NON-BIOLOGICAL DECOMPOSITION OF·NITRITE IN FOREST SOILS

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CONTENTS

The use of fertilizers in forest nurseries is a common practice and interest in using them in forest stands is increasing. In North America, urea is generally used as the source of N for treating stands, although ammonium nitrate is used extensively in the nurseries. Under nursery conditions, urea and other ammoniacal forms of nitrogen are nitrified causing temporary accumulations of nitrite (NO_2^-) and nitrate (NO_3^-).

Since NO_2^- and NO_3^- are subject to chemical breakdown in acid soils (Reuss and Smith 1965), the products of NO_2^- decomposition were investigated in acid forest soils with various pH and organic matter contents. The effect of adding some of the intermediate products (nitrogen dioxide and trioxide, NO_2 and N_2O_3) of NO_2^- decomposition to the soils on the formation of nitrous oxide (N_2O) and molecular nitrogen (N_2) is also reported here.

MATERIALS AND METHODS

Materials

Some properties of the soils used in this study are presented in Table 1. Soils 1 and 3 were collected from separate white spruce stands with very thin humus layers. Soil 6 was collected at a depth of 3 to 6 inches from a burned jack pine stand. These soils were collected near Petitcodiac, N.B. Soils 23 to 26 were collected from a black spruce stand at the Acadia Forest Experiment Station. Soils 23 and 24 were composed of the interphase layers of H and Ae horizons of a typical podzol soil, the only difference between them being the vegetation on the surface layer: Soil 23 had sphagnum mosses (*Sphagnum* spp.), while Soil 24 had feather mosses (*Pleurozium* spp. and *Hypnum* spp.) on the surface. Soils 25 and 26 were prepared by mixing 90% by weight of mineral soil (Ae) from an area with a mat of feather mosses and 10% by weight of F layer raw humus from feather and sphagnum mats respectively.

Table 1. Some properties of the soil samples used in this investigation

Sample no.	Tree species[a]	Horizon	Thickness, inches	pH	Organic matter, %	Moisture content, %	
						Air-dry	0.3 bar
1	wS	Ae	3-6	4.6	3.5	2.3	28
3	wS	Ae	3-6	4.2	4.9	2.4	32
6	jP	Bf	3-6	5.2	5.5	3.0	18
23	bS	H-Ae	5-6	3.3	19.2	4.5	25
24	bS	H-Ae	5-6	3.1	19.2	4.4	25
25	bS	Ae + F[b]		3.1	27.4	5.1	25
26	bS	Ae + F[b]		3.2	28.6	5.2	25

a. wS = white spruce; jP = jack pine; and bS = black spruce.

b. Composed of 90% Ae and 10% F.

Experimental

Since decomposition of NO_2^- is a fast reaction, the NO_2^- must not react with the medium until all the samples are ready for incubation or gaseous products will be lost. Therefore, NO_2^- must be added to all the samples within a minimum of time. Preliminary experiments were carried out to determine the most suitable method of adding NO_2^- to the samples to achieve this goal.

Each sample was incubated at room temperature (22-24°C) in a 250-ml filtering flask fitted with a rubber stopper and serum cap in the center (Fig. 1) after the air had been replaced with 80:20 helium-oxygen mixture by repeated evacuation (Reuss and Smith 1965). Continuous displacement was not used because it is considerably slower and large volumes of gas mixture are required.

An air-dry sample equivalent to 50 g oven-dry soil was weighed into a wax-coated paper cup and moistened with distilled water (5 ml less than the moisture content at 0.3 bar tension (Table 1) minus air-dry moisture content). After equilibration for 0.5 hour, the soil was put into the incubation chamber and a glass vial containing 2 N alkaline potassium permanganate solution was placed with its top slightly above the soil to allow for maximum absorbtion of the oxides of N evolved (Reuss and Smith 1965).
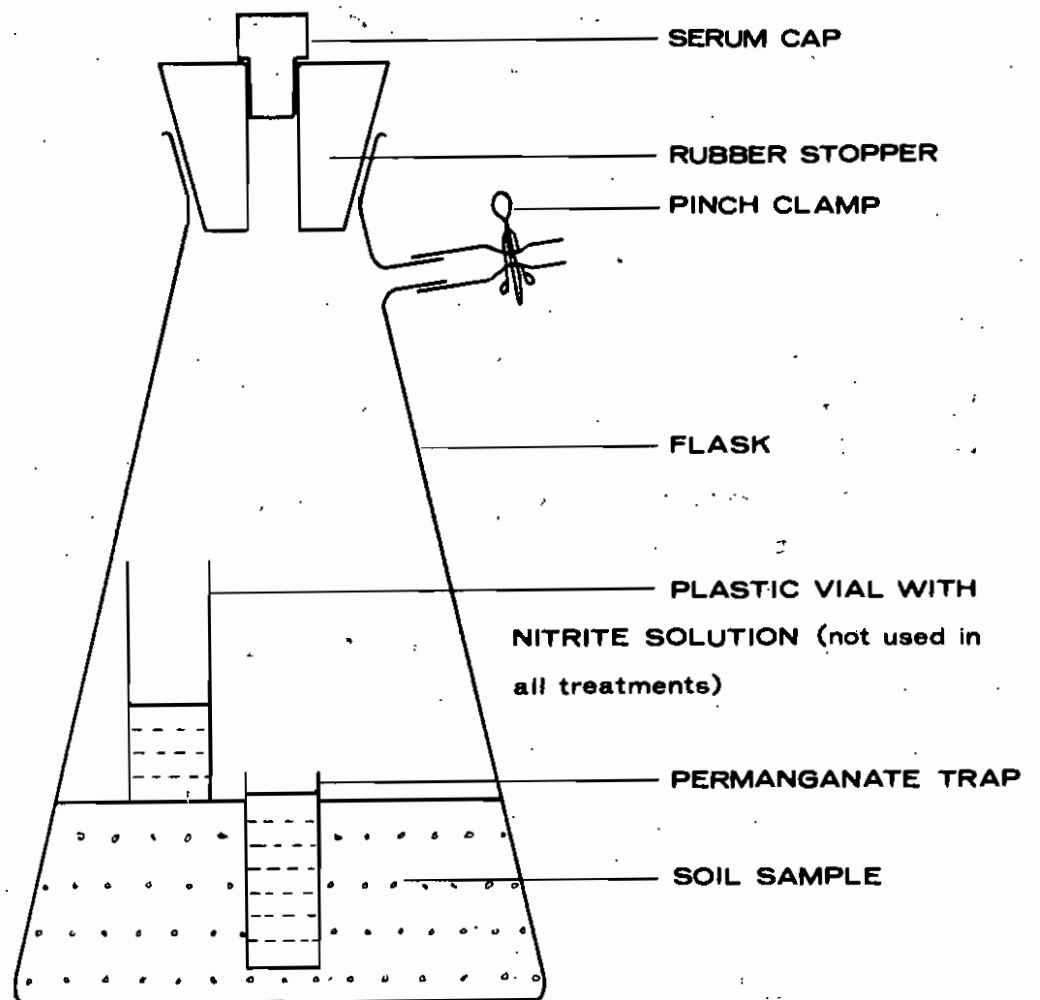
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Fig. 1. Schematic presentation of glassware used in this investigation.

