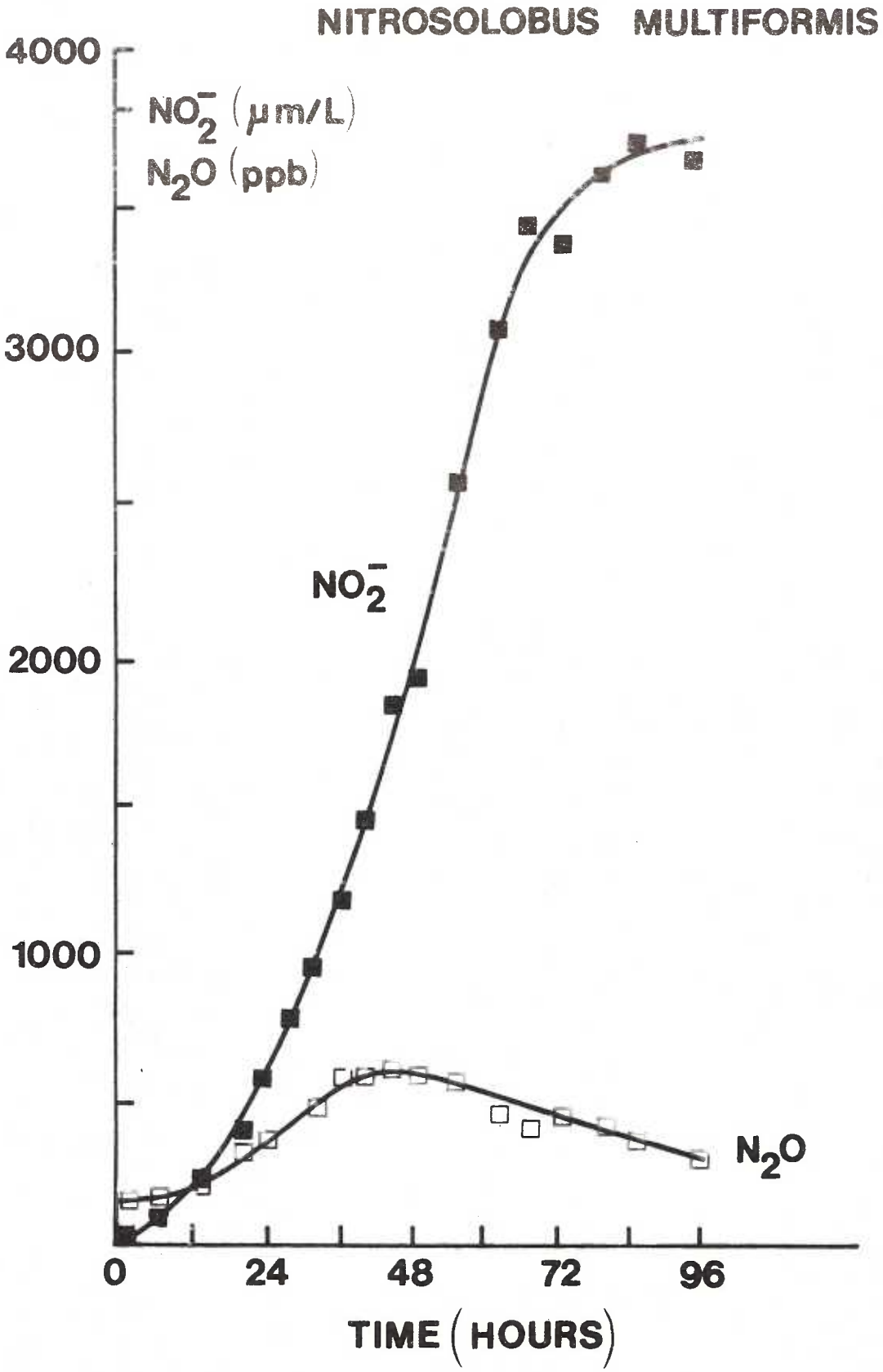

NITROSOMONAS MARINA
NO_2^- (μm/l)
N_2O (ppb)
10,000
1000
100
10
NO_2^-
N_2O
YIELD
1%
YIELD
0.1%
0
50
100
150
200
250
TIME (HOURS)

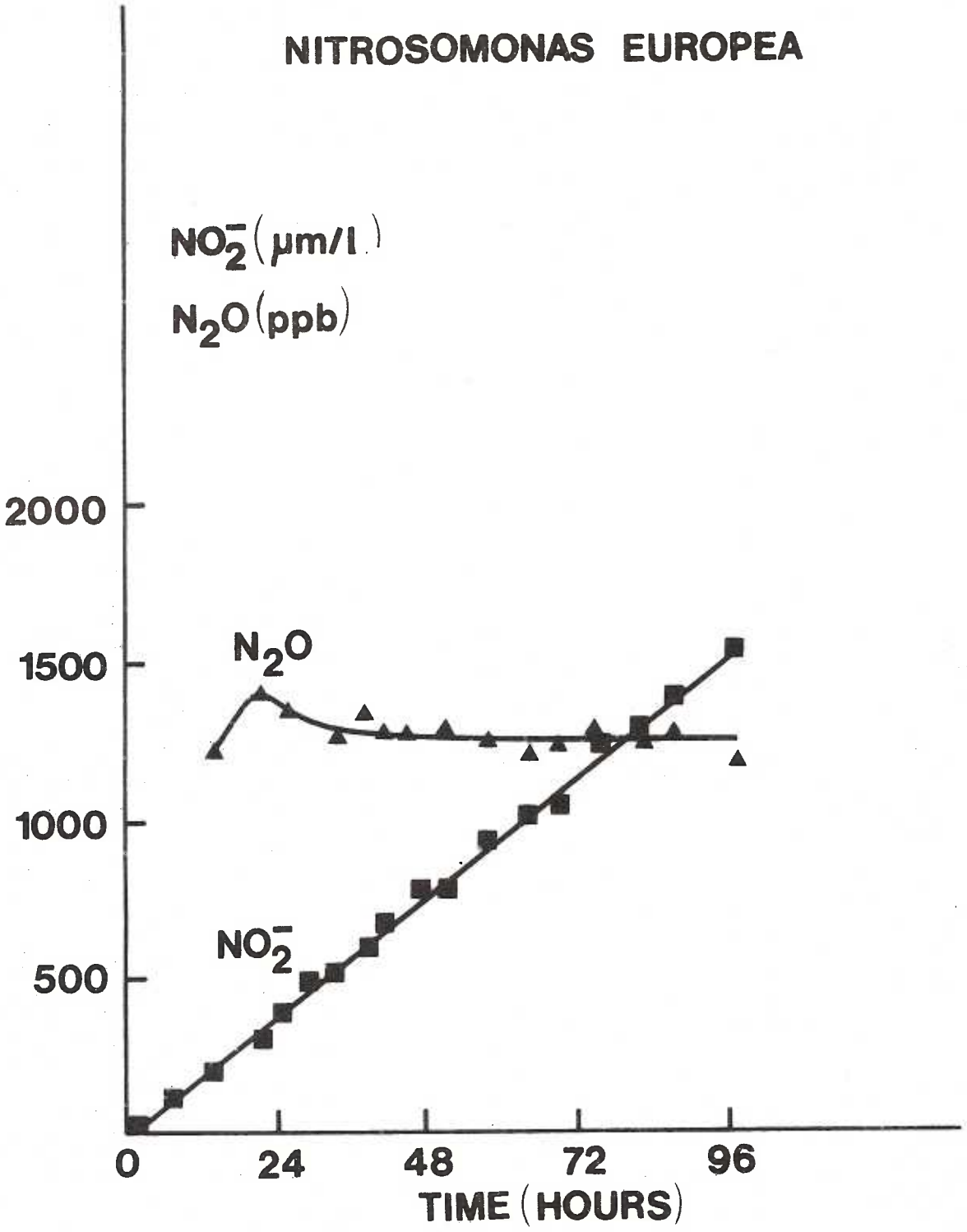

MEAN LOGARITHMIC GROWTH RATE (day^{-1})
0.3
0.2
0.1
0
0
5
10
15
20
O_2 (% of carrier gas)

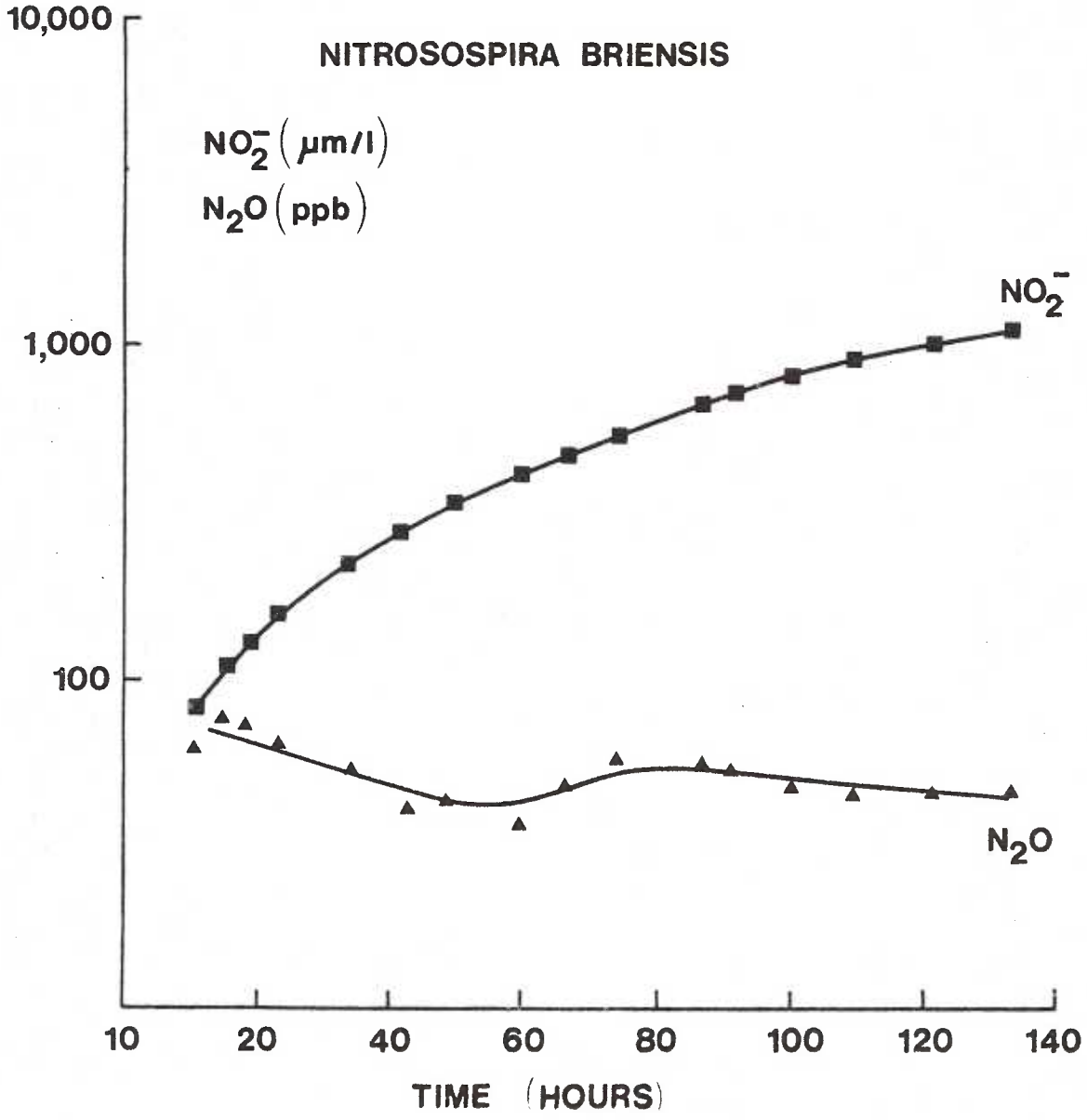
Other nitrifying organisms (ammonia oxidizers)

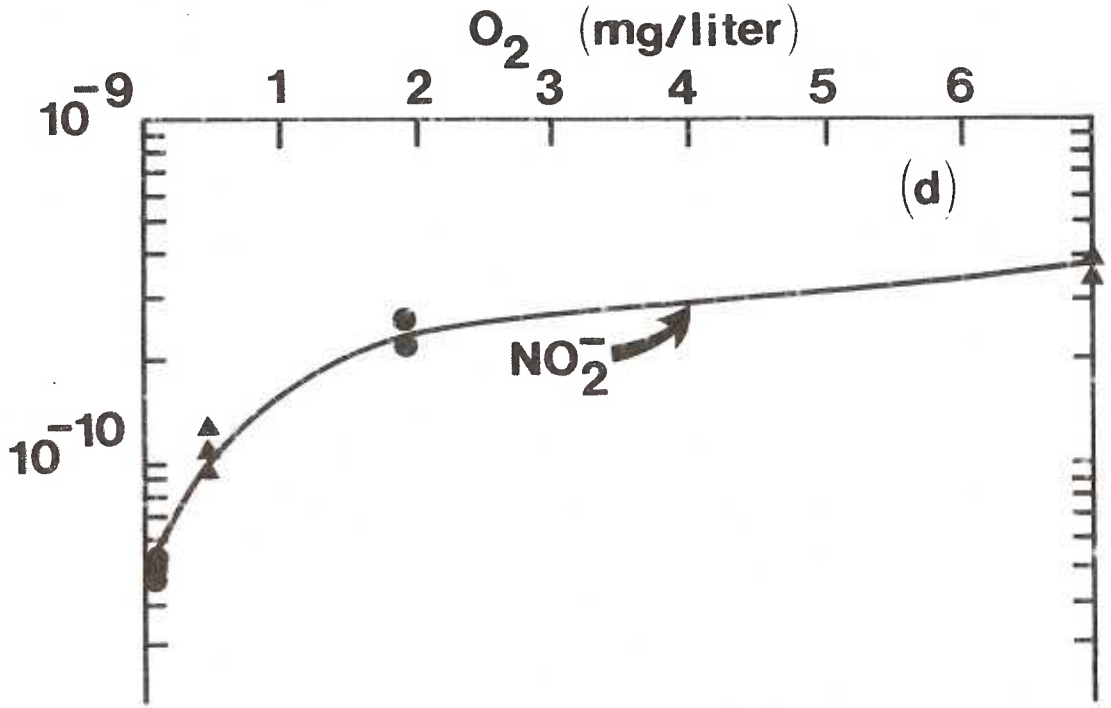
Date	Flask#	NO_2^- (mM)	N_2O Yield (%)	Organism	Source of Isolate
May 79	1-2-3-5	4.2	0.21±0.08	Nitrosomonas sp.	Gulf of Maine (Watson and Remsen, 1969)
May 79	1-2-3-4-5-6	3.0	0.47±0.1	Nitrosomonas europaea	Soils
Feb 79	1-2-3	2.0	0.26±0.1	Nitrosococcus oceanus	Western Atlantic Ocean (Watson, 1965)
May 79	3-4-5	2.7	0.09±0.02	Nitrosolobus multiformus	Soils (Surinam) (Watson et al., 1971)
May 79	2-4-5-6	1.5	0.11±0.04	Nitrosospira briensis	Soils (Switzerland) (Watson, 1971)

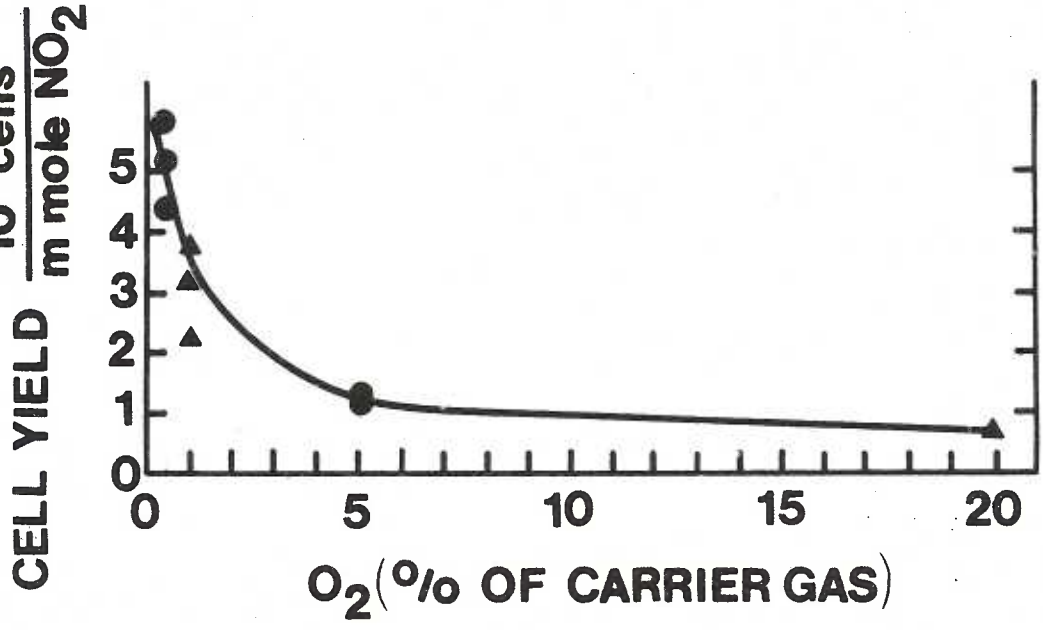
Temperature = 26°, Oxygen = 21%, pH = 7.5

