

TRANSFORMATION AND MOVEMENT
OF BAND-APPLIED NITROGEN FERTILIZERS IN
SEVERAL MANITOBA SOILS

A Thesis
Submitted to
the Faculty of Graduate Studies and Research
The University of Manitoba

In Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

by
Patrick Chi-Kee Pang
December , 1973



TRANSFORMATION AND MOVEMENT
OF BAND-APPLIED NITROGEN FERTILIZERS IN
SEVERAL MANITOBA SOILS

BY

PATRICK CHI-KEE PANG

A dissertation submitted to the Faculty of Graduate Studies of
the University of Manitoba in partial fulfillment of the requirements
of the degree of

DOCTOR OF PHILOSOPHY

© 1973

Permission has been granted to the LIBRARY OF THE UNIVERSITY OF MANITOBA to lend or sell copies of this dissertation, to the NATIONAL LIBRARY OF CANADA to microfilm this dissertation and to lend or sell copies of the film, and UNIVERSITY MICROFILMS to publish an abstract of this dissertation.

The author reserves other publication rights, and neither the dissertation nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

TABLE OF CONTENTS

CHAPTER	PAGE
I. INTRODUCTION.	1
II. LITERATURE REVIEW	4
Forms of nitrogen	4
Sources of mineral nitrogen	5
Fates of mineral nitrogen	9
Nitrogen fertilizers.	27
III. MATERIALS AND METHODS	32
IV. RESULTS AND DISCUSSIONS	48
Experiment I. Transformation and movement of band-applied $(\text{NH}_2)_2\text{CO}$, NH_4OH and $(\text{NH}_4)_2\text{SO}_4$ during incubation in three soils.	48
Experiment II. Effect of rate of nitrogen application on transformation of band-applied N fertilizers in neutral Wellwood II soil.	69
Experiment III. Effect of temperature on the transformation and movement of band-applied nitrogenous fertilizer in neutral Wellwood II soil.	84
Experiment IV. Effect of pH on the transformation of band-applied urea in Keld and Wellwood II soils	97
Experiment V. The recovery of banded KNO_3 and KNO_2 in presence of added urea in Wellwood I soil.	106
Experiment VI. A mass spectrometric investigation on denitrification from nitrite treated soil	110
Experiment VII. Recovery of band-applied $(^{15}\text{NH}_2)\text{CO}$, $^{15}\text{NH}_4\text{OH}$ and $(^{15}\text{NH}_4)_2\text{SO}_4$ from Wellwood II soil as measured by the nitrogen-15 tracer technique.	120
Experiment VIII. Nitrification of urea in soil suspension and the nitrifier populations.	126
V. SUMMARY AND CONCLUSIONS	135
VI. BIBLIOGRAPHY.	140

LIST OF TABLES

<u>Tables</u>	<u>Page</u>
1. Subgroup designation, legal location and physical and chemical characteristics of the soils.	33
2. Nitrogen treatments in acid Wellwood I soil.	37
3. Percent recovery of applied N as total inorganic nitrogen from soils incubated for various times at 20C.	64
4. Calculated diffusion coefficient of NH_4^+ -N from three nitrogen carriers in three soils at 20C.	65
5. Calculated diffusion coefficient of NH_4^+ -N from three nitrogen carriers banded at two rates of N in neutral Wellwood II soil at 20C.	81
6. Calculated diffusion coefficient of NH_4^+ -N from three nitrogen carriers banded in Wellwood II soil at 10 and 20C	89
7. Percent recovery of applied N as total inorganic nitrogen from Wellwood II soil incubated at various temperatures and times.	90
8. Concentration of ionic species of nitrogen and percent recovery as total inorganic nitrogen of band-applied KNO_3 and KNO_2 in the presence of urea.	107
9. Mass to charge ratio (m/e) of some gases.	110
10. Possible gaseous products having m/e 28 through 48 obtained during incubation of Wellwood I soil to which nitrogen-15 labelled NaNO_2 was applied.	113
11. Possible gaseous products having m/e 28 through 48 obtained during incubation of Wellwood I soil to which non-labelled NaNO_2 was applied.	114
12. Percent recovery of total nitrogen (inorganic + organic fractions) from N-15 labelled nitrogen carriers banded in Wellwood II soil (rate = 200 kg N/ha)	121
13. Percent recovery of total inorganic nitrogen from N-15 labelled nitrogen carriers banded in Wellwood II soil (rate = 200 kg N/ha).	123
14. Percent N loss by immobilization and volatilization from three nitrogenous materials banded in Wellwood II soil (rate = 200 kg N/ha).	124