Treatments

1. To determine the most appropriate method of preventing the NO_2^- from reacting with the sample until the atmosphere in the sample chamber had been replaced, NO_2^- was added by three methods: (a) as a salt mixed with the soil; (b) as a solution added to the soil with a syringe; and (c) as a solution added to the soil from a plastic vial in the incubation chamber. When the salt was mixed with the soil, the dry sample was transferred to the flask and water was added with a syringe. When NO_2^- was added as a solution, the concentration was adjusted to contain the desired quantities of N in 5 ml, which was either introduced into the flask with a syringe or pipetted into a plastic vial and placed on the soil inside the flask. In the latter case, the solution was added to the soil by carefully tilting the flask immediately before incubation and after the atmosphere in the flask was free of N_2.

2. To confirm the non-biological nature of NO_2^- decomposition reactions, sterilized soil samples were used in some preliminary studies. The samples were sterilized either chemically using sodium salt of 4-chloromercuribenzoic acid (0.10 g per 100 g of soil) or with steam by autoclaving them in the filtering flasks at $121^\circ C$ for 0.5 hour. Distilled water, sterilized by passage through Millipore filters, was used for moistening the soil and preparing the NO_2^- solution.

3. NO_2^- decomposition after treatment with various levels of NO_2^--N was investigated in several soils. The concentrations of NO_2^--N used were 0, 100, 200, 400, and 800 ppm N on a dry-weight basis.

4. To establish the nature of the decomposition reaction, some of the intermediate products were added to the samples. Nitrogen trioxide and dioxide (N_2O_3 and NO_2) gases were injected using gas-tight syringes (Hamilton syringes) into the sample container.

Analysis

To estimate the extent to which each of the possible steps of the NO_2^- decomposition reaction had progressed, N in the forms of NO_2^-, NO_3^-, N_2O, and N_2 were determined. In addition, NH_4^+-N was also determined.

Gaseous Products

Gases were analyzed using a Perkin Elmer 800 series gas chromatograph fitted with a hot wire detector and a dual column system. A Sargeant recorder with disc integrator was used to determine the areas under each peak (curve). An activated charcoal packed column; (80-100 mesh, 180 cm long) was used for determining N_2O and $NO + NO_2$. A molecular sieve 13A packed column; (80-100 mesh, 180 cm long) was used for measuring N_2 and O_2 levels in the sample container. Helium flowing at 35 ml min^{-1} was used as the carrier gas. Other settings on the chromatographic systems were similar to those reported by Reuss and Smith (1965) and Stevenson *et al.* (1966). A 1-ml gas sample was used for the determination of O_2 and N_2; and a 5-ml sample was used for N_2O and $NO + NO_2$. Because of difficulties in calibrating the chromatograph for $NO + NO_2$, it could only be used qualitatively to determine the reaction sequence. Part of the $NO + NO_2$ was absorbed by the alkaline permanganate trap in the sample flask.

Soil Samples

Inorganic forms of N in the soil samples were determined after extraction with 100 ml of saturated calcium hydroxide solution. For extraction, a 10-g sample was weighed into a 250-ml Erlenmyer flask and 100 ml of $Ca(OH)_2$ solution added. The mixture was shaken on a wrist-action shaker for 0.5 hour and filtered through Whatman No. 1 filter paper. A portion of the extract was analyzed for NO_2^- and NO_3^- by alkaline distillation using Devarda alloy (Bremner 1965). After 5 minutes of distillation for NH_4^+, about 0.2 g of Devarda alloy was added and distillation was continued for another 8 minutes. Complete recovery of up to 10 mg N as NO_2^- and NO_3^- was possible during the 8 minutes of distillation.

Alkaline Permanganate Traps

The alkaline permanganate traps, placed within the sample container, absorbed oxides of nitrogen ($NO + NO_2$) and oxidized them to NO_3^-. As a result, the traps were analyzed colorimetrically (Jackson 1958) for NO_3^- after decolorizing the permanganate and removing the managanese sulfate precipitate (see Appendix I).

RESULTS AND DISCUSSION

Testing Different Methods of Adding Nitrite

Soil from one of the white spruce stands (No. 3) treated with 200 ppm N was used to determine the best method of adding NO_2^-. Experiments with different methods of NO_2^- addition were repeated twice and NO_2^-, N_2O, and N_2 were determined to estimate the extent to which NO_2^- decomposition had progressed. Since N_2 does not react with the medium, the sample that produces the largest amount of N_2 can be considered to have reacted to the fullest extent.

In the samples treated with dry salt and water or by injecting NO_2^- solution, large quantities of NO_3^- and negligible quantities of gaseous products were produced (Table 2). When NO_2^- solution was added by the tilting method, however, large quantities of end-products of NO_2^- decomposition (N_2) and small amounts of NO_3^- were recovered. From these results, it was considered necessary to add NO_2^- to the sample by the tilting method.

Table 2. Effects of adding 200 ppm N as NO_2^- by different methods to Soil 3 on its decomposition products determined after 48 hours of incubation at room temperature (22-24°C) (Average of four determinations)

Treatment	Concentration of products, ppm		
	Nitrate (NO_3^-)	Nitrous oxide (N_2O)	N gas (N_2)
Control[a]	4.6	0.0	6.8
Injected solution	55.6	3.2	4.8
Tilted solution	19.4	3.3	26.0
Dry salt + water	80.4	3.3	11.4

a. The water was added to the sample before transferring it to the container.

In addition to the large difference between the quantities of N_2 evolved from the samples treated by adding salt and by tilting the NO_2^- solution, NO_2^- and NO_3^- determined in the soil showed rather large variations in the salt method, apparently because weighing and mixing of

extremely small quantities of salt with soil samples results in uneven distribution of NO_2^-.

Nitrite Decomposition in Sterilized Samples

The effects of sterilizing the samples with the sodium salt of 4-chloromercuribenzoic acid were determined in all the samples and only one soil was sterilized by autoclaving. The quantity of N_2 produced was used to determine the influence of sterilizing on NO_2^- decomposition. Sterilized samples produced similar quantities of N_2 to the unsterilized samples with and without added NO_2^- (Fig. 2). Chemical and heat sterilization had similar effects. On the basis of these findings, further experiments were conducted using nonsterile soil samples.