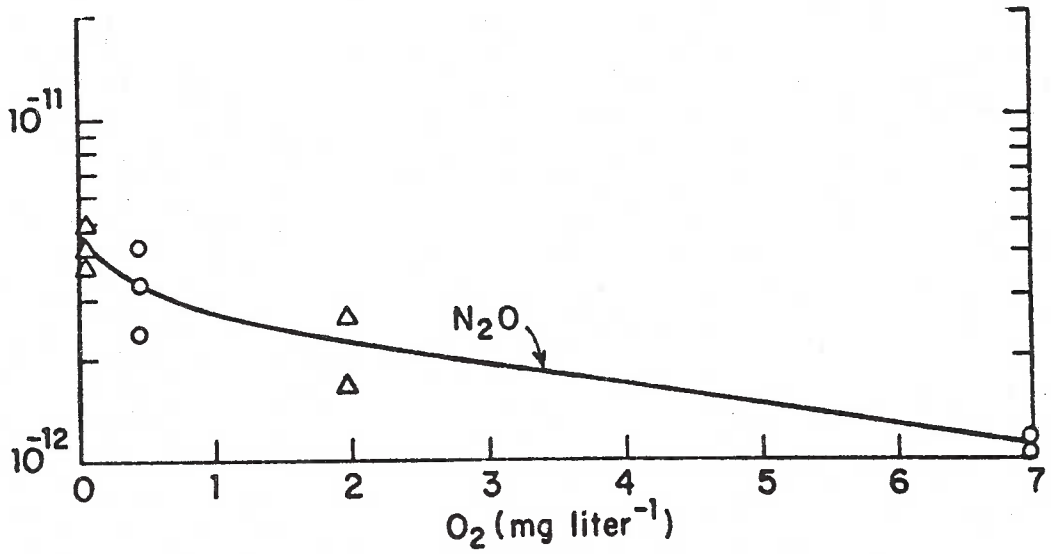

NITROSOLOBUS MULTIFORMIS
4000
3000
2000
1000
NO_2^- (µm/L)
N_2O (ppb)
NO_2^-
N_2O
0
24
48
72
96
TIME (HOURS)

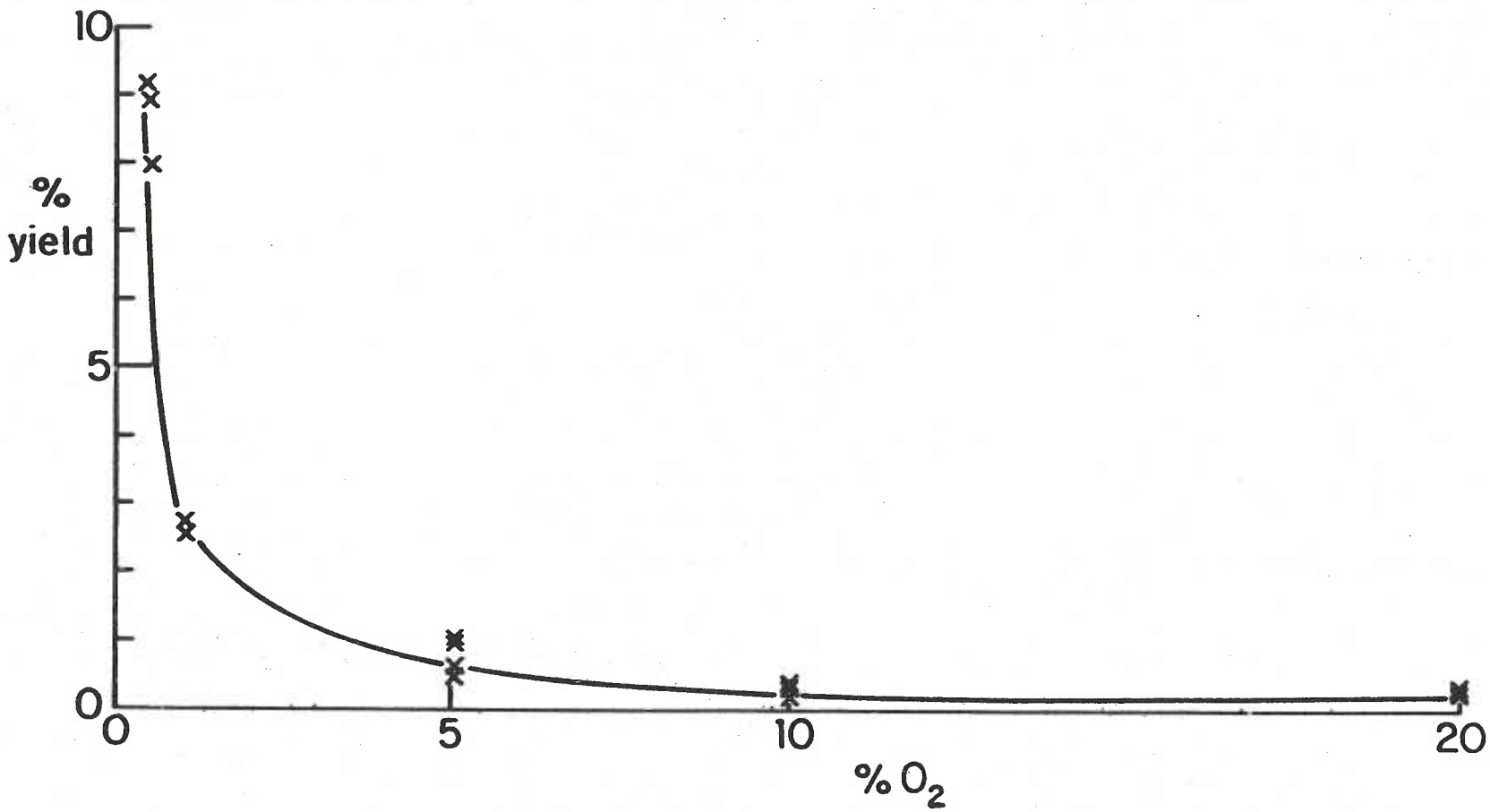

NITROSOMONAS EUROPEA
NO_2^- (µm/l)
N_2O (ppb)
2000
1500
1000
500
N_2O
NO_2^-
0
24
48
72
96
TIME (HOURS)

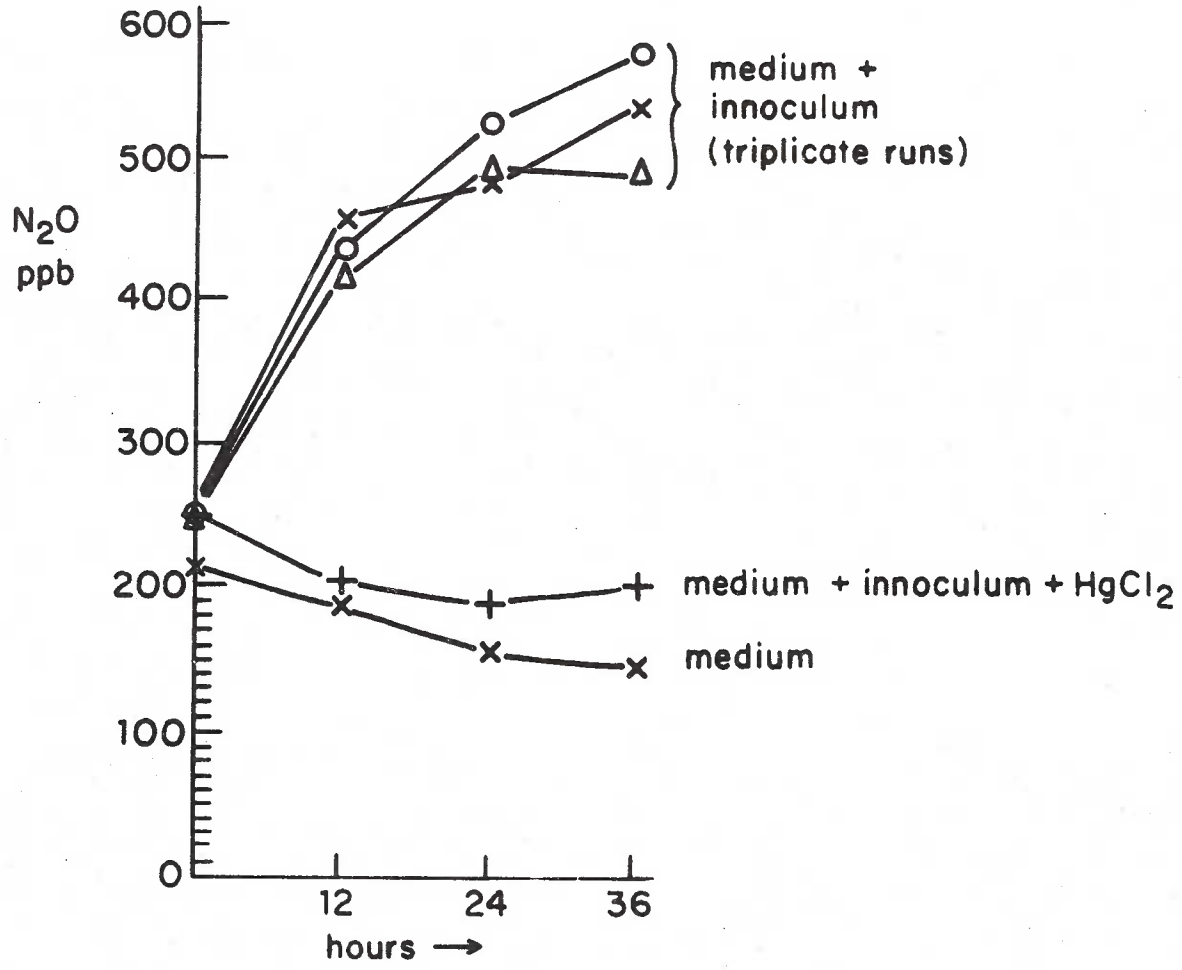

NITROSOSPIRA BRIENSIS
NO_2^- (μm/l)
N_2O (ppb)
10,000
1,000
100
NO_2^-
N_2O
10
20
40
60
80
100
120
140
TIME (HOURS)

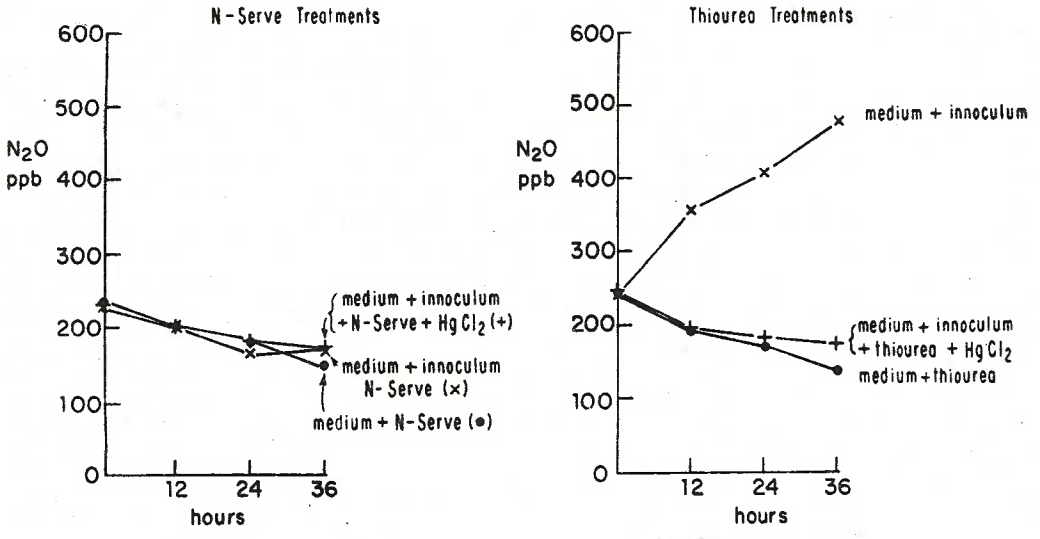

O_2 (mg/liter)
1
2
3
4
5
6
10^{-9}
10^{-10}
(d)
NO_2^-


CELL YIELD $\frac{10^6 \text{cells}}{\text{m mole } NO_2}$
0
1
2
3
4
5
0
5
10
15
20
O_2(% OF CARRIER GAS)


10^{-11}
10^{-12}
N_2O
0
1
2
3
4
5
6
7
O_2 (mg liter^{-1})