Tables

Page

15. pH of soil suspension treated with urea after one to four weeks incubation at 20C. 127
16. Nitrifier numbers in Keld, Wellwood II and Morton soils . 132

LIST OF FIGURES

<u>Figures</u>	<u>Page</u>
1. Diagrammatic scheme of soil nitrogen sources and transformations and fate of the end products.	20
2. Incubation column.	34
3. Incubation apparatus for mass spectrometric analysis of gaseous products due to denitrification.	38
4. Apparatus for preparation of $^{15}\text{NH}_4\text{OH}$ (52.20 atom % ^{15}N)	41
5. Incubation vessel for the study of nitrification in soil suspension	42
6. Changes in pH due to 92 mg N band-applied $(\text{NH}_2)_2\text{CO}$, $(\text{NH}_4)_2\text{SO}_4$ and NH_4OH in acid Keld (A), neutral Wellwood II (B) and alkaline Morton (C) soils at 20C.	49
7. Distribution of total inorganic N, $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ in the acid Keld soil (pH 5.4) from 92 mg N band-applied urea at 20C.	51
8. Distribution of total inorganic N, $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ in the acid Keld soil (pH 5.4) from 92 mg N band-applied NH_4OH at 20C.	52
9. Distribution of total inorganic N, $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ in the acid Keld soil (pH 5.4) from 92 mg N band-applied $(\text{NH}_4)_2\text{SO}_4$ at 20C.	53
10. Distribution of total inorganic N, $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ in the neutral Wellwood II soil (pH 6.6) from 92 mg N band-applied $(\text{NH}_2)_2\text{SO}$ at 20C.	55
11. Distribution of total inorganic N, $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ in the neutral Wellwood II soil (pH 6.6) from 92 mg N band-applied NH_4OH at 20C.	56
12. Distribution of total inorganic N, $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ in the neutral Wellwood II soil (pH 6.6) from 92 mg ^{15}N band-applied $(\text{NH}_4)_2\text{SO}_4$ at 20C.	57
13. Distribution of total inorganic N, $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ in the alkaline Morton soil (pH 8.2) from 92 mg N band-applied $(\text{NH}_2)_2\text{CO}$ at 20C	59
14. Distrubition of total inorganic N, $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ in the alkaline Morton soil (pH 8.2) from 92 mg ^{15}N band-applied NH_4OH at 20C.	60

FiguresPage

15. Distribution of total inorganic N, $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ in the alkaline Morton soil ($\text{pH} 8.2$) from 92 mg N band-applied $(\text{NH}_4)_2\text{SO}_4$ at 20C. 61
16. Distribution of total inorganic N, $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ in the Wellwood II soil from 11.5 mg N band-applied $(\text{NH}_2)_2\text{CO}$ at 20C. 70
17. Distribution of total inorganic N, $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ in the Wellwood II soil from 11.5 mg N band-applied NH_4OH at 20C 71
18. Distribution of total inorganic N, $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ in the Wellwood II soil from 11.5 mg N band-applied $(\text{NH}_4)_2\text{SO}_4$ at 20C 72
19. Change in soil pH due to 11.5 mg N band-applied nitrogenous substances in neutral Wellwood II soil at 20C 73
20. Distribution of total inorganic N, $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ in the neutral Wellwood II soil from 23 mg N band-applied $(\text{NH}_2)_2\text{CO}$ at 20C. 75
21. Distribution of total inorganic N, $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ in the neutral Wellwood II soil from 23 mg N band-applied NH_4OH at 20C. 76
22. Distribution of total inorganic N, $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ in the neutral Wellwood II soil from 23 mg N band-applied $(\text{NH}_4)_2\text{SO}_4$ at 20C 77
23. Recovery of total mineral nitrogen from band-applied $(\text{NH}_2)_2\text{CO}$, NH_4OH and $(\text{NH}_4)_2\text{SO}_4$ at three rates of N 80
24. Distribution of total inorganic N, $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ in neutral Wellwood II soil from band-applied N in presence of 100 ppm N-serve at 20C after 2 weeks incubation (rate = 800 kg N/ha). 82
25. Distribution of total inorganic N, $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ in the neutral Wellwood II soil from 92 mg N band-applied $(\text{NH}_2)_2\text{CO}$ at 10C. 85
26. Distribution of total inorganic N, $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ in the neutral Wellwood II soil from 92 mg N band-applied $(\text{NH}_2)_2\text{CO}$ at 15C. 86
27. Change in pH due to 92 mg N band-applied $(\text{NH}_2)_2\text{CO}$ in neutral Wellwood II soil at three temperatures 88
28. Distribution of total inorganic N, $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ in the neutral Wellwood II soil from 92 mg N band-applied NH_4OH at 10C. 91