Effects of Nitrite Concentration on Decomposition Products

Recovery of Ammoniacal-nitrogen

In Soils 1, 3, and 25, with 3.5, 4.9, and 27.4% organic matter respectively (Table 1), slightly larger quantities of NH_4^+ were released when treated with higher dosages of NO_2^- (Fig. 3). This may be caused by the displacement from exchange sites of NH_4^+ ions with sodium ions. In the remaining two soils, both with 19.2% organic matter, NH_4^+ levels did not tend to change with NO_2^- treatment. The organic matter content of the samples apparently did not influence the quantities of NH_4^+ released.

Recovery of Nitrite-nitrogen

In Soil 3, most of the added NO_2^- was decomposed within 48 hours of incubation (Fig. 4). In all the other samples, recovery of NO_2^- was higher than that in Soil 3 and the differences between the quantities recovered were small. Several workers (Reuss and Smith 1965; Nelson and Bremner 1969) reported that NO_2^- decomposition increases with increases in organic matter content and decreases in pH of the soil. Some factors other than organic matter content, percent N, and moisture content, therefore seem to be responsible for the higher degree of NO_2^- decomposition in Soil 3. Trace elements catalyse NO_2^- decomposition (Wullstein and Gilmour 1966) and they may be high in Soil 3. Percentages of added NO_2^- recovered from Soils 23, 24, 25, and 26 were much higher
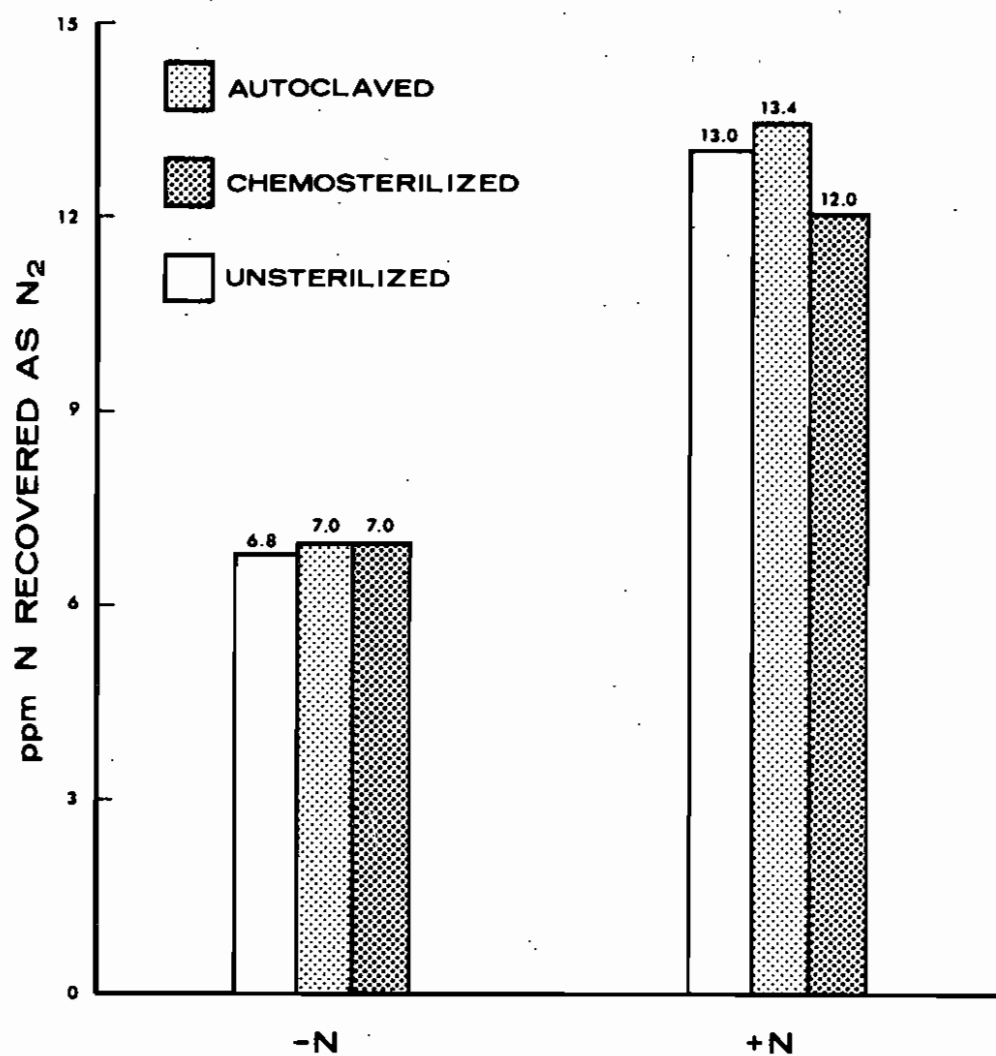
Fig. 2. Effect of sterilization on the formation of N_2 in soil 3 incubated with (+N) and without (-N) nitrogen (+N = 200 ppm N added as NO_2^-).
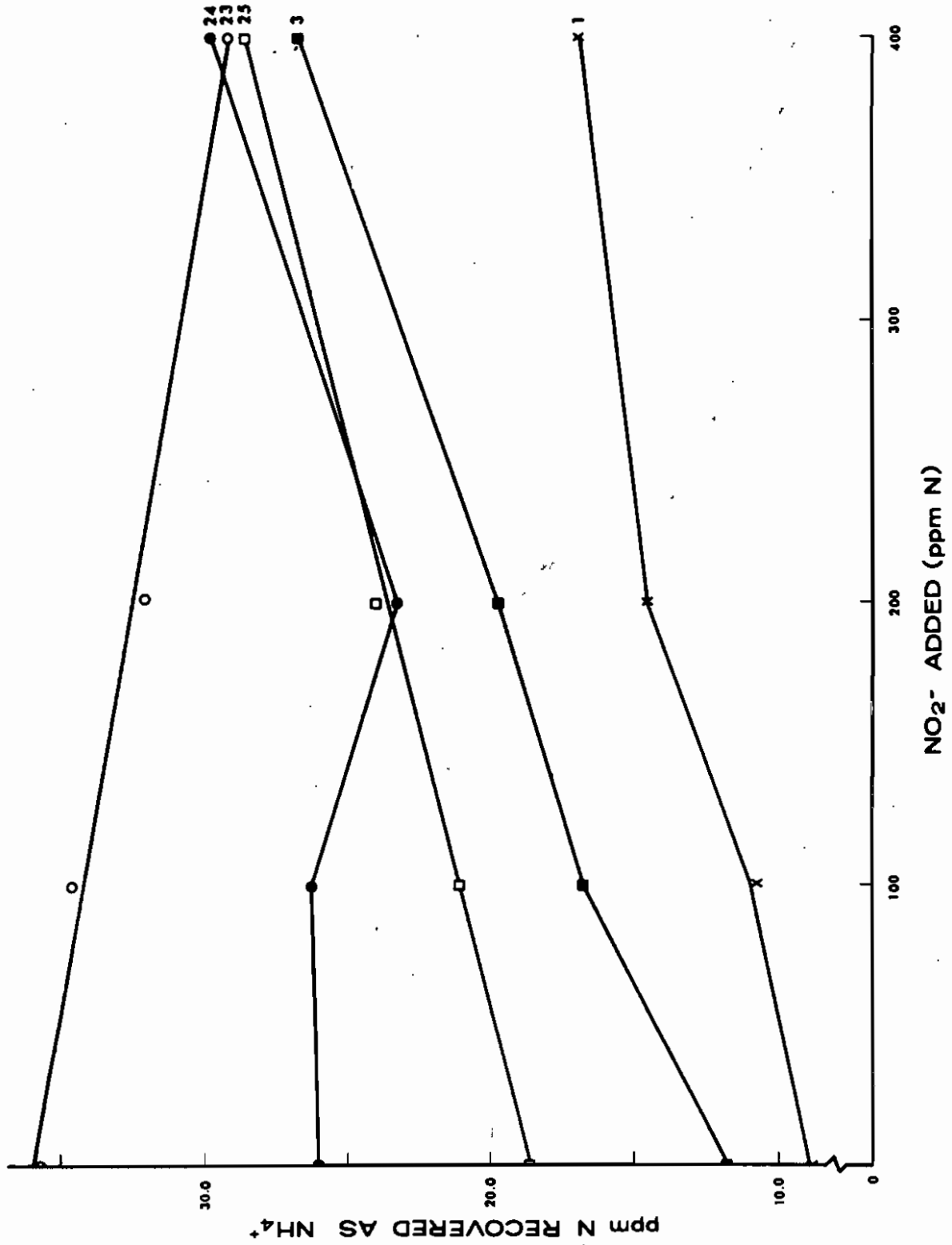
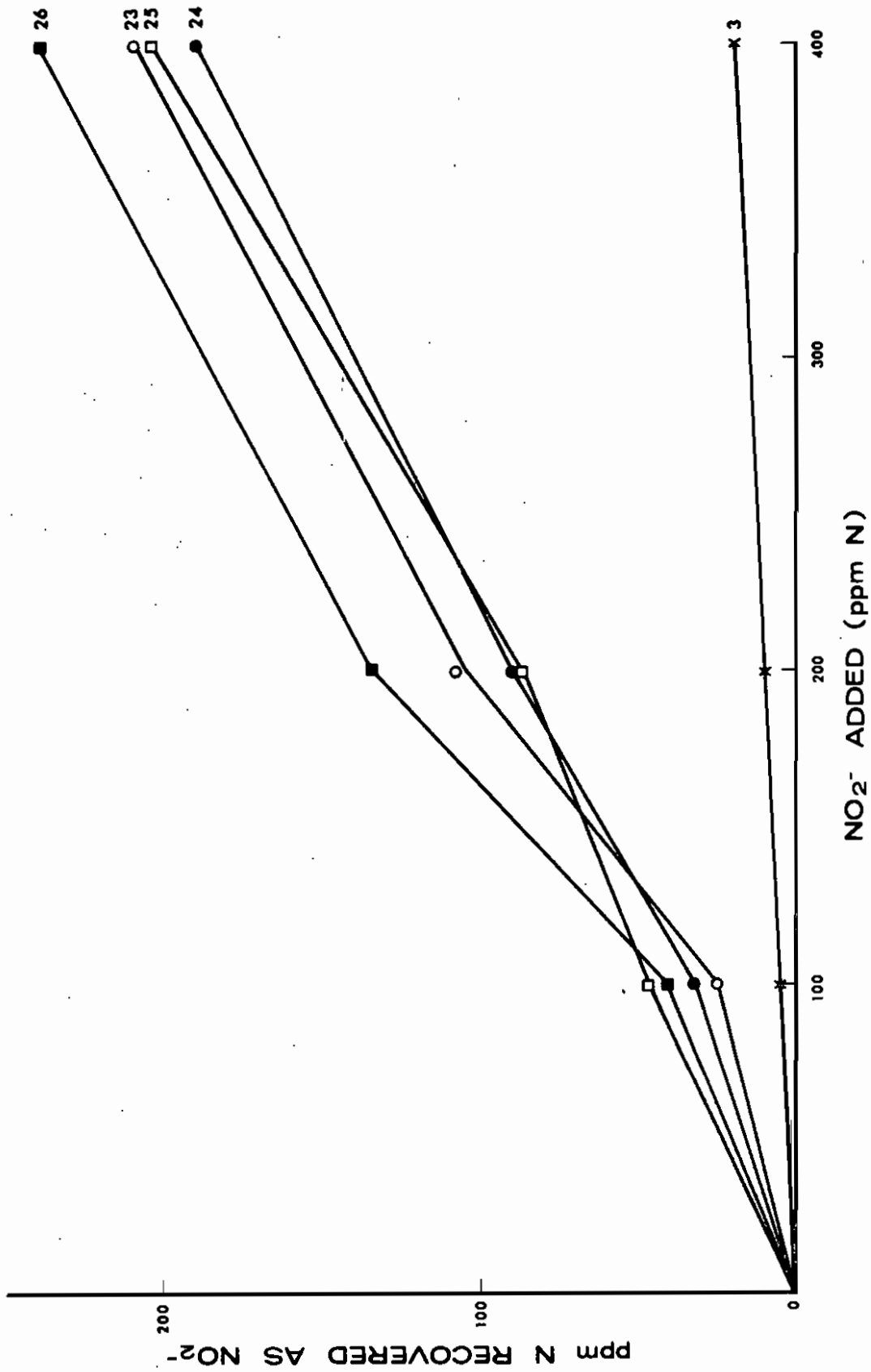Fig. 3. Quantities of NH_4^+-N (as ppm N) formed in soil samples treated with various levels of NO_2^--N and incubated for 48 hours.