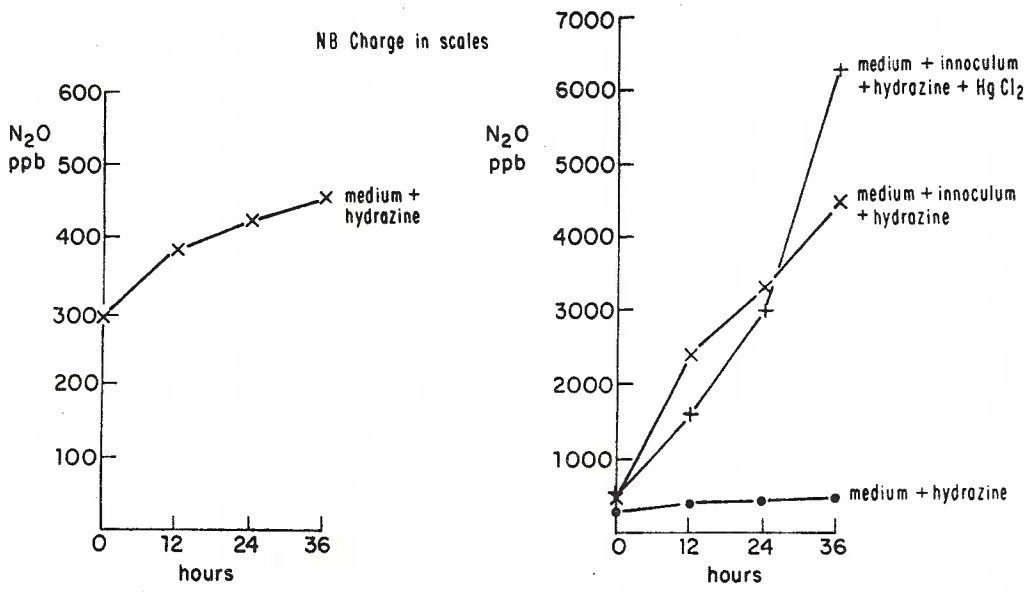

10
%
yield
5
0
0
5
10
20
% O_2

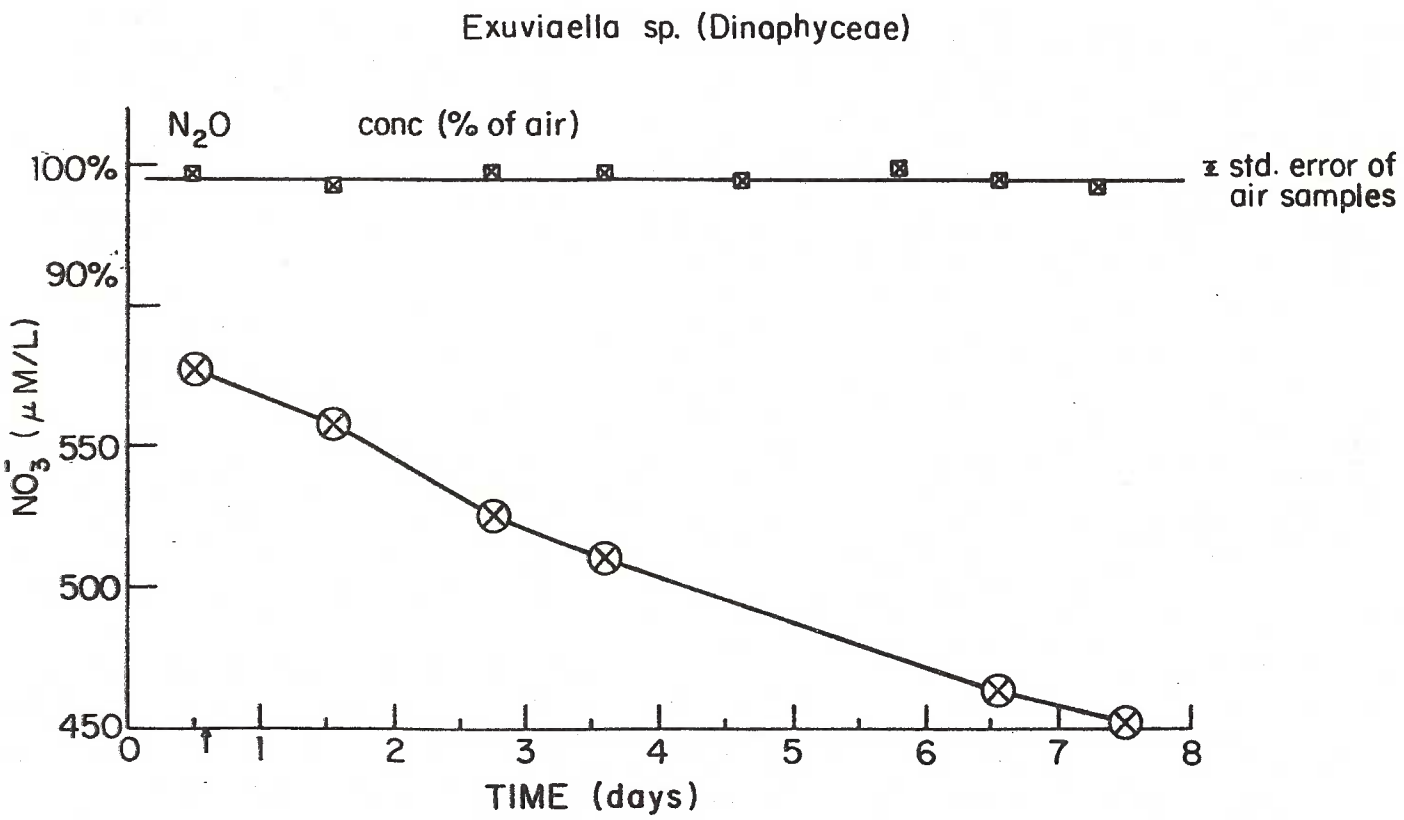

600
500
400
300
200
100
0
N_2O
ppb
12
24
36
hours →
medium +
innoculum
(triplicate runs)
medium + innoculum + $HgCl_2$
medium

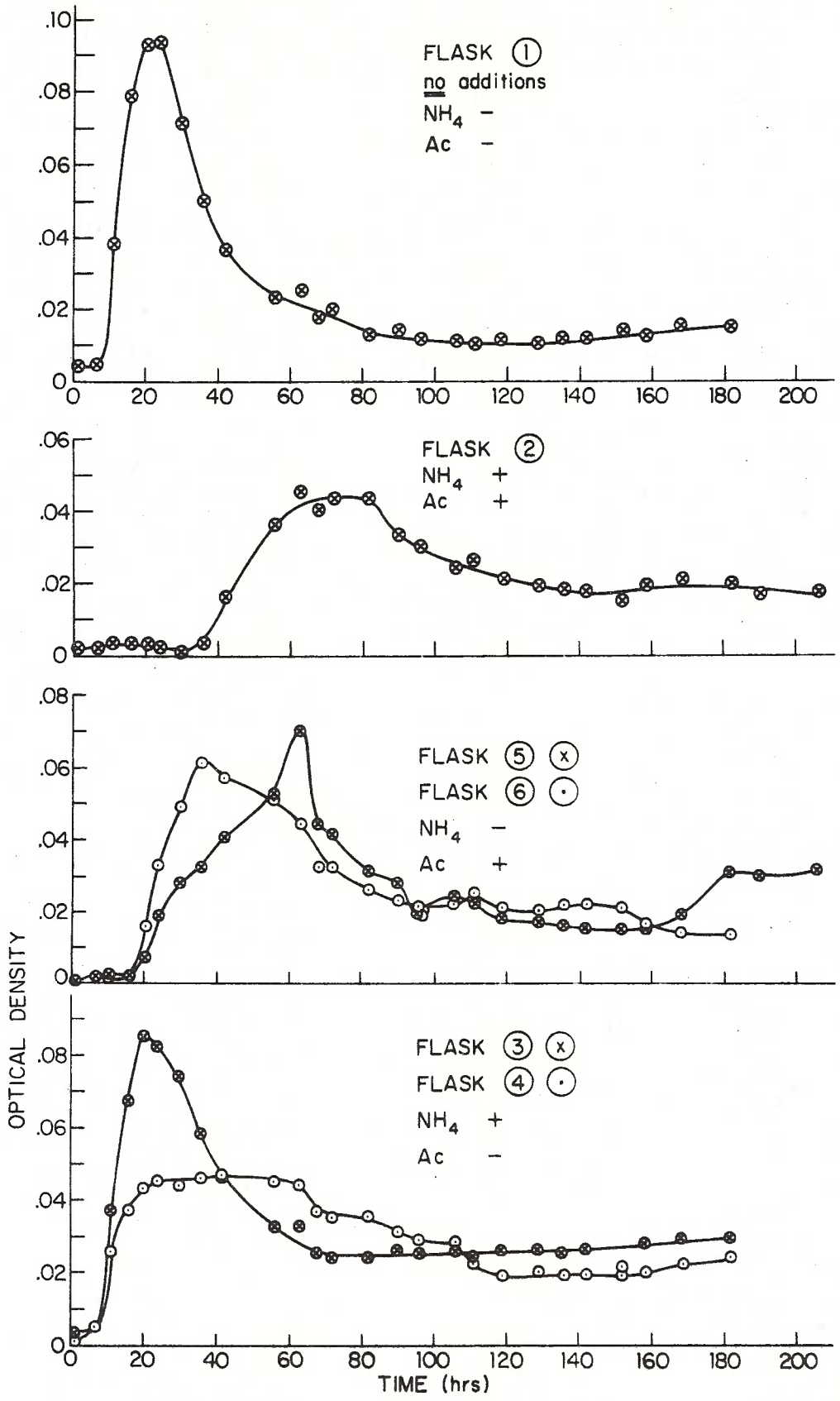


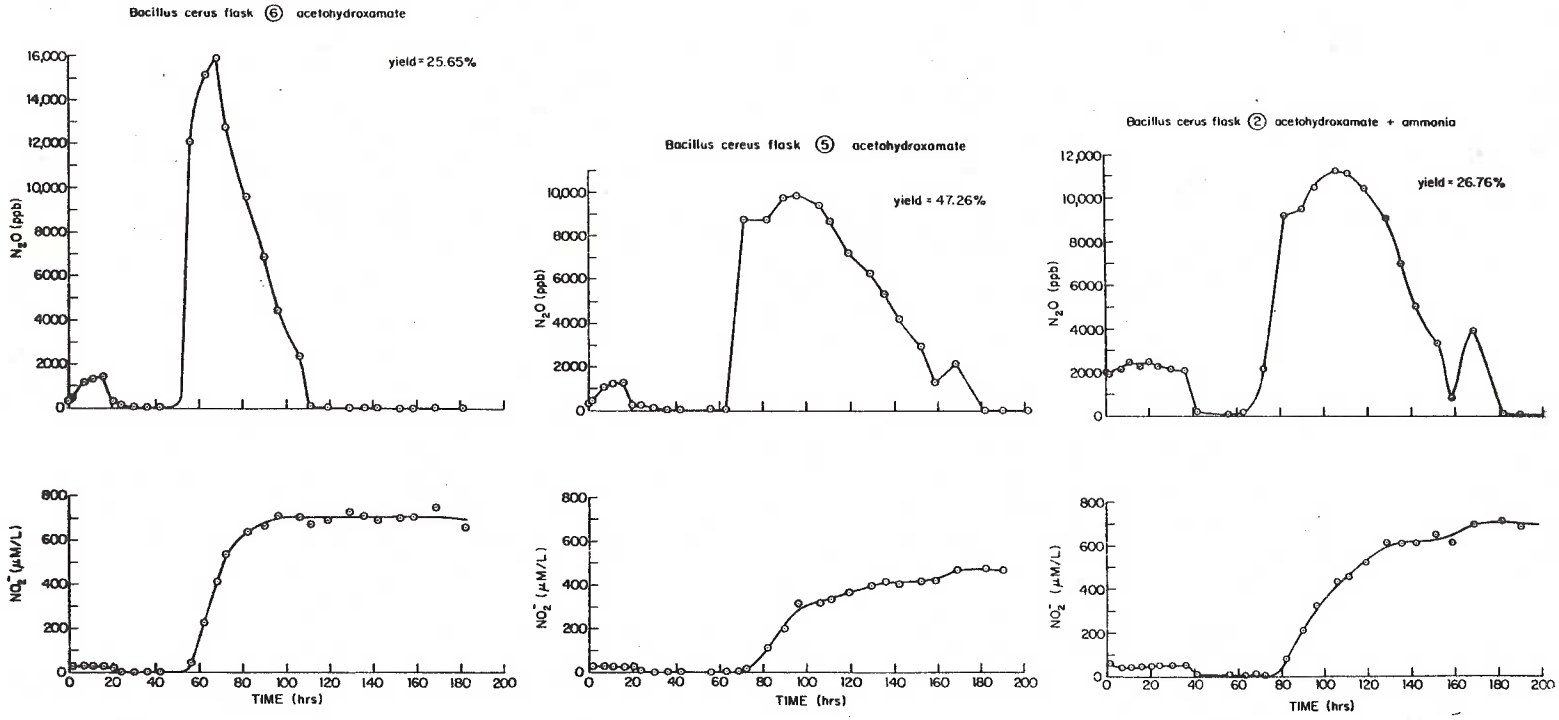
Nitrocystis Oceanus Inhibitor Experiments

Hydrazine Treatments

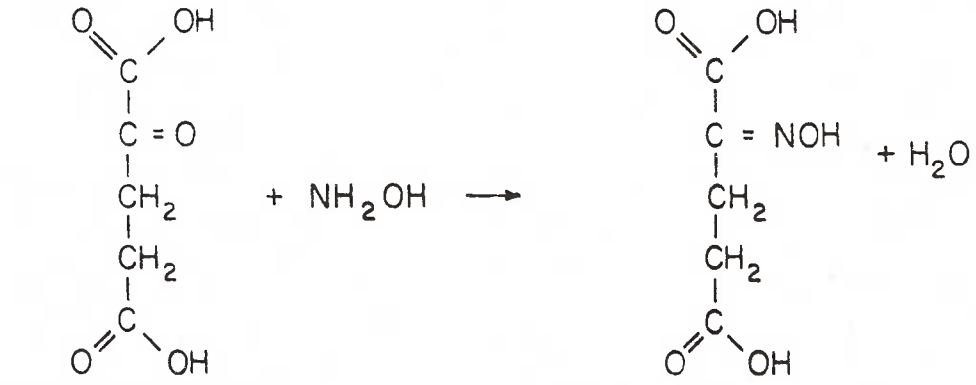



Exuviaella sp. (Dinophyceae)
N_2O conc (% of air)
100%
90%
± std. error of air samples
NO_3^- (μM/L)
550
500
450
0
1
2
3
4
5
6
7
8
TIME (days)

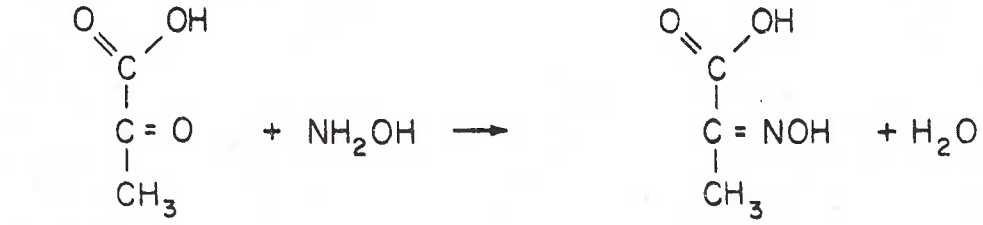

FLASK ①
no additions
NH4 −
Ac −
FLASK ②
NH4 +
Ac +
FLASK ⑤ ⊗
FLASK ⑥ ⊙
NH4 −
Ac +
FLASK ③ ⊗
FLASK ④ ⊙
NH4 +
Ac −
OPTICAL DENSITY
TIME (hrs)
0
.02
.04
.06
.08
.10
0
20
40
60
80
100
120
140
160
180
200


Bacillus cerus flask ⑥ acetohydroxamate
yield = 25.65%
Bacillus cereus flask ⑤ acetohydroxamate
yield = 47.26%
Bacillus cerus flask ② acetohydroxamate + ammonia
yield = 26.76%
N_2O (ppb)
NO_2^- (μM/L)
TIME (hrs)

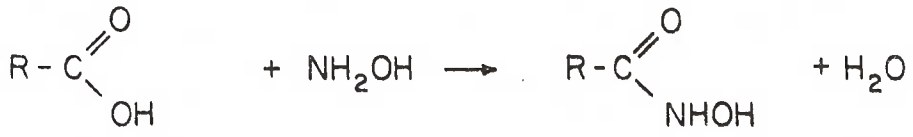
FORMATION OF OXIMES AND HYDROXAMATES



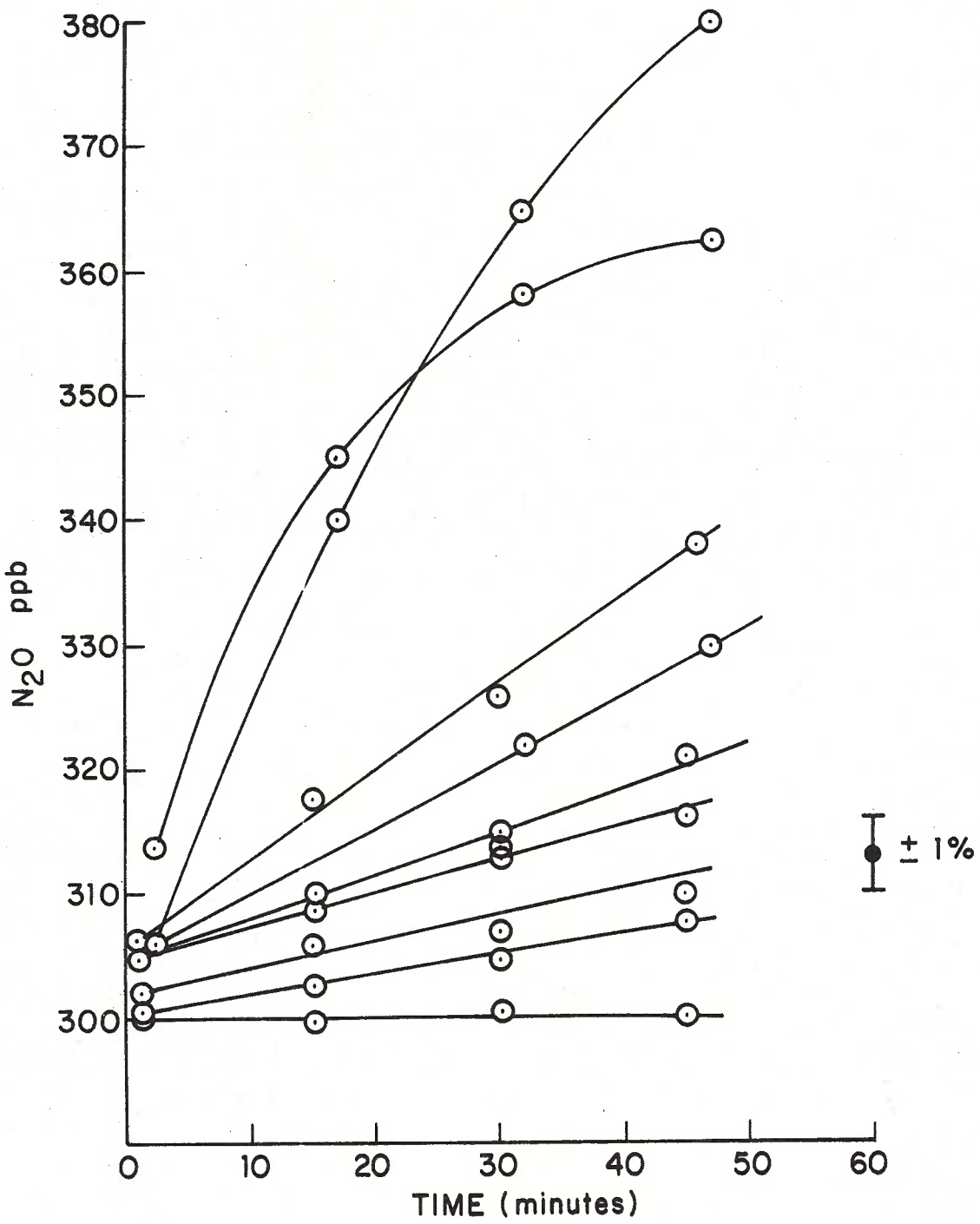
alphaketoglutarate — alphaketoglutarate oxime