Figures

Page

29.	Distribution of total inorganic N, NH_4^+ -N, NO_2^- -N and NO_3^- -N in the neutral Wellwood II soil from 92 mg N band-applied NH_4OH at 15C.	92
30.	Distribution of total inorganic N, NH_4^+ -N, NO_2^- -N and NO_3^- -N in the neutral Wellwood II soil from 92 mg N band-applied $(\text{NH}_4)_2\text{SO}_4$ at 10C.	93
31.	Distribution of total inorganic N, NH_4^+ -N, NO_2^- -N and NO_3^- -N in the neutral Wellwood II soil from 92 mg N band-applied $(\text{NH}_4)_2\text{SO}_4$ at 15C.	94
32.	Change in pH due to 92 mg N band-applied $(\text{NH}_4)_2\text{SO}_4$ in neutral Wellwood II soil at three temperatures	95
33.	Distribution of total inorganic N, NH_4^+ -N, NO_2^- -N and NO_3^- -N in acidified Wellwood II soil (pH 5.5) from band-applied $(\text{NH}_2)_2\text{CO}$ at 20C	98
34.	Distribution of total inorganic N, NH_4^+ -N, NO_2^- -N and NO_3^- -N in limed acid Keld soil (pH 6.5) from 92 mg N band-applied $(\text{NH}_2)_2\text{CO}$ at 20C	100
35.	Distribution of total inorganic N, NH_4^+ -N, NO_2^- -N and NO_3^- -N in limed Keld soil (pH 7.3) from 92 mg N band-applied $(\text{NH}_2)_2\text{CO}$ at 20C.	102
36.	Distribution of total inorganic N, NH_4^+ -N, NO_2^- -N and NO_3^- -N in limed Keld soil (pH 7.3) inoculated with 1% Wellwood II soil from 92 mg N band-applied $(\text{NH}_2)_2\text{CO}$ at 20C.	103
37.	Distribution of total inorganic N, NH_4^+ -N, NO_2^- -N and NO_3^- -N in limed Keld soil (pH 7.3) inoculated with <u>Nitrosomonas europaea</u> culture from 92 mg N band-applied $(\text{NH}_2)_2\text{CO}$ at 20C	105
38.	The mass spectrometric scan of air and gas samples from $\text{Na}^{15}\text{NO}_2$ treated soil.	111
39.	Concentration of total mineral N, NH_4^+ -N, NO_2^- -N and NO_3^- -N in Keld soil suspension treated with urea after one to four weeks incubation at 20C	128
40.	Concentration of total mineral N, NH_4^+ -N, NO_2^- -N and NO_3^- -N in Wellwood II soil suspension treated with urea after one to four weeks incubation at 20C	130
41.	Concentration of total mineral N, NH_4^+ -N, NO_2^- -N and NO_3^- -N in alkaline Morton soil suspension treated with urea after one to four weeks incubation at 20C.	131

ACKNOWLEDGEMENT

I would like to express my gratitude to Dr. Robert A. Hedlin and Dr. Chai Moo Cho for their valuable advice and constructive criticism during the course of this investigation and the preparation of the manuscript.

I also wish to thank Mrs. Marlene Goodwin and Mr. Chi Chang for the computer programming.

ABSTRACT

The transformation, effect on soil pH and movement of nitrogen from banded nitrogen materials (urea, ammonium sulfate and ammonium hydroxide) were studied in three Manitoba soils of different initial soil pH values. The greatest effect on soil pH took place near the fertilizer zone, but the magnitude of the change varied greatly depending on soil characteristics. Irrespective of the fertilizer materials, the oxidation of ammonium form of nitrogen was the slowest with acid Keld soil (pH 5.4). Nitrification with neutral Wellwood II soil (pH 6.6) was rapid, with a high accumulation of nitrite taking place, when band-applied with 800 kg N/ha of urea and NH_4OH . With alkaline Morton soil (pH 8.2), oxidation of the ammonium form of nitrogen to nitrate was rapid without any accumulation of nitrite. The oxidation of band-applied $(\text{NH}_4)_2\text{SO}_4$ was slow in these three soils. The difference in nitrifying capacity among the three soils was related to the initial nitrifier numbers whose activities were affected by the initial soil pH.