Fig. 4. Quantities of undecomposed $NO_2^- -N$ (as ppm N) recovered from soil samples treated with various levels of $NO_2^- -N$ and incubated for 48 hours.
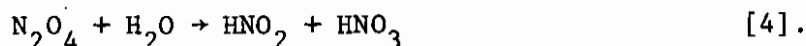
than the values reported by Führ and Bremner (1964) who worked with soil containing slightly lower organic matter content. These workers found higher NO_2^- fixing capacity in soils with higher organic matter level. The forest soil samples under study seem to differ from agricultural soils with respect to NO_2^- decomposition under acidic conditions.

Nitrate-nitrogen Formed

Formation of NO_3^- from NO_2^- was reported by Temple (1914) who proposed the sequence of reactions as

$$3HNO_2 \rightarrow HNO_3 + 2NO + H_2O \qquad\qquad [1].$$
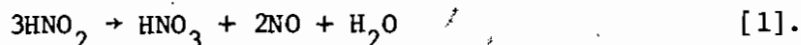
The above equation, however, describes NO_2^- decomposition under anaerobic conditions. In the presence of O_2, nitric oxide (NO) reacts further

$$2NO + O_2 \rightarrow 2NO_2 \qquad\qquad [2]$$

$$2NO_2 \rightarrow N_2O_4 \qquad\qquad [3]$$

$$N_2O_4 + H_2O \rightarrow HNO_2 + HNO_3 \qquad\qquad [4].$$

Reactions responsible under anaerobic conditions for the formation of NO_3^- from NO_2^- are also operative under aerobic conditions (Sneed and Brasted 1958).

The initial step involved in the decomposition of NO_2^- is therefore:

$$2HNO_2 \rightarrow N_2O_3 + H_2O \qquad\qquad [5]$$

and N_2O_3 breaks down to yield equimoles of NO and NO_2 (Sneed and Brasted 1958). Thus one of the sequences of NO_2^- decomposition would be:

$$NO_2^- \rightarrow N_2O_3 \rightarrow NO + NO_2 \rightarrow N_2O_4 \rightarrow NO_3^-$$
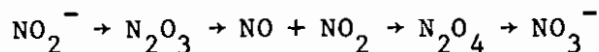
According to this sequence of reactions, one would expect an inverse relationship between the undecomposed portion of NO_2^- added and the NO_3^- formed. The results do not show such a trend (Fig. 5), although Soil 3, which had the least quantity of NO_2^- remaining, yielded the largest quantity of NO_3^-. Since part of NO and NO_2 are absorbed by the alkaline permanganate traps and some other gaseous products are formed, the relationship may not hold true. The samples containing more organic matter produced considerably smaller quantities of NO_3^-. This may be due to the fact that the influence of organic matter on NO_2^- decomposition was different in each soil. The difference was greatest in the set of samples treated with 400 ppm N as NO_2^-.
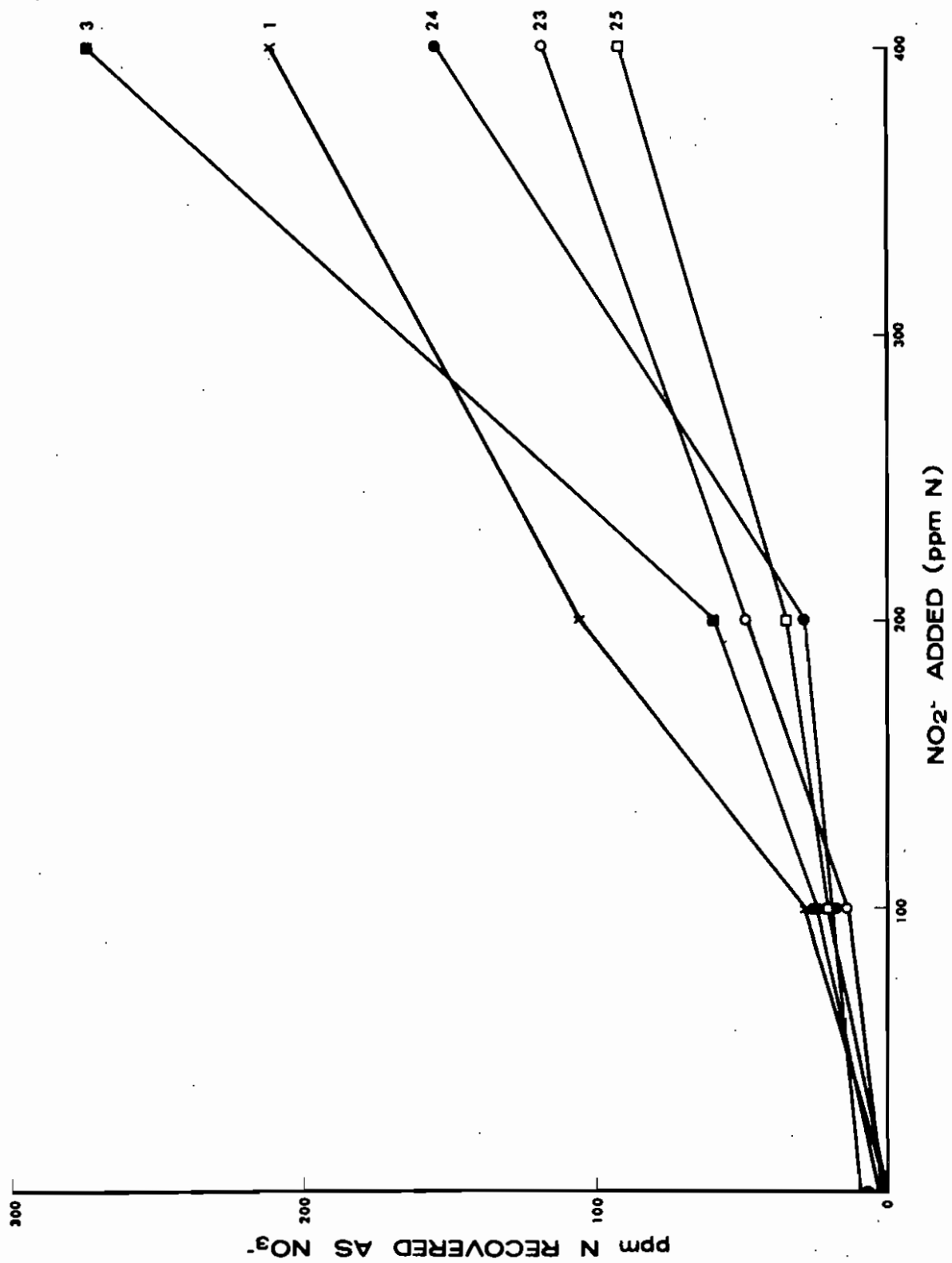
Fig. 5. Quantities of NO_3^- (as ppm N) formed in soil samples treated with various levels of NO_2^--N and incubated for 48 hours.

Gaseous Products

NO, NO_2, and N_2O are the three inorganic gaseous oxides of N produced from NO_2^- decomposition (Yost and Russell 1944). Methyl nitrite (CH_3ONO) can also be formed in soils (Stevenson and Swaby 1964). These gases, undergo further reactions and an alkaline permanganate solution (trap) can partly absorb them (Stevenson and Swaby 1964; Reuss and Smith 1965; Nelson and Bremner 1969). NO and NO_2 can be reabsorbed into the soil sample and be converted to NO_2^- and NO_3^- according to reactions 2, 3, and 4. N_2O can be further converted to N_2 (Austin 1961).