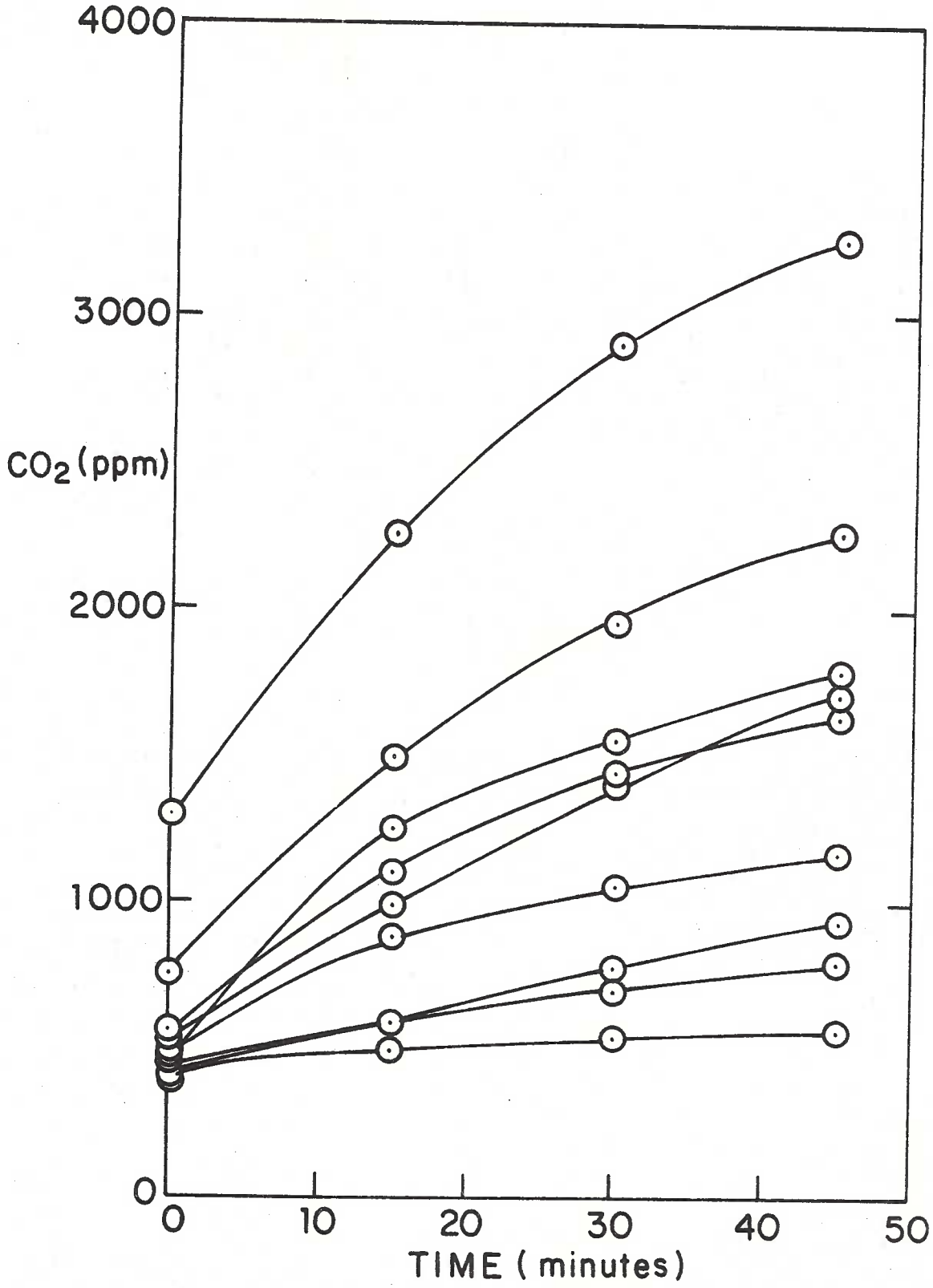


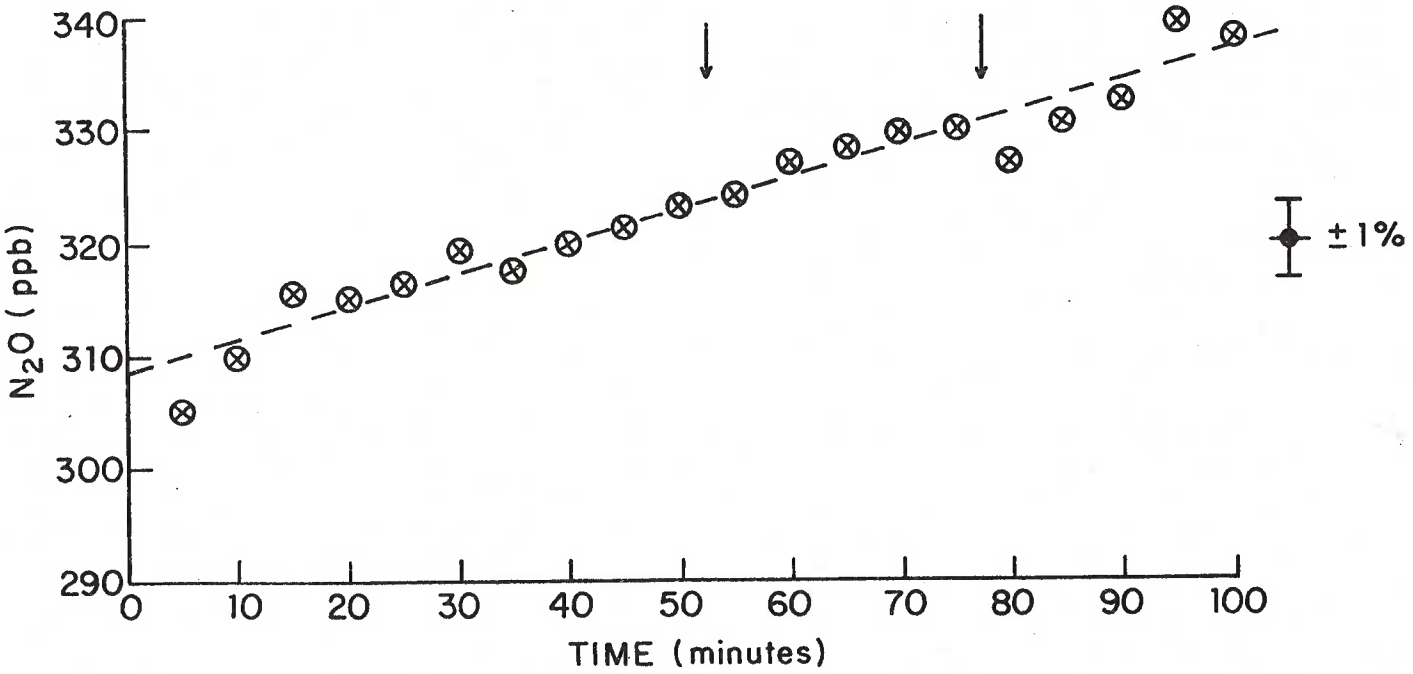
pyruvate — pyruvic oxime

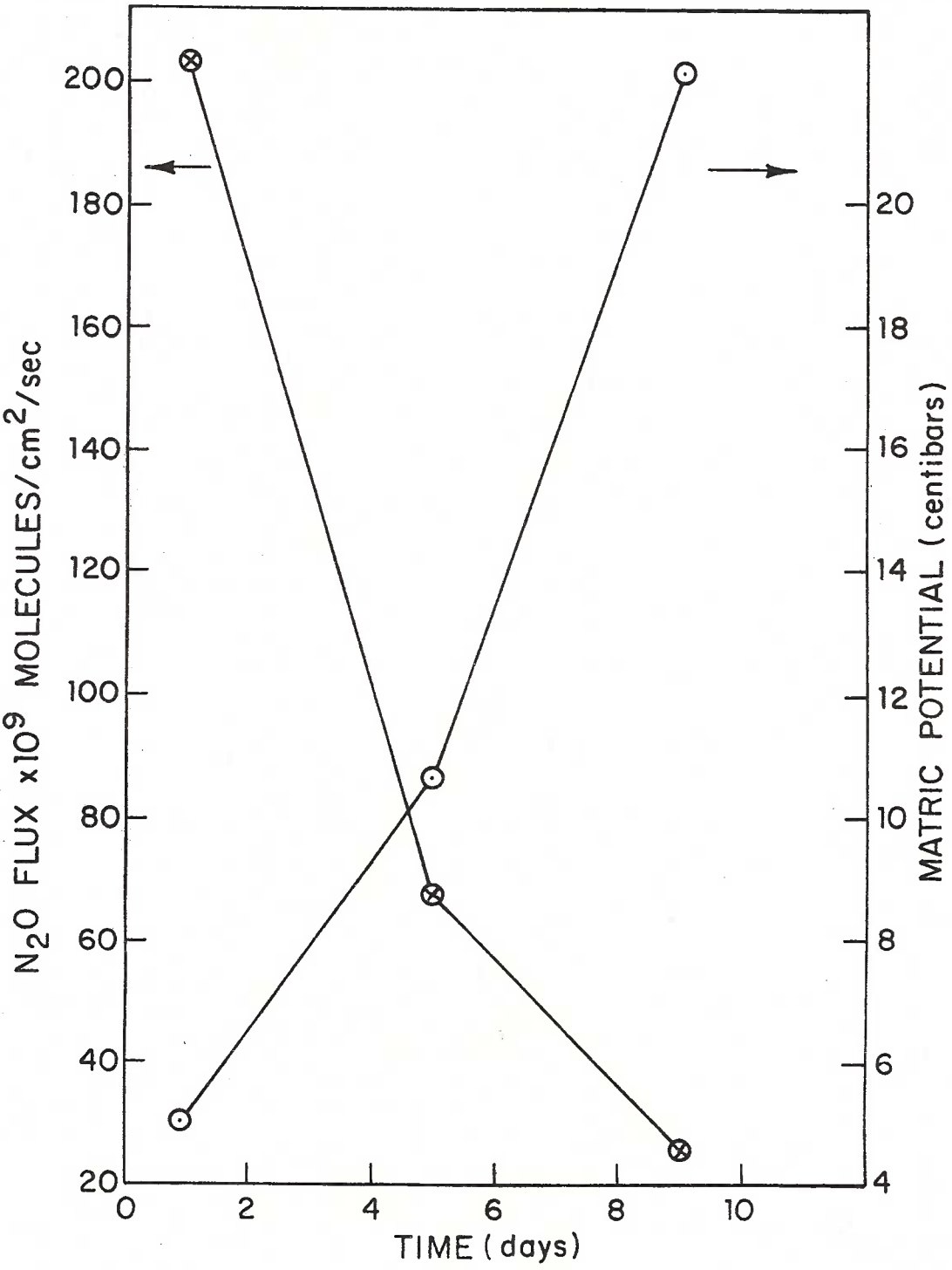


carboxy acid — hydroxamate

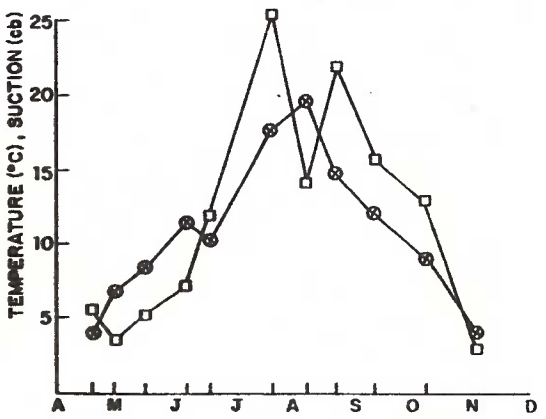

380
370
360
350
340
330
320
310
300
N_2O ppb
0
10
20
30
40
50
60
TIME (minutes)
± 1%


4000
3000
2000
1000
0
CO_2 (ppm)
0
10
20
30
40
50
TIME (minutes)

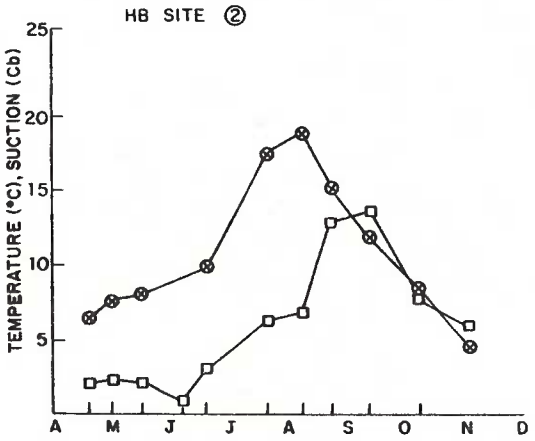

340
330
320
310
300
290
N_2O (ppb)
0
10
20
30
40
50
60
70
80
90
100
TIME (minutes)
±1%


200
180
160
140
120
100
80
60
40
20
0
2
4
6
8
10
20
18
16
14
12
10
8
6
4
TIME (days)
N_2O FLUX $\times 10^9$ MOLECULES/cm^2/sec
MATRIC POTENTIAL (centibars)

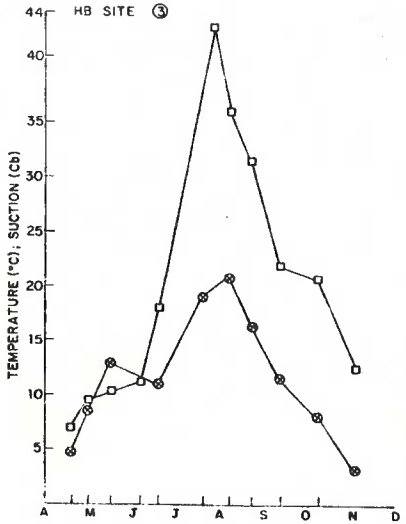
HB SITE ①



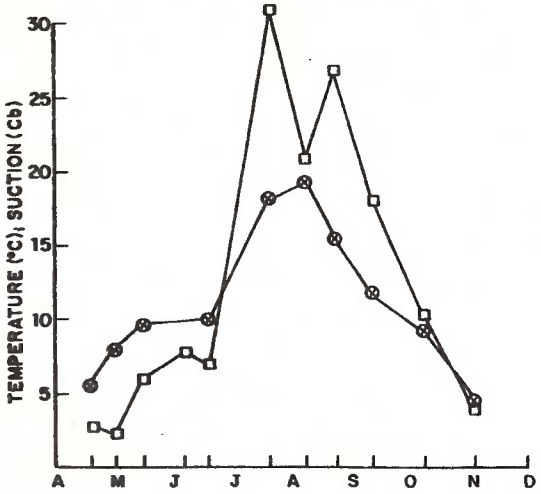
HB SITE ②



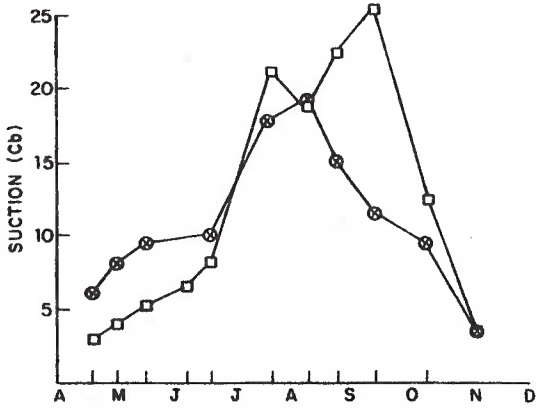
HB SITE ③



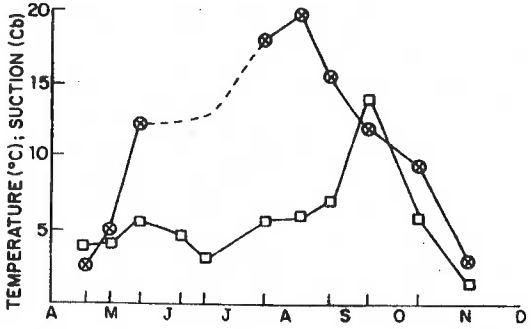
HB SITE ④

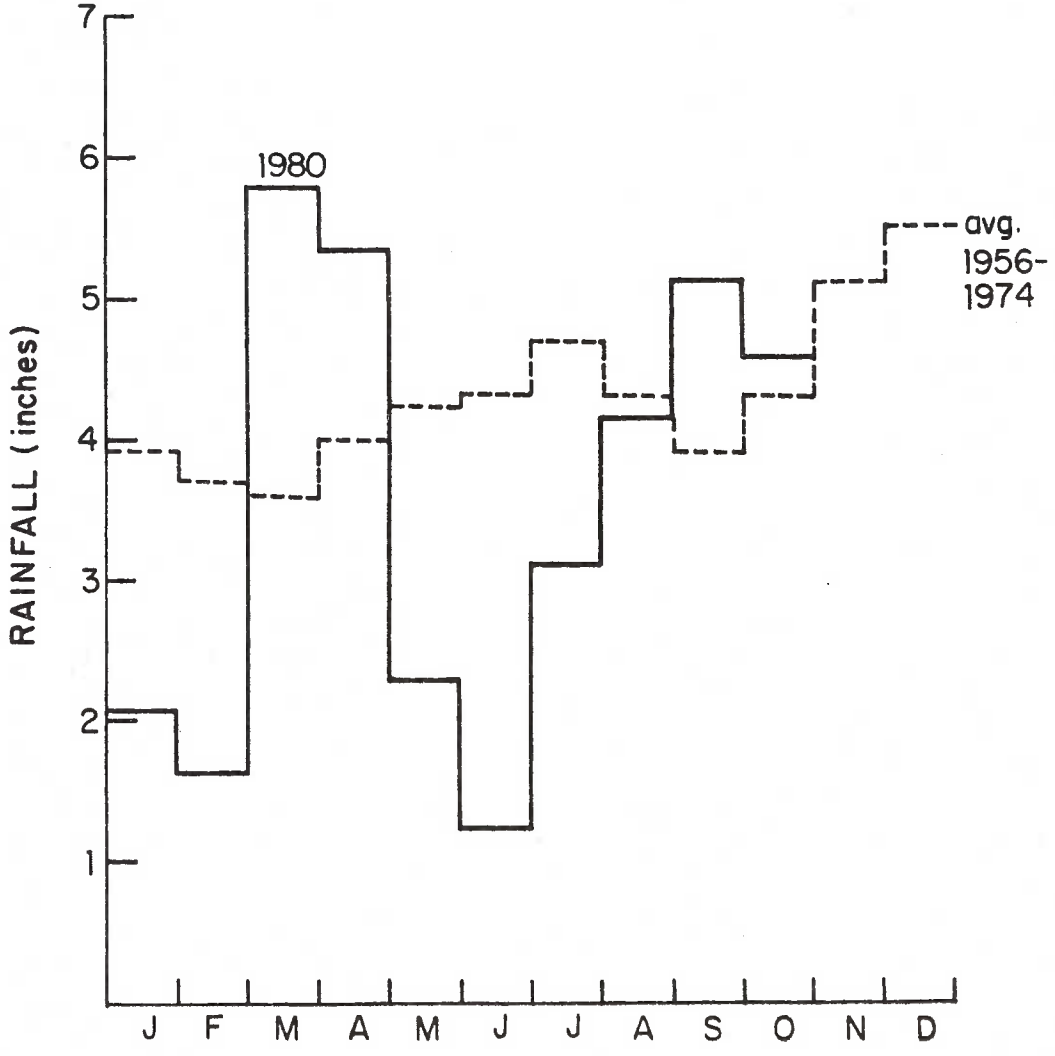


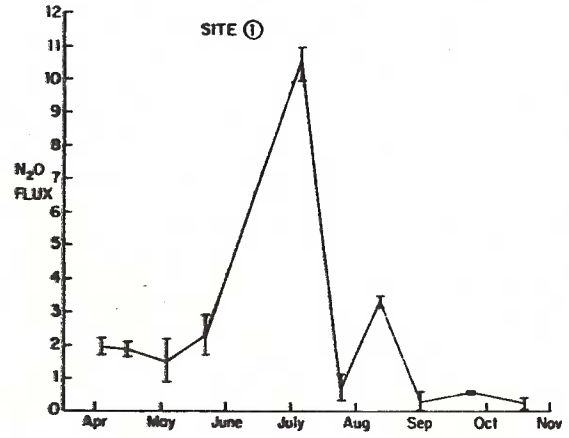
HB SITE ⑤

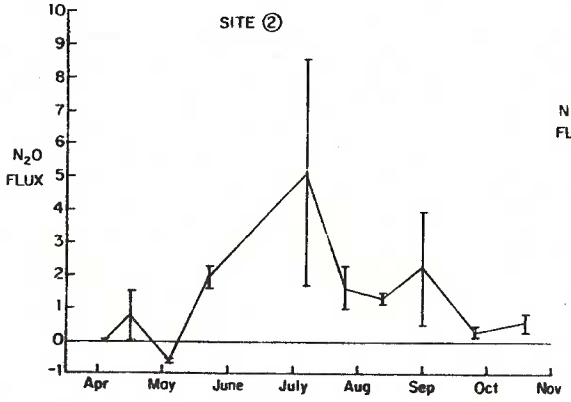


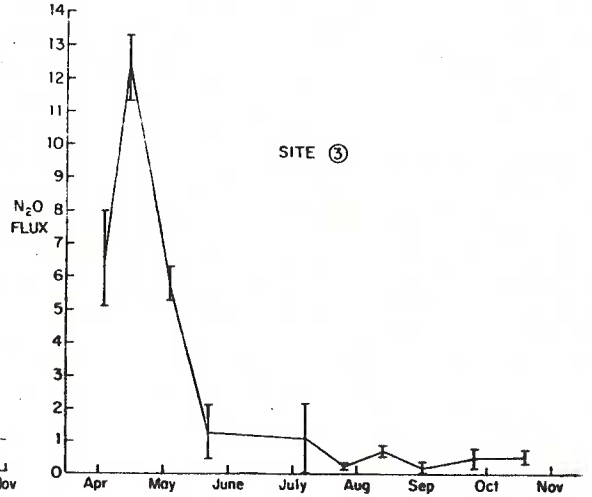
HB SITE ⑥

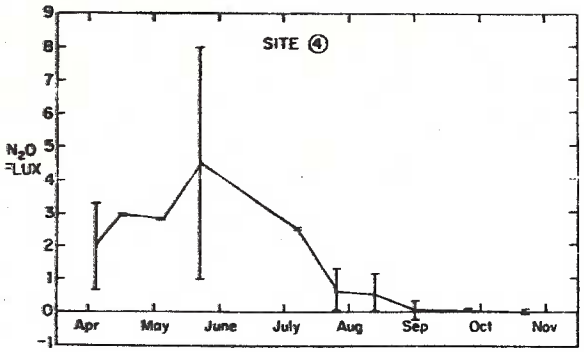


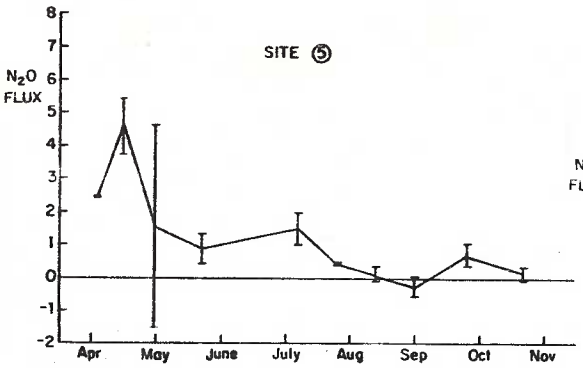

RAINFALL (inches)
7
6
5
4
3
2
1
1980
avg.
1956-
1974
J
F
M
A
M
J
J
A
S
O
N
D

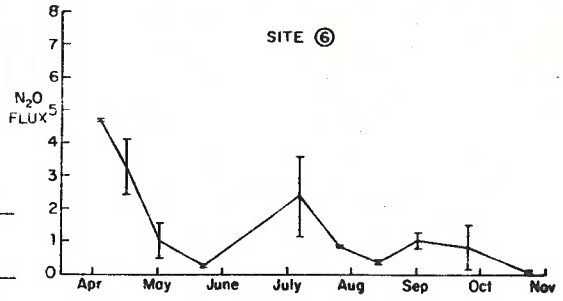

SITE ①
N_2O FLUX
Apr
May
June
July
Aug
Sep
Oct
Nov


SITE ②
N_2O FLUX
Apr
May
June
July
Aug
Sep
Oct
Nov


SITE ③
N_2O FLUX
Apr
May
June
July
Aug
Sep
Oct
Nov


SITE ④
N_2O FLUX
Apr
May
June
July
Aug
Sep
Oct
Nov


SITE ⑤
N_2O FLUX
Apr
May
June
July
Aug
Sep
Oct
Nov


SITE ⑥
N_2O FLUX
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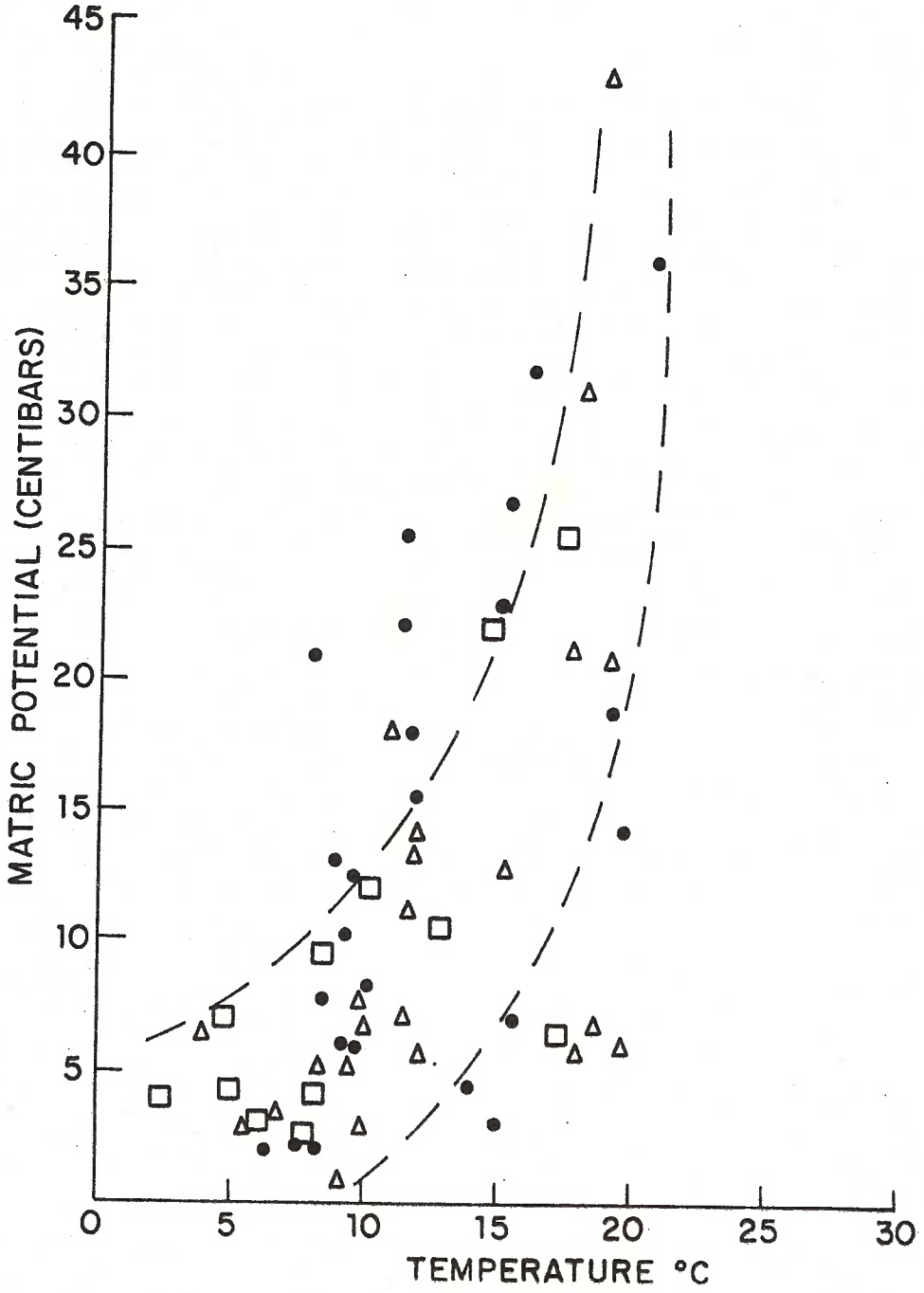
MATRIC POTENTIAL (CENTIBARS)
45
40
35
30
25
20
15
10
5
0
5
10
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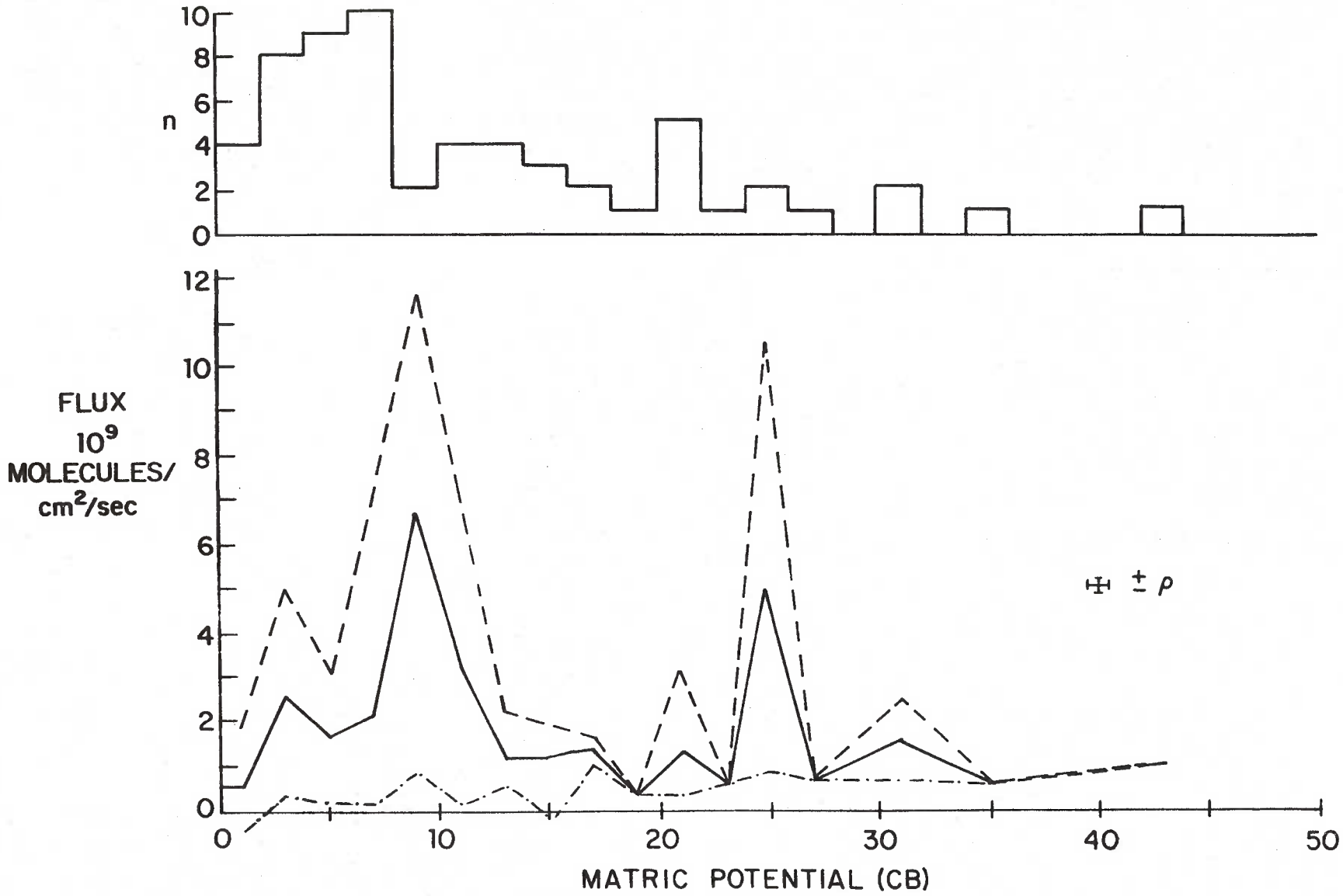
20
25
30
TEMPERATURE °C