Since alkaline permanganate solution does not absorb all the NO and NO_2 produced in the system, the traps were analysed only in the preliminary experiments. The quantities of NO and NO_2 absorbed varied considerably. A thick crust was formed on the surface of most of the traps and probably decreased the ability of the solution to absorb oxides of N.

N_2O, determined in different samples treated with various concentrations of NO_2^- showed an apparent relationship between the quantities of NO_2^- decomposed and the quantities of N_2O produced (Fig. 6). Soil 3, which had the smallest quantity of NO_2^- remaining after incubation (Fig. 6), produced the largest quantity of N_2O except at the 400 ppm N level, and Soil 26 yielded the smallest quantity of N_2O but the highest concentration of NO_2^- at the end of incubation. These results, however, are contrary to the findings reported for agricultural soils in which the quantity of N_2O produced depended upon the organic matter content of the sample (Stevenson and Swaby 1964; Reuss and Smith 1965; Nelson and Bremner 1969). This discrepancy cannot be explained on the basis of the data presented here unless the nature of the organic matter in these soils is understood. Reaction sites (functional groups) exposed on the surface of different organic compounds influence the sequence of NO_2^- decomposition reactions and their end-products (Stevenson *et al.* 1970).

N_2 formed from NO_2^- can certainly be considered as a loss of fertilizer-N. Formation of N_2 in the soils treated with NO_2^- and incubated under aerobic condition was demonstrated with labelled ^{15}N by Broadbent as early as 1962. The reaction mechanism involved, however, has not yet been elucidated clearly. Allison (1963) suggested that the
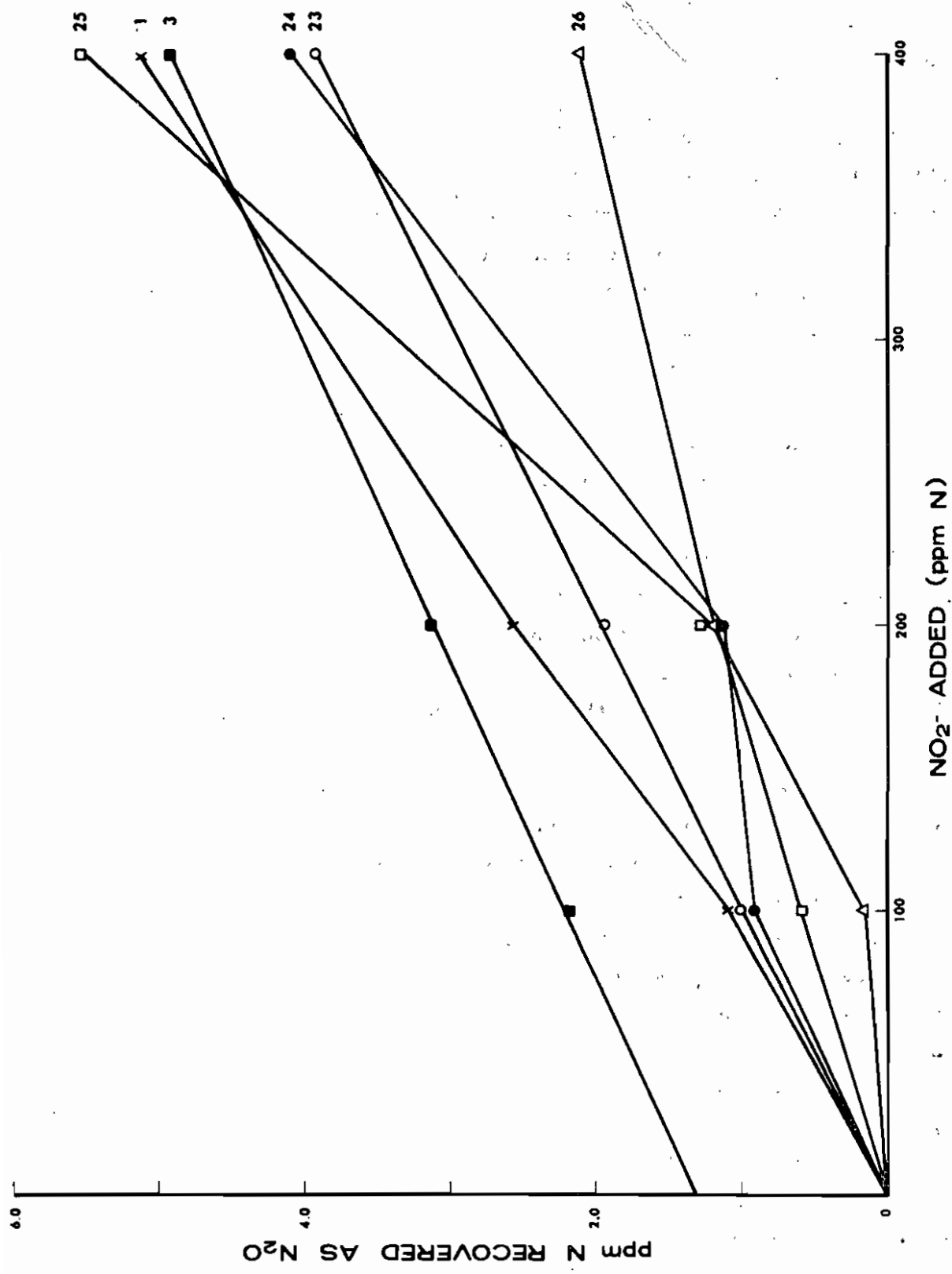
Fig. 6. Quantities of N_2O (as ppm N) evolved from soil samples treated with various levels of NO_2^--N and incubated for 48 hours.

reaction of NO_2^- with α -amino acids (Van Slyke reaction) would be a major pathway for production of N_2. Wullstein (1969) reported that N_2 can be produced from NO_2^- in acid medium in the absence of organic compounds, but there is the need for high concentration of iron (over 10,000 ppm Fe). Stevenson and Swaby (1964) recorded the formation of N_2 from NO_2^- when systems were treated with different humic and fulvic compounds. Reuss and Smith (1965) and Nelson and Bremner (1969) have certainly proven the importance of organic matter in NO_2^- decomposition in soils. Obviously then, N_2 can be produced in acid soils through all three mechanisms in the soils under study, but the role of organic matter was not the same in all the samples (Fig. 7). Soils 25 and 26, which had about 28% organic matter, produced less N_2 than Soils 23 and 24 which contained only 19% organic matter. The latter two soils contained organic matter that was illuviated from the upper organic horizons and it probably was absorbed onto the soil particles. In Soils 25 and 26, however, organic matter from the surface layer was mixed with the alluvial layer. Thus one can expect differences in the activities of organic matter depending on the nature of its absorption and retention in the soil. In Soil 3 where most of the added NO_2^- was decomposed within 48 hours (Fig. 4), large quantities of N_2 were produced as compared to Soil 26 (Fig. 7), in which a large proportion of the added NO_2^- was recovered after 48 hours incubation. The influence of organic matter and other factors in soils appears to be of complex interaction. Wullstein (1969) reported that both organic matter and transition metals have similar direct influence on the decomposition of NO_2^- as long as they are absorbed onto the surface of particles, colloidal or otherwise.