n
10
8
6
4
2
0
FLUX
10^9
MOLECULES/
cm^2/sec
12
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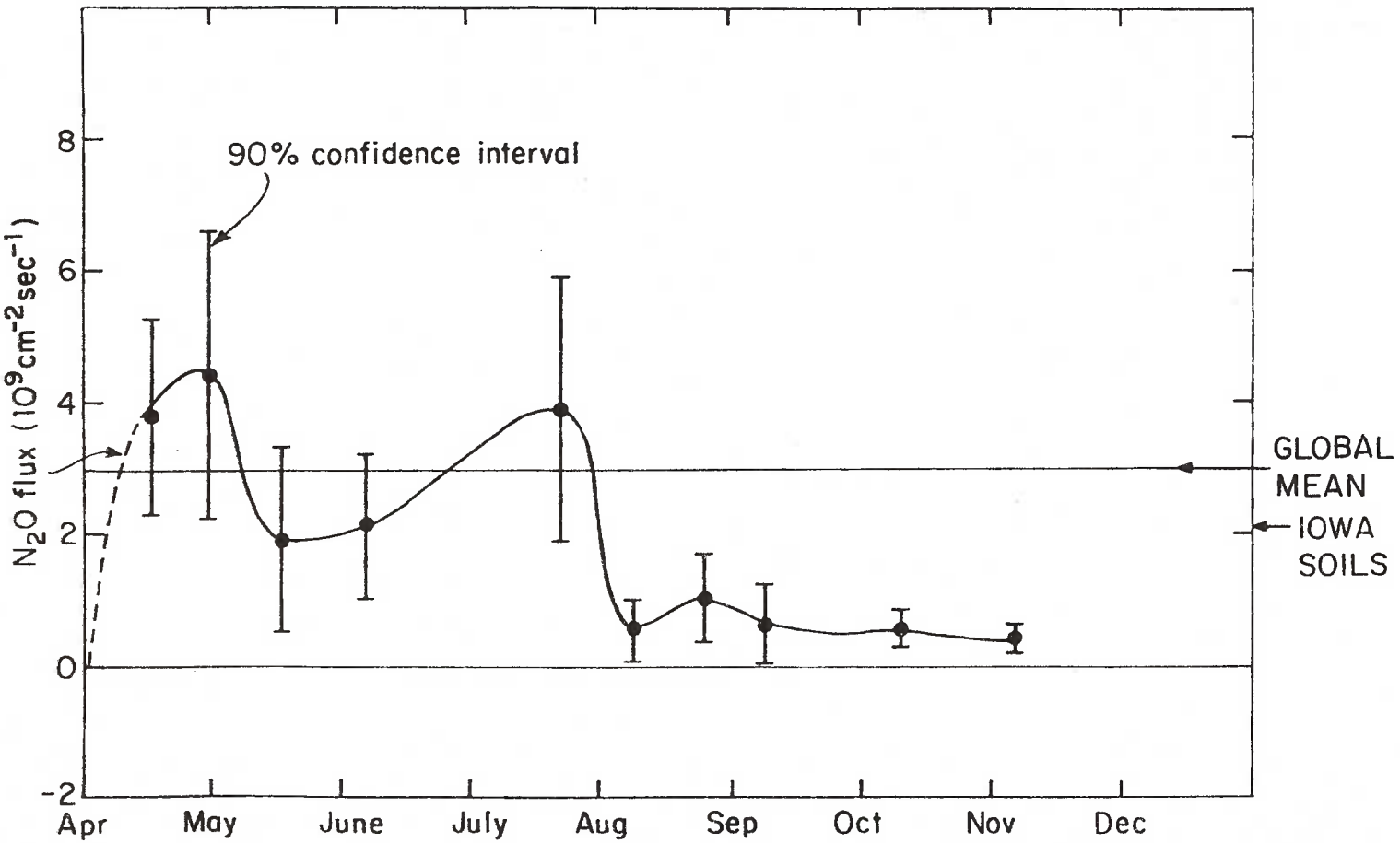
8
6
4
2
0
0
10
20
30
40
50
MATRIC POTENTIAL (CB)
H ± ρ


90% confidence interval
N_2O flux ($10^9 cm^{-2} sec^{-1}$)
8
6
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-2
Apr
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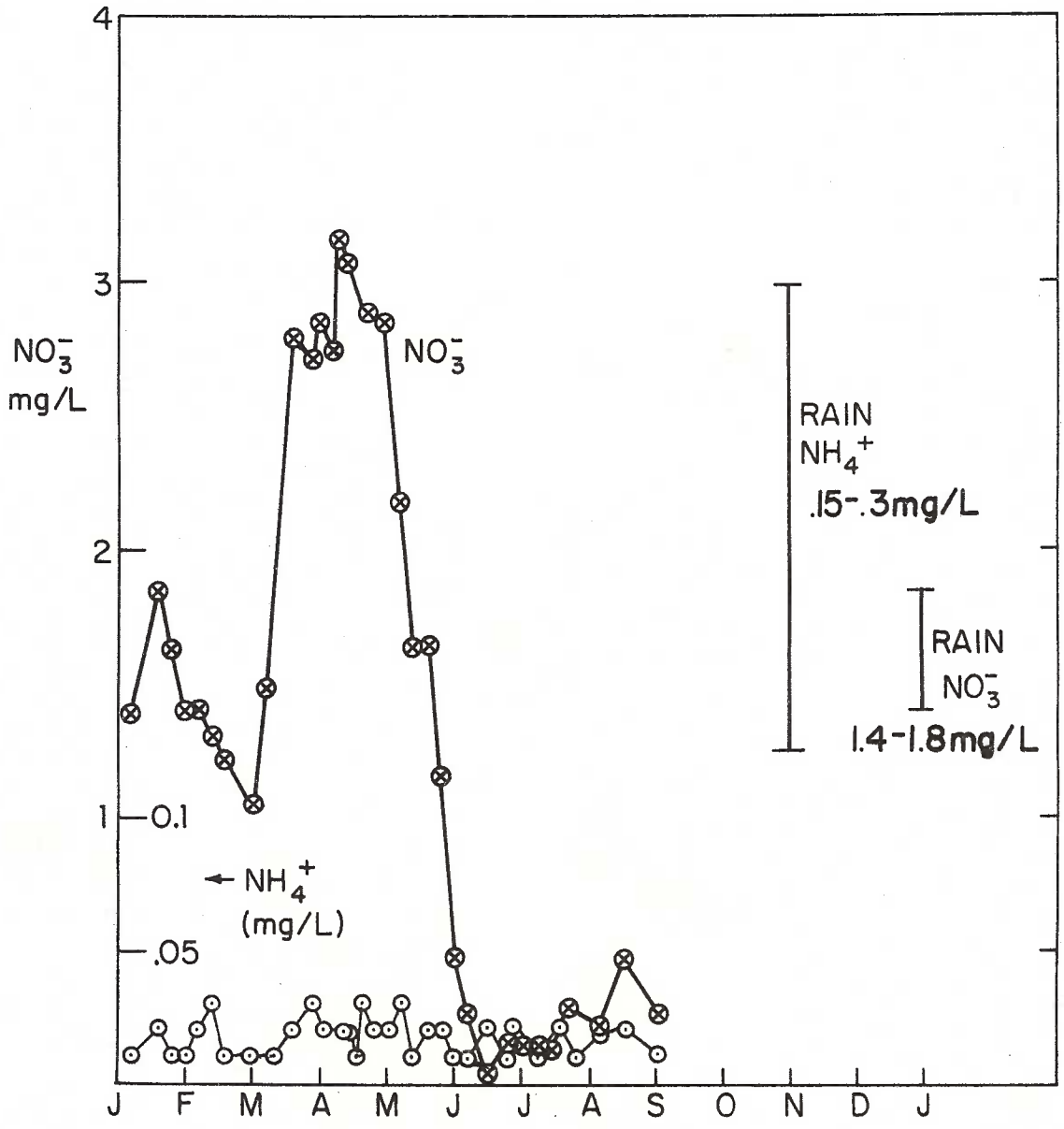
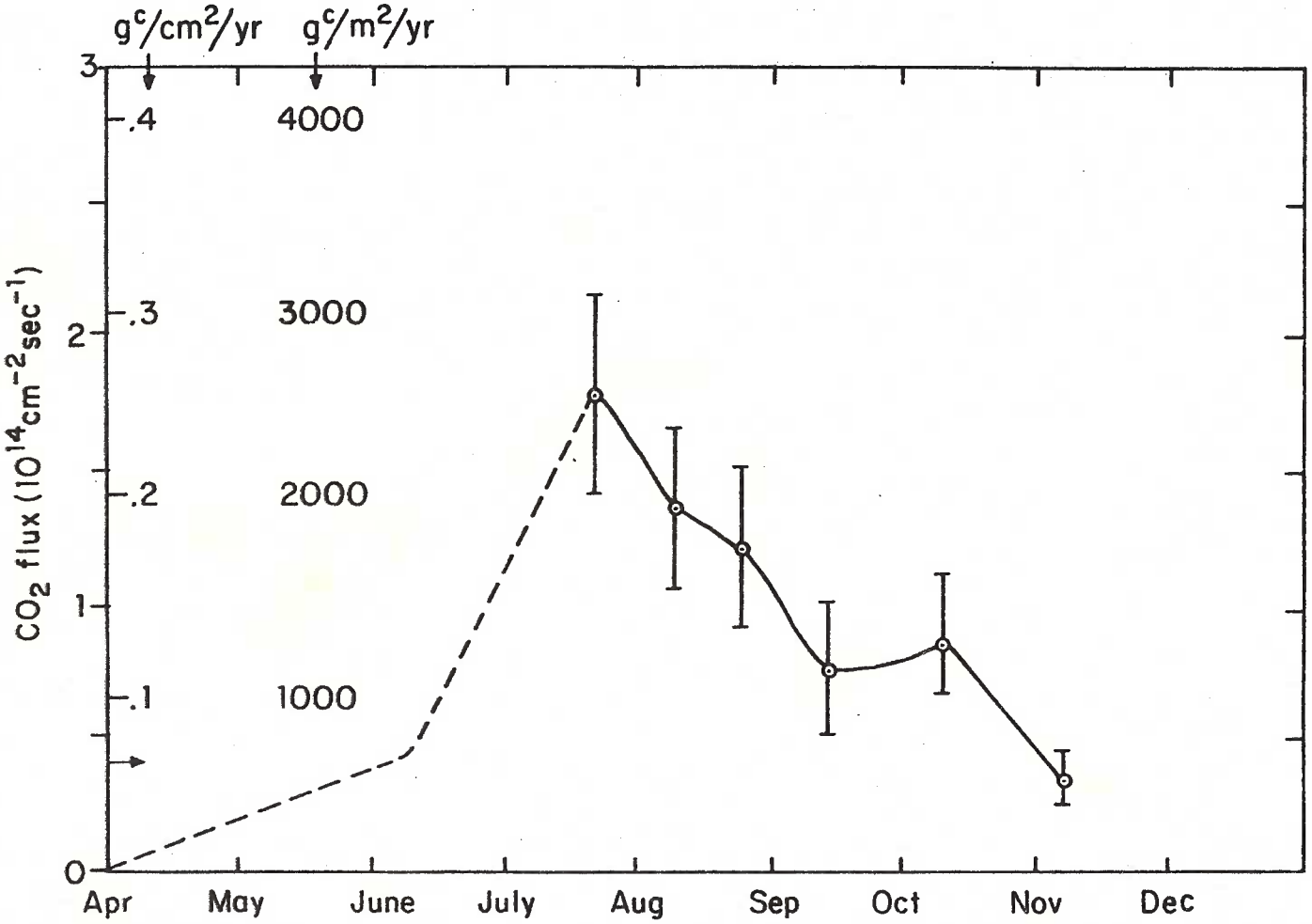
Dec
GLOBAL MEAN
IOWA SOILS


4
3
2
1
0.1
.05
NO_3^- mg/L
NO_3^-
NH_4^+ (mg/L)
RAIN NH_4^+ .15-.3mg/L
RAIN NO_3^- 1.4-1.8mg/L
J F M A M J J A S O N D J


g^c/cm^2/yr
g^c/m^2/yr
3
.4
4000
.3
3000
2
.2
2000
1
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CO2 flux (10^14 cm^-2 sec^-1)
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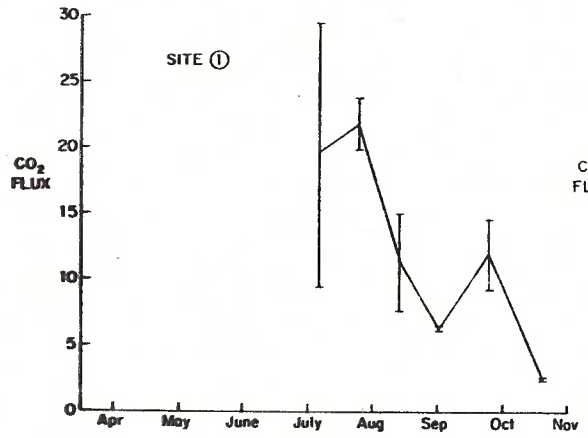
SITE ①
CO_2 FLUX
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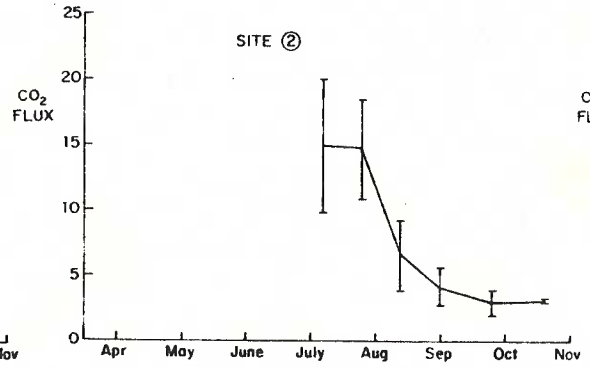
SITE ②
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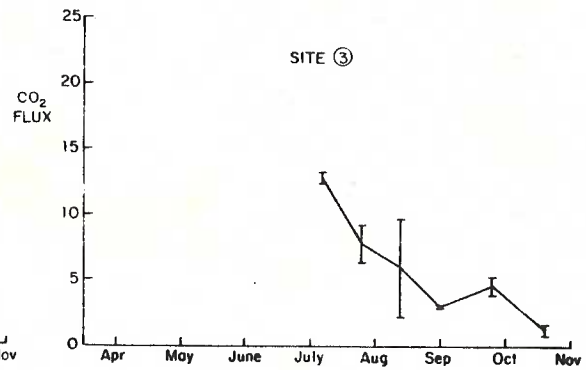
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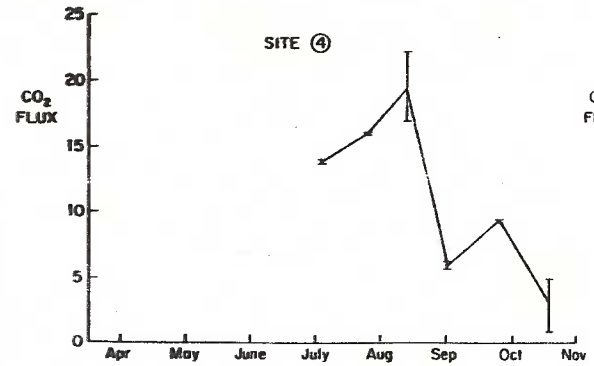
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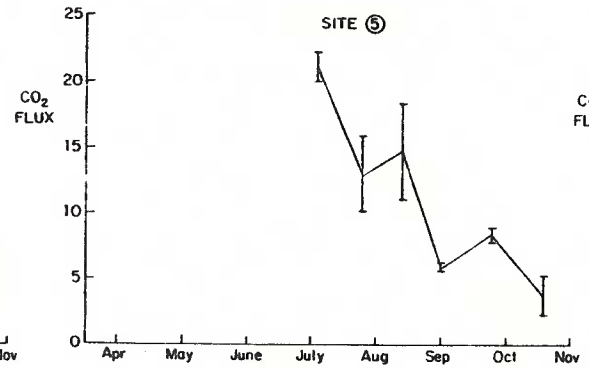
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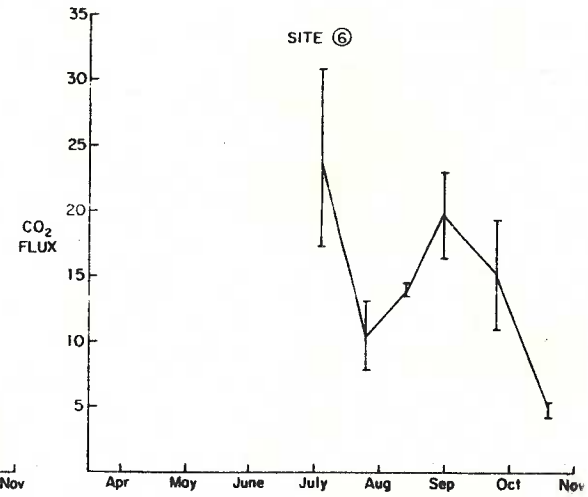
SITE ⑥
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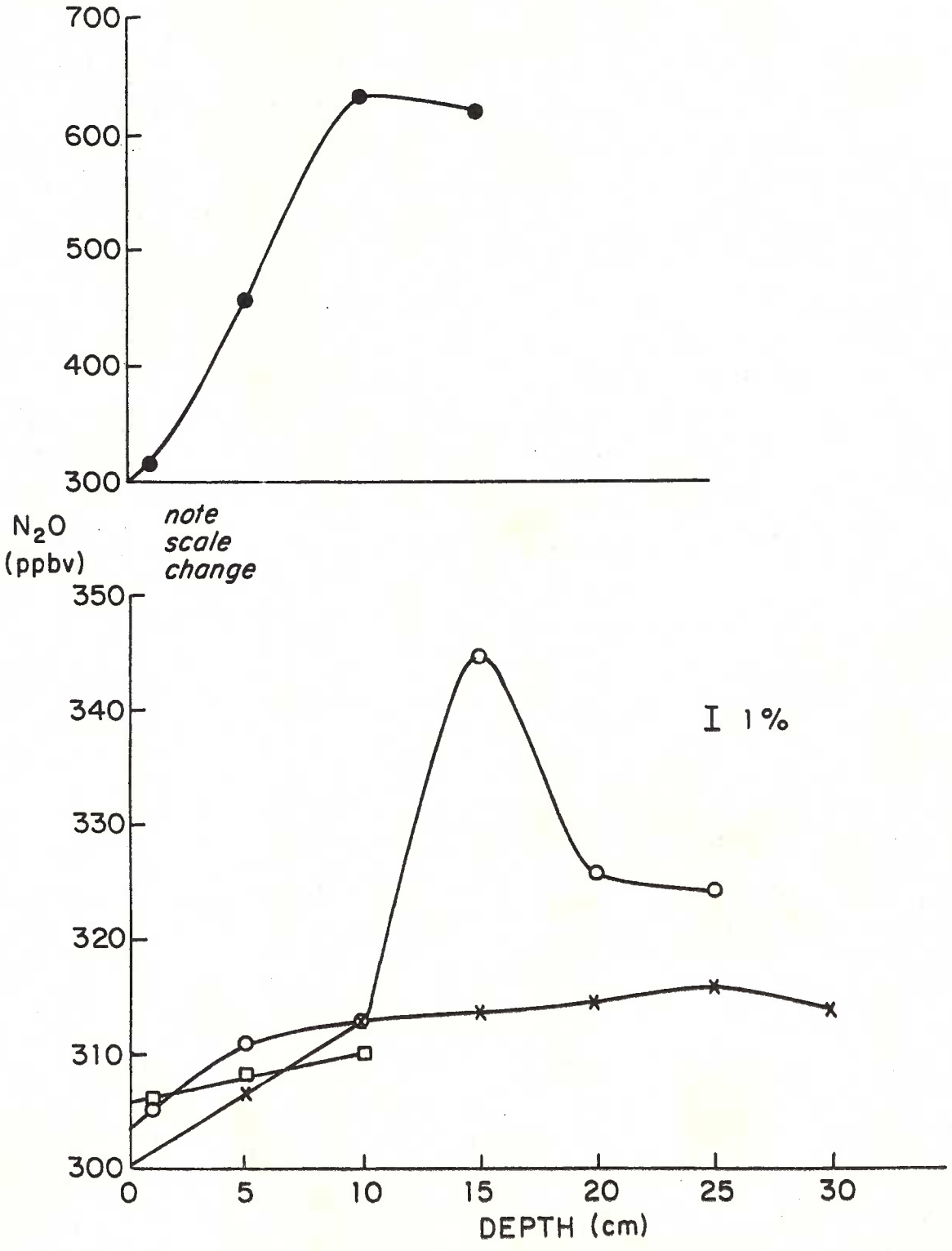
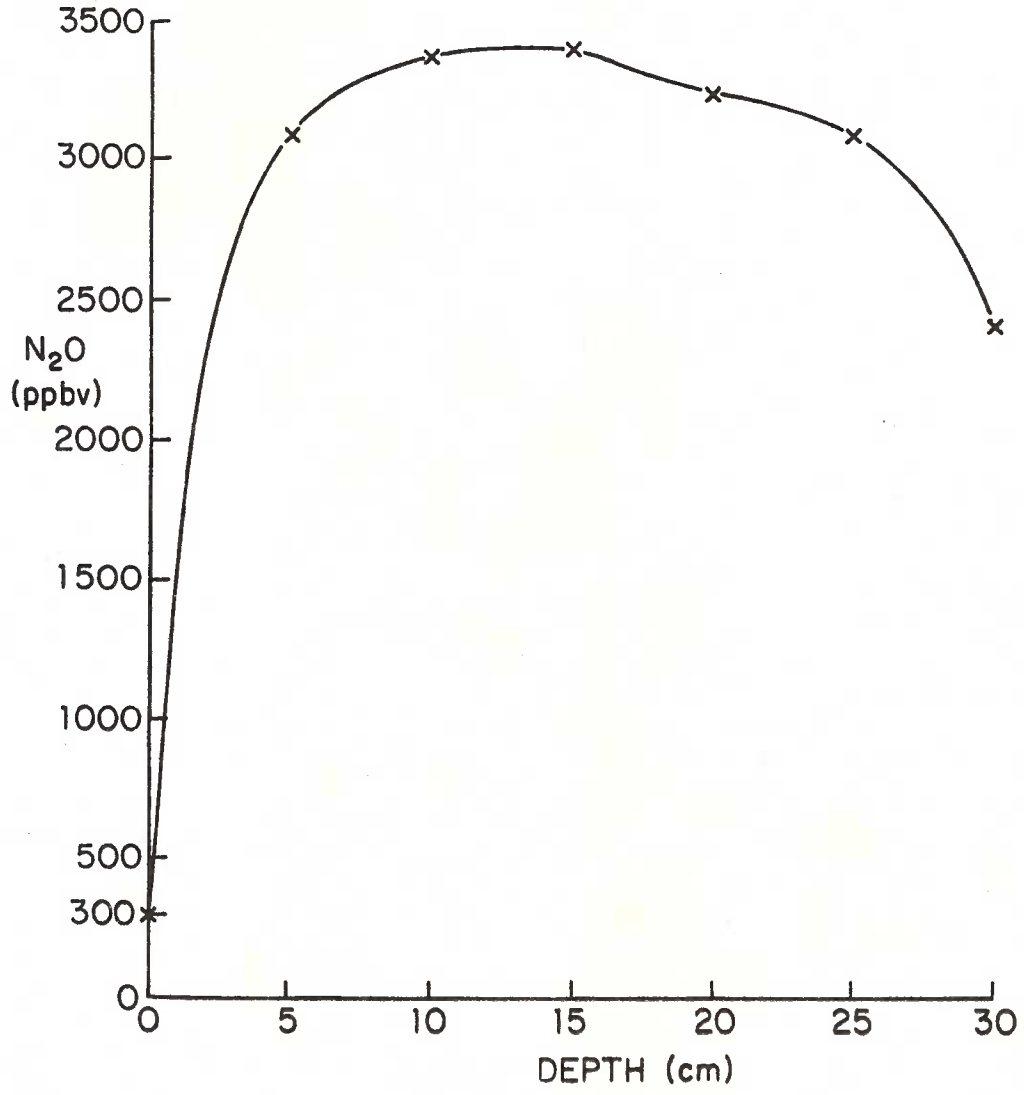

700
600
500
400
300
N_2O
(ppbv)
note
scale
change
350
340
330
320
310
300
I 1%
0
5
10
15
20
25
30
DEPTH (cm)


3500
3000
2500
N_2O
(ppbv)
2000
1500
1000
500
300
0
0
5
10
15
20
25
30
DEPTH (cm)

References

Ajtay, G.L., P. Ketner, and P. Duvigneaud, 1979. Terrestrial primary production and phytomass, in B. Bolin, E.T. Degens, S. Kempe and P. Ketner (eds.) The Global Carbon Cycle, SCOPE Rept. 13, John Wiley & Sons, Chicester.

Alexander, M., 1977. Introduction to Soil Microbiology, 2nd ed. Wiley & Sons, New York.

Anderson, J.H., 1963. The formation of nitrous oixde from hypo-nitrite. Analyst 88, 494-499.

Anderson, J.H., 1964. The metabolism of hydroxylamine to nitrite by Nitrosomonas. Biochem. J. 91, 8-17.

Anderson, J.H., 1964. Studies on the oxidation of ammonia to hydroxylamine by Nitrosomonas. Biochem. J. 92, 1c-3c.

Anderson, J.H., 1965. Estimation of the nitric oxide formed from hydroxylamine by Nitrosomonas. Biochem. J. 94, 236-239.

Anderson, J.H., 1965. Studies on the oxidation of ammonia by Nitrosomonas. Biochem. J. 95, 688-698.

Anderson, J.H., 1965. Studies on the formation of nitrogenous gas from hydroxylamine by Nitrosomonas. Biochim. Biophys. Acta 97, 337-339.

Beevers, L., 1976 Nitrogen metabolism in plants. Arnold, London.

Belser, L.W. and E.L. Schmidt, 1978. Nitrification in soils. In Microbiology-1978, D. Schlesinger (ed.), Am. Soc. Microbiol., Washington, D.C.

Belser, L.W. and E.L. Schmidt, 1980. Growth and oxidation kinetics of three genera of ammonia oxidizing nitrifiers. FEMS Microbiol. Lett. 7, 213-216.

Belser, L.W., 1979. Population ecology of nitrifying bacteria. Ann. Rev. Microbiol. 33, 309-333.

Billen, G., 1976. Evaluation of nitrifying activity in sediments by dark ^{14}C-bicarbonate incorporation. Water Res. 10, 51-57.

Bormann, F.H., G.E. Likens, D.W. Fisher and R.S. Pierce, 1968. Nutrient loss accelerated by clear-cutting of a forest ecosystem. Science 159, 882-884.

Bormann, F.H., T.G. Siccama, G.E. Likens and R.H. Whittaker, 1970. The Hubbard Brook ecosystem study: composition and dynamics of the tree stratum. Ecol. Monogr. 40, 373-388.

Borman, F.H., G.E. Likens, T.G. Siccama, R.S. Pierce and J.S. Eaton, 1974. The export of nutrients and recovery of stable conditions following deforestation at Hubbard Brook. Ecol Monogr. 44, 255-277.

Bormann, F.H., G.E. Likens, and J.M. Melillo, 1977. Nitrogen budget for and aggrading northern hardwood forest ecosystem. Science 196, 981-983.

Bormann, F.H. and G.E. Likens, 1979. Pattern and process in a forested ecosystem. Springer-Verlag, New York.

Brady, N.C., 1974. The nature and properties of soil, 8th ed. MacMillan, New York.

Breitenbeck, G.A., A.M. Blackmer, and J.M. Bremner, 1980. Effects of different nitrogen fertilizers on emission of nitrous oxide from soil. Geophys. Res. Lett. 7, 85-88.

Bremner, J.M. and A.M. Blackmer, 1978. Nitrous oxide: emission from soils during nitrification of fertilizer itrogen. Science 199, 295-296.

Bremner, J.M., S.G. Robbins, and A.M. Blackmer, 1980. Seasonal variability in emission of nitrous oxide from soil. Geophys. Res. Lett. 7, 641-644.

Carlucci, A.F. and P.M. McNally, 1960. Nitrification by marine bacteria in low concentrations of substrate and oxygen. Limnol. Oceanogr. 19, 136-139.

Carlucci, A.F. and J.D. Strickland, 1968. The isolation, purification and some kinetic studies of marine nitrifying bacteria. J. Exp. Mar. Biol. Ecol. 2, 15o-166.

Cohen, Y., 1978. Consumption of dissolved nitrous oxide in an anoxic basin, Saanich Inlet, British Columbia. Nature 272.

Cohen, Y. and L.I. Gordon, 1978. Nitrous oxide in the oxygen minimum of the eastern tropical north Pacific: evidence for its consumption during denitrification and possible mechanisms for its production. Deep-Sea Res. 25, 509-524.

Cohen, Y. and L.I. Gordon, 1979. Nitrous oxide production in the ocean. J. Geophys. Res. 84, 347-353.

Cole, J.A. and C.M. Brown, 1980. Nitrite reduction to ammonia by fermentative bacteria: a short circuit in the biological nitrogen cycle. FEMS Microbiol. Lett. 7, 65-72.

Commoner, B., 1972. The environmental cost of economic growth in S.M. Schurr (ed.) Energy, Economic Growth, an the Environment, Johns Hopkins Univ. Press, Baltimore.

Corbet, A.S., 1934. The formation of hyponitrous acid as an intermediate compound in the biological or photochemical oxidation of ammonia to nitrous acid I. chemical reactions. J. Biochem. 28, 1575-1582.

Corbet, A.S., 1935. The formation of hyponitrous acid as an intermediate compound in biological or photochemical oxidation of ammonia to nitrous acid II. Microbiological oxidation. J. Biochem. 29, 1086-1096.

Crutzen, P.J., L.E. Heidt, J.P. Krasnec, W.H. Pollack, and W. Seiler 1979. Biomass burning as a source of atmospheric gases CO, H_2, N_2O, NO, CH_3Cl and COS. Nature 282, 253-256.

Csaky, T.Z., 1948. On the estimation of bound hydroxylamine in biological materials. Acta Chimica Scandinavica 2, 450-454.

Curtis, E.J.C., K. Durrant, and M. Harman, 1975. Nitrification in rivers of the Trent Basin. Water Res. 9, 255-268.

Delwiche, C.C., 1970. The nitrogen cycle. Sci. Am. 223, 136-146.

Dommergues, Y.R., L.W. Belser, and E.L. Schmidt, 1978. Limiting factors for microbial growth and activity in soil. Adv. Microb. Ecol. 2, 49-104.

Doxtader, K.G. and M. Alexander, 1966. Nitrification by heterotrophic soil microorganisms. Soil Sci. Soc. Amer. Proc. 30, 351.

Edwards, N.T. and P. Sollins, 1973. Continuous measurement of carbon dioxide evolution from partitioned forest floor components. Ecology 54, 406-412.

Edwards, N.T., 1975. Effects of temperature and moisture on carbon dioxide evolution in a mixed deciduous forest floor. Soil Sci. Soc. Amer. Proc. 39, 361-365.

Elkins, J.W., 1978. Aquatic sources and sinks for nitrous oxide. Unpublished thesis, Harvard University.

Elkins, J.W., S.C. Wofsy, M.B. McElroy, C.E. Kolb, and W.A. Kaplan, 1978. Aquatic sources and sinks for nitrous oxide. Nature 275, 602-606.

Elkins, J.W., S.C. Wofsy, M.B. McElroy, and W.A. Kaplan, 1980

Elliott, W.H., 1948. Adenosinetriphosphate in glutamine synthesis. Biochemical J. 42, v-vi.

Engel, M.S. and M. Alexander, 1958. Growth and autotrophic metabolism of Nitrosomas europaea. J. Bacteriol. 76, 217-222.

Falcone, A.B., A.L. Shug, and D.J.D. Nicholas, 1962. Oxidation of hydroxylamine by particles from Nitrosomonas. Biochem. Biophys. Res. Comm. 9, 126-131.

Falcone, A.B., A.L. Shug and D.J.D. Nicholas, 1963. Some properties of a hydroxylamine oxidase from Nitrosomonas europaea. Biochim. Biophys. Acta 77, 199-208.