Conversion of Intermediate Products to N_2O and N_2

Larger quantities of N_2O were found in samples treated with N_2O_3 than in these that received either NO_2 or NO_2^- (Fig. 8). Conversely, more N_2 was evolved in the samples treated with NO_2 (Fig. 8). These results indicate that the pathways involved in the formation of these end-products may possibly be different.
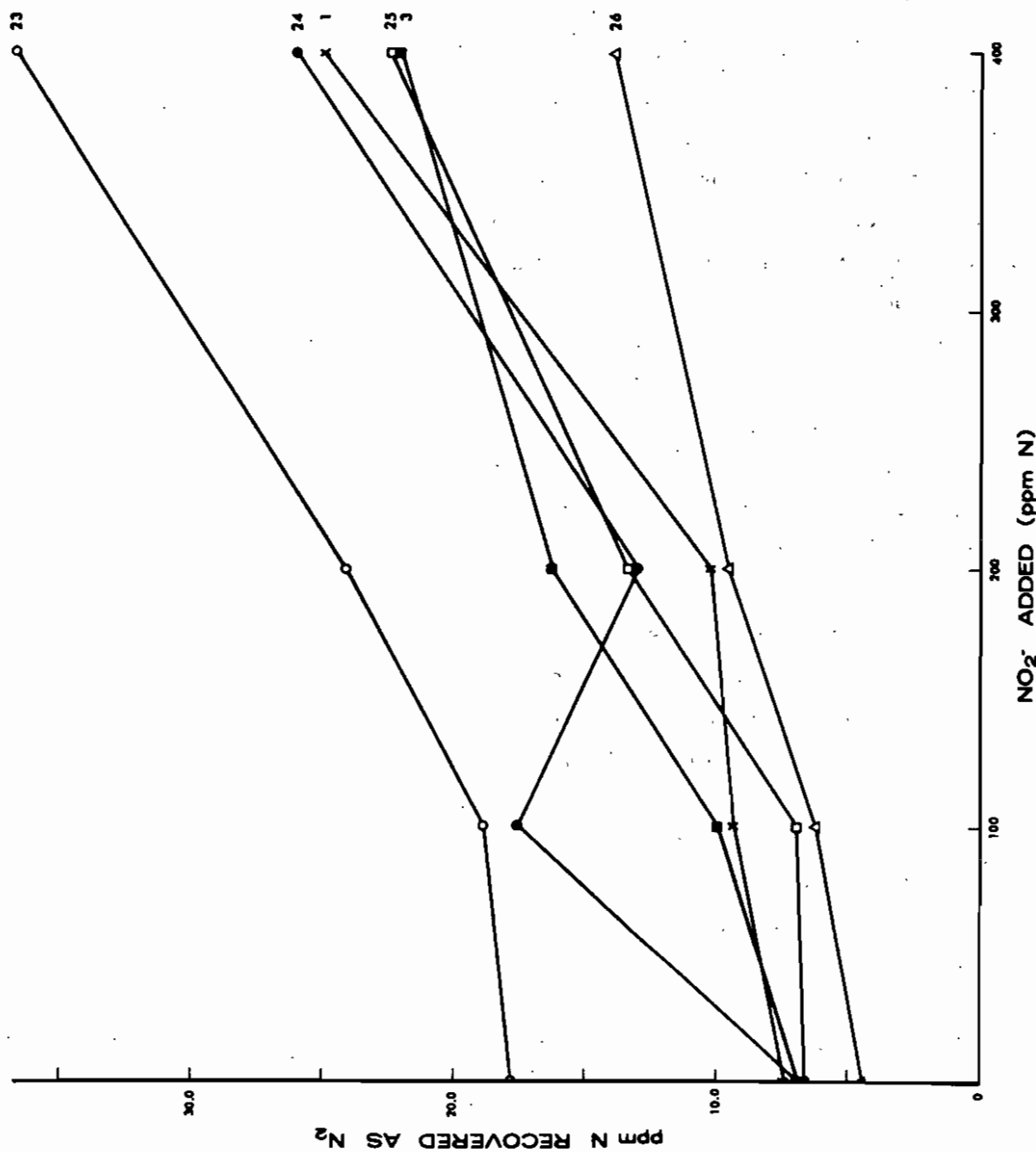
Fig. 7. Quantities of N_2 (as ppm N) evolved from soil samples treated with various levels of NO_2^--N and incubated for 48 hours.
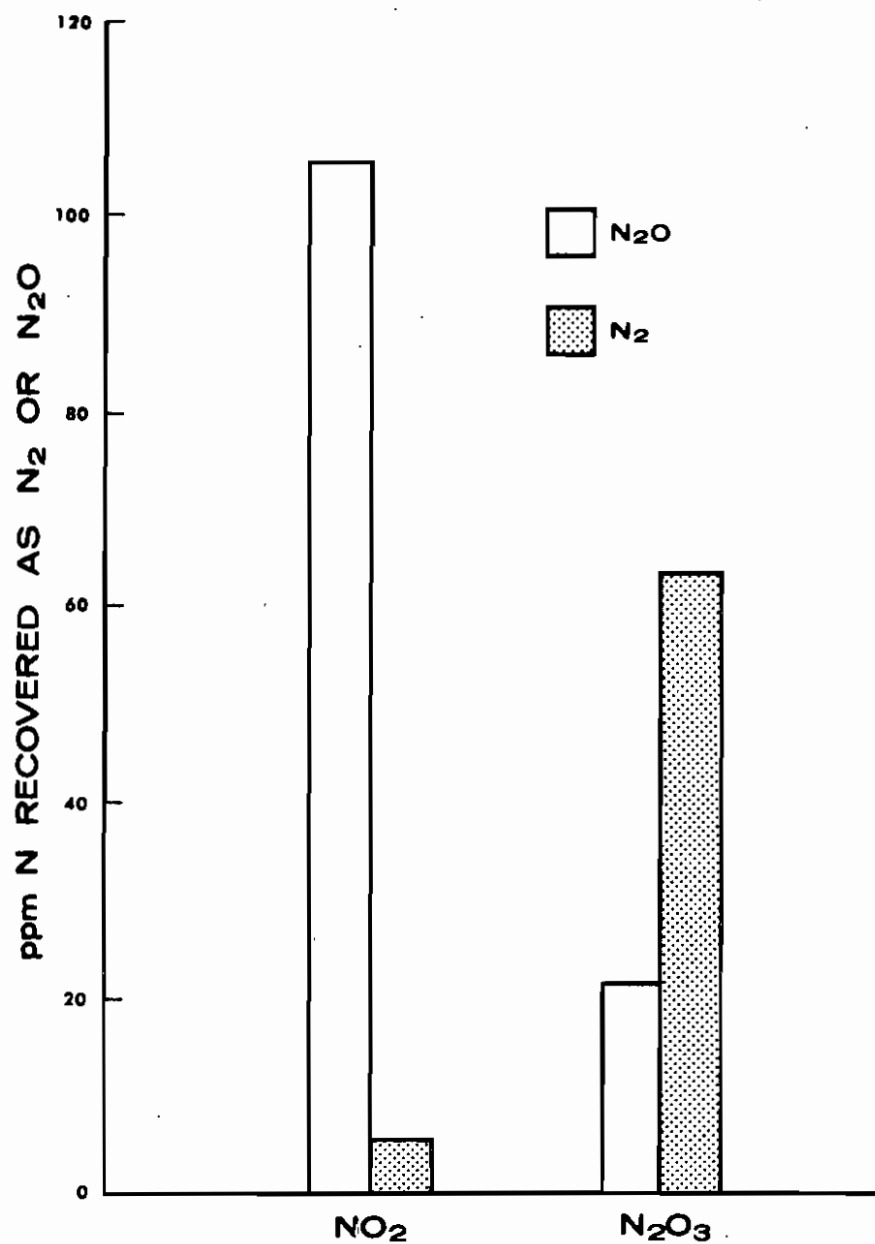
Fig. 8. Quantities of N_2O and N_2 (as ppm N) formed in Soil 24 when treated with about 500 ppm N in the form of NO_2 and N_2O_3 gases.