Focht, D.D. and W. Verstraete, 1977. Biochemical ecology of nitrification and denitrification. In M. Alexander (ed.) Advances in Microbial Ecology, vol.1, 135-214. Plenum Press, New York.

Freney, J.R., O.T. Denmead, and J.R. Simpson, 1978. Soil as a source or sink for atmospheric nitrous oxide. Nature 273, 530-532.

Freney, J.R., O.T. Denmead and J.R. Simpson, 1979. Nitrous oxide emission from soils at low moisture contents. Soil Biol. Biochem. 11, 167-173.

Froment, A., 1972. Soil respiration in a mixed oak forest. Oikos 23, 273-277.

Garrett, H.E. and G.S. Cox, 1973. Carbon dioxide evolution from the floor of an oak-hickory forest. Soil Sci. Soc. Amer. Proc. 37, 641-644.

Gomez, R., 1979, personal communication.

Goreau, T.J., W.A. Kaplan, S.C. Wofsy, M.B. McElroy, F.W. Valois and S.W. Watson, 1980. Production of NO_2^- and N_2O by nitrifying bacteria at reduced concentrations of oxygen. Appl. Environ. Microbiol. 40, 526-532.

Gosz, J.R., G.E. Likens, and F.H. Bormann, 1972. Nutrient content of litter fall on the Hubbard Brook Experimental Forest, New Hamp. Ecology 53, 769-784.

Gosz, J.R., G.E. Likens, and F.H. Bormann, 1973. Nutrient release from decomposing leaf and branch litter in the Hubbard Brook Forest, New Hamp. Ecol. Monogr. 43, 173-191.

Gosz, J.R., G.E. Likens, and F.H. Bormann, 1976. Organic matter and nutrient dynamics of the forest and forest floor in the Hubbard Brook Forest. Oecologia 22, 305-320.

Grossowicz, N., E. Wainfan, E. Borek, and H. Waelsch, 1950. The enzymatic formation of hydroxamic acids from glutamine and asparagine. J. Biol. Chem. 187, 111-125.

Gunderson, K., A.F. Carlucci, and K. Bostrom, 1966. Growth of some chemoautotrophic bacteria at different oxygen tensions. Experientia 22, 229-230.

Hahn, J., 1974. The North Atlantic as a source of atmospheric N_2O. Tellus 26, 160-168.

Hahn, J., 1979. The cycle of atmospheric nitrous oxide. Phil. Trans. Roy. Soc. London A 290, 495-504.

Haynes, R.J. and K.M. Goh, 1978. Ammonium and nitrate nutrition of plants. Biol. Rev. 53, 465-510.

Harms, H., H-P. Koops, and H. Wehrmann, 1976. An ammonia-oxidizing bacterium Nitrosovibrio tenuis nov. gen. nov. sp. Arch. Microbiol. 108, 105-111.

Hewitt, E.J., 1975. Assimilatory nitrate-nitrite reduction. Ann. Rev. Plant. Physiol. 26, 73-100.

Hewitt, E.J., and T.A. Smith, 1974. Plant mineral nutrition. Wiley, New York, 298 p.

Hobbie, J.E., R.J. Daley and S. Jasper, 1977. Use of Nuclepore filters for counting bacteria by fluorescence microscopy. Appl. Environ. Microbiol. 33, 225-228.

Hooper, A.B., 1978. Nitrogen oxidation and electron transport in ammonia-oxidizing bacteria. In Schlessinger, D. (ed.) Microbiology 1978. Am. Soc. Microbiol., Washington, D.C.

Hooper, A.B. and A. Nason, 1965. Characterization of hydroxylamine-cytochrome c reductase from the chemautotrophs Nitrosomonas europaea and Nitrosocystis oceanus. J. Biol. Chem. 240, 4044-4057.

Hooper. A.B. and K.R. Terry, 1979. Hydroxylamine oxioreductase of Nitrosomonas production of nitric oxide from hydroxylamine. Biochim. Biophys. Acta 571, 12-20.

Hudson, R.T. and E.T. Reed, 1979. The stratosphere: present and future. NASA Ref. Publ. 1049, Washington, D.C.

Hutchinson, G.L. and A.R. Mosier, 1979. Nitrous oxide emissions from an irrigated cornfield. Science 205, 1125-1127.

~~Hynes, and R. Knowles, 1978.~~

Jones, U.S., 1979. Fertilizers and soil fertility. Reston Publ. Co., Reston, Va.

Jordan, C.F. and R. Herrera, 1981. Tropical rain forests: are nutrients really critical? Am. Nat. 117, 167-180.

Jordan, C.F., R.L. Todd and G. Escalante, 1979. Nitrogen conservation in a tropical rain forest. Oecologia 39, 123-128.

Kaplan, W.C., J. Elkins, C. Kolb, M.B. McElroy, S.C. Wofsy and A.C. Duran, 1978. Nitrous oxide in freshwater systems: an estimate for the yield of atmospheric N_2O from disposal of human waste. Pure Appl. Geophys. 116, 423-438.

Kira, T., 1978. Community architecture and organic matter dynamics in tropical lowland rain forest of southeast Asia with special reference to Pasoh Forest, West Malaysia. In P.B. Tomlinson and M.H. Zimmerman (eds.) Tropical trees as living systems. Cambridge University Press, Cambridge.

Kluyver, A.J. and H.J.L. Donker, 1926. Die sinheit in der biochemie. Chem. zelle Gewebe 13, 134.

Larcher, W., 1980. Physiological plant ecology. Springer-Verlag, New York.

Levitt, M.D., 1980. Intestinal gas production - recent advances in flatology. New Eng. J. Medicine 302, 1474-1475.

Likens, G.E., F.H. Bormann, and N.M. Johnson, 1969. Nitrification: importance to nutrient losses from a cutover forested ecosystem. Science 163, 1205-1206.

Likens, G.E., F.H. Bormann, N.M. Johnson, D.W. Fisher, and R.S. Pierce, 1970. Effects of forest cutting and herbicide treatment on nutrient budgets in the Hubbard Brook watershed-ecosystem. Ecol. Monogr. 40, 23-47.

Likens, G.E., F.H. Bormann, R.S. Pierce, J.S. Eaton, and N.M. Johnson, 1977. Biogeochemistry of a forested ecosystem. Springer-Verlag, New York.

Likens, G.E., F.H. Bormann, R.S. Pierce, and W.A. Reiners, 1978. Recovery of a forested ecosystem. Science 199, 492-496.

Loveless, J.E. and H.A. Painter, 1968. The influence of metal ion concentration and pH value on the growth of Nitrosomonas strain isolated from activated sludge. J. Gen. Microbiol. 52, 1-14.

Malhi, S.S., F.D. Cook and M. Nyborg, 1979. Inhibition of nitrite formation by thiourea in pure cultures of Nitrosomonas. Soil Biol. Biochem. 11, 431-432.

Mariakulandai, A. and S.M. Manickam, 1975. Chemistry of Fertilizers and Manures, Asia Publishing House, New York.

McElroy, M.B., 1976. Chemical processes in the solar system: a kinetic perspective. IN D.R. Herschbach, ed., Chemical Kinetics, Ser. 2, 9, 127-211.

McElroy, M.B., 1980. Sources and sinks for nitrous oxide. Proc. NATO Adv. Study Inst. Portugal.

McElroy, M.B., S.C. Wofsy, and Y.L. Yung, 1977. The nitrogen cycle: perturbations due to man and their impact on atmospheric N_2O and O_3. Phil. Trans Roy. Soc. B 277, 159-181.

McKenny, D.J., D.L. Wade and W.I. Findlay, 1978. Rates of N_2O evolution from N-fertilized soil. Geophys. Res. Lett. 5, 777-780.

Melillo, J.M., 1977. Mineralization of nitrogen in northern forest ecosystems. Ph.D. thesis, Yale University, New Haven, 136 pp.

Middleton, K.R. and G.S. Smith, 1979. A comparison of ammoniacal and nitrate nutrition of perennial ryegrass through a thermodynamic model. Plant and Soil 53, 487-504.

Miflin, B.J., 1980. Nitrogen metabolism and amino acid biosynthesis in crop plants. In P.S. Carlson (ed.), The Biology of Crop Productivity, Academic Press, New York.

Mortimer, C.H., 1941, 1942. The exchange of dissolved substances between mud and water in lakes. J. Ecol. 29, 280-329, 30i, 147-201.

National Academy of Sciences, 1972. Soils of the humid tropics. Washington, D.C.

National Academy of Sciences, 1977. Methane generation from human, animal, and agricultural wastes. Washington, D.C.

National Academy of Sciences, 1977. Nitrogen oxides. Medical and biologic effects of environmental pollutants. Washington, D.C.

National Academy of Sciences, 1978. Nitrates: an environmental assessment. Washington, D.C.

National Academy of Sciences, 1980. Research priorities in tropical biology. Washington, D.C.

National Research Council, 1979. Ammonia. University Park Press, Baltimore.

Nicholas, D.J.D. and O.T.G. Jones, 1960. Oxidation of hydroxylamine in cell-free extracts of Nitrosomonas europaea. Nature 185, 512-514.

Nielsen, D.R. and J.G. MacDonald, 1978. Nitrogen in the Environment. Academic Press, New York.

Nye, P.H. and D.J. Greenland, 1960. The soil under shifting cultivation, Technical communication no. 51, Commonwealth Bureau of Soils, Commonwealth Agricultural Bureaux, Farnham Royal, Bucks, U.K.

Nye, P.H. and P.B. Tinker, 1977. Solute Movement in the Soil-Root System. Blackwell, Oxford.

Painter, R , 1970. A review of literature on inorganic nitrogen metabolism in microorganisms. Water Res. 4, 393-450.

Rees, M.K., 1968. Studies of the hydroxylamine metabolism of Nitrosomonas europaea, I. purification of hydroxylamine oxidase. Biochem. 7, 353-366.

Rees, M.K., 1968. Studies of the hydroxylamine metabolism of Nitrosomonas europaea. II. Molecular properties of the electron-transport particle, hydroxylamine oxidase. Biochem. 7, 366-372.

Reichle, D.E., B.E. Dinger, N.T. Edwards, W.F. Harris and P. Sollins 1973. Carbon flow and storage in a forest ecosystem. In G.M. Woodwell and E.V. Pecan (eds.) Carbon and the Biosphere, USAEC, Washington, D.C.

Reiners, W.A., 1968. Carbon dioxide evolution from the floor of three Minnesota forests. Ecology 49, 471-483.

Ritchie, G.A.F. and D.J.D. Nicholas, 1972. Identification of the sources of nitrous oxide produced by oxidative and reductive processes in Nitrosomonas europaea. Biochem. J. 126, 1181-1191.

Roberts, J.D. and M.C. Caserio, 1964. Basic Principles of Organic Chemistry. W.A. Benjamin, Inc., New York.

Russell, E.W., 1973. Soil Conditions and Plant Growth, 10th ed. Longman, London.

Schulze, 1967. Soil respiration of tropical vegetation types. Ecology 48, 652-653.

Seifert, J., 1969. The influence of moisture on the degree of nitrification in soil. ACTA Univ. Carolina Biolog., 353-360.

Seifert, J., 1970. The influence of moisture on the degree of nitrification in soil II. ACTA Univ. Carolina Biolog., 467-470.

Smith, P.A.S., 1965. The chemistry of open-chain organic nitrogen compounds, 2 vols. W.A. Benjamin Inc., New York.

Smith, W.H., F.H. Bormann, and G.E. Likens, 1968. Response of chemoautotrophic nitrifiers to forest cutting. Soil Sci. 106, 471-473.

Soderlund, R. and B.H. Svensson, 1976. The global nitrogen cycle. In Svensson, B.H. and R. Soderlund (eds) Nitrogen, Phosphorus, and Sulphur-Global Cycles. SCOPE Rept. 7, Ecol. Bull. (Stockholm) 22, 23-73.

Somville, M., 1978. A method for the measurement of nitrification rates in water. Water Res. 12, 843-848.

Stainton, M.P., M.J. Capel, and F.A.J. Armstrong, 1977. The Chemical Analysis of Fresh Water, 2nd ed. Can. Fish Mar. Serv. Misc. Spec. Publ. 25: 180 p.

Stalfelt, M.G., 1972. Stalfelt's Plant Ecology. Longman, London.

Stark, N.M. and C.F. Jordan, 1978. Nutrient retention by the root mat of an Amazonian rain forest. Ecology 59, 434-437.

Stumpf, P.K. and W.D. Loomis, 1950. Observations on a plant amide enzyme system requiring manganese and phosphate. Arch. Biochem. 25, 451-453.

Thomas, W.L., 1956. (ed.) Man's Role in Changing the Face of the Earth, 2 vols. University of Chicago Press, Chicago.

Tuffy, T.J., J.V. Hunter, and V.A. Matulewich, 1974. Zones of nitrification. Water Res. Bull. 10, 555-564.

Virtanen, A.I. and H. Jarvinen, 1951. On the formation of Bound hydroxylamine in Azotobacter. Acta Chemica Scand. 5, 22-226.

Waelsch, H., E. BOrek, N. Grossowicz and M. Schou, 1950. Glutomo-and asparto- transferases. Federation Proc. 9, 242.

Wallace, W. and D.J.D. Nicholas, 1969. Glutamate dehydrogenase in Nitrosomonas europaea and the effect of hydroxylamine oximes and related compounds on its activity. Biochim. Biophys. Acta 171 229-237.

Watson, S.W., 1965. Characteristics of a marine nitrifying bacterium Nitrosocystis oceanus sp. N. Limn. & Oceanogr. 10, suppl. R274-R289.

Watson, S.W., 1971. Reisolation of Nitrosospira briensis W. Winogradsky and H. Winogradsky 1933. Archiv. fur Mikrobiol. 75, 179-188.

Watson, S.W., 1974. Gram-negative chemolithotropic bacteria. In R.E. Buchanan and N.E. Gibbons (eds) Bergey's Manual of Determinative Bacteriology, 8th ed. Williams and Wilkins Co., Baltimore.

Watson, S.W., L.B. Graham, C.C. Remsen and F.W. Valois, 1971. A lobular, ammonia-oxidizing bacterium, Nitrosolobus multiformis Nov. Gen. Nov. Sp. Archiv. fur Mikrobiol. 76, 183-203.

Watson, S.W. and C.C. Remsen, 1969. Macromolecular subunits in the walls of marine nitrifying bacteria. Science 163, 685-686.

Weiss, R.F., 1981. The temporal and spatial distribution of tropospheric nitrous oxide. J. Geophys. Res., in press.

Weiss, R.F. and H. Craig, 1976. Production of atmospheric nitrous oxide by combustion. Geophys. Res. Lett. 3, 751-753.

Whittaker, R.H. and G.E. Likens, 1973. Carbon in the biota, in G.M. Woodwell and E.V. Pecan (eds) Carbon and the Biosphere. AEC Symp. Ser. 30, Springfield, Va.

Whittaker, R.H., F.H. Bormann, G.E. Likens and T.G. Siccama, 1974. The Hubbard Brook ecosystem study: forest biomass and production. Ecol. Monogr. 44, 233-252.

Woodwell, G.M. and D.B. Botkin, 1973. Metabolism of terrestrial ecosystems by gas exchange technique: the Brookhaven approach. In D.E. Reichle (ed) Analysis of Temperate Forest Ecosystems. Springer-Verlag, New York, 73-85.

Woodwell, G.M. and W.R. Dykeman, 1966. Respiration of a forest measured by CO_2 accumulation during temperature inversions. Science 154, 1031-1034.

Yoshida, T. and M. Alexander, 1970. Nitrous oxide formation by Nitrosomonas europaea and heterotrophic microorganisms. Soil Sci. Soc. Amer. Proc. 34, 880-882.

Yoshida, T. and M. Alexander, 1971. Hydroxylamine oxidation by Nitrosomnas europaea. Soil Sci. 3, 307-312.

Yoshinari, T., 1976. Nitrous oxide in the sea. Mar. Chem. 4, 189-202.

Yoshinari, T., 1980. N_2O reduction by Vibrio succinogenes. Appl. and Env. Microbiol. 39, 81-84.

Yung, Y.L., W.C. Wang, and A.A. Lacis, 1976. Greenhouse effect due to atmospheric nitrous oxide. Geophys. Res. Lett. 3, 619-621.

Zobel, C.E., 1935. Oxidation-reduction potentials and the activity of marine nitrifiers. J. Bacteriol. 29, 78-88.