CONCLUSIONS

Soil organic matter certainly plays an important role in NO_2^- decomposition. Its effects on the nature of products formed, however, depend on the reaction sites exposed. It appears that contrary to experience in agricultural soils in which the quantity of N_2O produced is dependent on the organic matter content, N_2O formation in forest soils is dependent on the quantities of NO_2^- decomposed. The influence of the organic matter in alluvial and illuvial layers on the quantities of N_2 formed was different. In forest soils, in which temporary accumulation of NO_2^- occurs, loss of N in the form of N_2 is possible. Evolution of oxides of N in the forms of NO and NO_2 is also likely to occur, and their degree and significance needs to be understood.

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APPENDIX I. Analysis of Alkaline Permanganate Traps for Oxides of
Nitrogen.

The oxides of N that are absorbed in trap solutions were
oxidized to NO_3^- by the $KMnO_4$. Thus, this procedure is designed to
destroy the color of the $KMnO_4$ and permit colorimetric NO_3^- determina-
tion.

Reagents

1. Trapping solution of alkaline permanganate; 0.2 *M* $KMnO_4$ in 2 *N* KOH

Dissolve 112.2 g reagent grade KOH in about 800 ml of distilled
water. Add 31.7 g $KMnO_4$, warm, and stir until solution is complete.
Transfer to a 1-liter volumetric flask and make up to volume. Store in
brown bottle away from direct light.

2. Oxalate-sulfuric acid solution; 0.6 *M* $Na_2C_2O_4$ in 7 *N* H_2SO_4

Place about 1200 ml distilled water in a 2-liter beaker. Add
carefully 390 ml conc. H_2SO_4. Weigh out and add 160.6 g $Na_2C_2O_4$. Stir
until oxalate is completely dissolved, transfer to a 2-liter volumetric
flask, and make up to volume. Store in brown plastic bottle (the
oxalate will attack glass).

3. 10 *N* NaOH

Dissolve 400 g reagent grade NaOH in about 800 ml distilled
water, allow to cool, transfer to a 1-liter volumetric flask, and make
to volume.

Procedure

Wash the entire contents (5 ml) of the trap into a 100-ml
beaker but avoid using excess water. A blank of unused trap solution
should be included. From a burette, add 5 ml of Reagent 2 (acid and
oxalate). In a few minutes, the reaction should be complete and the
material colorless. Warming on the hot plate will facilitate the
reaction. Incomplete reaction may be the result of insufficient
acidity or insufficient oxalate.

After slight warming, add 8 ml of Reagent 3 (10 *N* NaOH) to
each beaker. Mn will precipitate as MnO_2 at this point and the base
must be in sufficient quantities to make the solution alkaline. Upon
standing, a slight brown scum of MnO_2 should form on the surface.
Failure of this to form may be due to insufficient base.

The precipitate should be digested with gentle warming for at least 0.5 hour. The material is then filtered into a 100-ml volumetric flask and washed several times with small portions of warm water. The volume is then made to 100 ml and an aliquot (2 ml) taken for the standard NO_3^- determination with phenoldisulphonic acid (Jackson 1958). The addition of ammonium sulfamate to destroy NO_2^- is unnecessary.

The blank carried through the entire procedure is used to set the colorimeter.

Standard Curve and Calculations

Dissolve exactly 1.44 g KNO_3 in distilled water in to a clean beaker then transfer to a 1-liter flask. Make up to volume. This solution has 200 ppm N.

Add 0, 1, 2, 3, ... 8 ml of KNO_3 standard to a series of beakers containing 5 ml $KMnO_4$ trap solution. Carry through the procedure as outlined taking a 5-ml aliquot for NO_3^- determination. Carry through the standard NO_3^- procedure making up the colored solution to 50 ml for reading on the spectrophotometer. There will now be 0, 10, 20 ... 80 µg N per 50 ml. Read on spectrophotometer and plot curve.

Determine from the standard curve, the ratio µg N to Optical density (OD), this is the inverse of the slope if OD is plotted on the y axis and µg N on the x axis.

For each sample, after reading OD, calculate N concentration from:
[Ratio x OD x 20]/1000 = [mg N]/Trap ... for a 5-ml aliquot,
[Ratio x OD x 50]/1000 = [mg N]/Trap ... for a 2-ml aliquot

Ratio obtained during the preparation of standard curve was 110.

Notes

Do not use excess water to wash the trap solution into the beaker or for washing the precipitate as the total volume must be less than 100 ml. The destruction of $KMnO_4$ requires high acidity and incomplete reaction at this point is often the result of insufficient acidity. If the traps are to be exposed to large amounts of CO_2 evolution, it may be necessary to use 4 *N* KOH or 10 ml trapping solution per trap or both. If more trapping solution is used, the amounts or the strengths of Reagents 2 and 3 may be increased accordingly. If 4 *N* KOH is used in the

trap solution, the acidity of Reagent 2 should be increased to 9 N.

After the residue from the filtration is exposed to the air, it should assume a dark brown color. If it remains gray, add 1 or 2 ml of NaOH to the filtrate. If precipitate is formed, refilter.