The concentration of nitrite accumulated near the zone of urea and NH_4OH application in the Wellwood II soil was high enough to be phytotoxic. Further investigations indicated that temperature, soil pH and rate of nitrogen application affected the transformation of band-applied nitrogen and thus resulted in nitrite accumulation. Large quantities of nitrite accumulated at temperatures of 10, 15 and 20C, when band-applied with urea and NH_4OH . Accumulation of nitrite was slow at 10C, but greater than 15% of the banded urea was present at 10C after 12 weeks incubation. Acidifying Wellwood II temporarily

decreased the rate of nitrogen transformation. Liming Keld soil did not increase the rate of nitrification. Nitrite accumulation was observed in limed Keld soil (pH 7.3) only when it was inoculated with Nitrosomonas culture. This further showed that the initial nitrifier numbers are very important if rapid nitrification is to take place. Higher rates of nitrogen banded in the soil resulted in a higher amount of nitrite being accumulated. At the rate of 100 kg N/ha, the conversion of ammonium form of nitrogen to nitrate was quite rapid without accumulation of nitrite in Wellwood II soil.

Nitrogen-15 tracer studies showed that incomplete recovery of added nitrogen in the inorganic form was due to immobilization and volatilization. The amount of N loss depended on the type of nitrogen materials. Substantial amounts of N_2 and small amounts of NO and N_2O were evolved when nitrite was added to Wellwood I (pH 6.0). The N_2 gas formed was the result of Van Slyke type reaction of nitrite with organic constituents containing nitrogen in the soils.

Diffusion coefficients of ammonium in the soils were dependent upon the types of soil, nitrogen material, temperature and rate of application. Generally, the smaller diffusion coefficient restricted movement of ammonium away from the zone of application and therefore retarded the transformation to nitrite and nitrate.

INTRODUCTION

Successful crop production often requires the addition of large quantities of nitrogen since the amount of nitrogen mineralized during the growing season is not usually adequate to produce a high yield of crops. Nitrogenous fertilizers, ammonium sulfate, urea, ammonium nitrate and anhydrous ammonia, are widely used for this purpose. Recovery of nitrogen in the crop is usually approximately 50% of that applied. Only a portion of the remainder is recovered in the soil as mineral and organic nitrogen.

The importance of nitrogen as a fertilizer and its relatively low recovery has led to numerous studies to determine the most efficient source, rate and method of N application. In such studies, urea has often been found to be less effective than other ammonium sources particularly when applied with the seed. Field studies conducted in Manitoba showed that urea, when applied in excess of 20 lbs N/acre, reduced seedling emergence and yield of barley (Toews, 1971). This was found to be the result of ammonia toxicity within the seed (Carefoot, 1971). He also showed that ammonium sulfate, and ammonium nitrate have a toxic effect on seedling emergence and yield when applied at high rates. Other effects might also have been involved. Nitrite accumulation had been observed in Manitoba soils when urea was applied at a high rate (Jones and Hedlin, 1970a). Nitrite is toxic to crops.

The importance of nitrogen fertilizers has led to studies of the nitrification of ammonium and ammonium-yielding fertilizers in soils. Most of these studies have been conducted in the laboratory

using a system in which the nitrogen is mixed uniformly with the soil. Hence the concentration of nitrogen has usually been quite low as compared to that which commonly occurs when the same amount of nitrogen is applied in the field. Under field conditions, nitrogen fertilizer is very seldom uniformly distributed throughout the surface soil since fertilizers are generally broadcast, placed in band or drilled in with the seed.

A nitrogenous fertilizer applied at the rate of 50 lbs N per acre in the field results in an average concentration of about 25 ppm in the Ap layer based on the assumption that a 6-inch acre contains two million pounds of soil. Since the fertilizer is not uniformly mixed, there are locations near the fertilizer pellets and granules where the concentration of nitrogen is far in excess of 25 ppm. This is particularly true of band applications or those drilled in with the seed. Because of this high concentration of nitrogen, many processes such as the transport phenomena, transformation of nitrogen and local interaction of ions during the transport may take place. As a result, non-uniformly applied nitrogen fertilizer results in a different environment for nitrogen transformation as compared to that of uniformly applied nitrogen. Besides, the high concentration of nitrogen may have adverse effects on plant growth. It is known, however, that these effects vary considerably depending upon the nitrogen fertilizer used and the properties of the soil to which it is applied.

With this in mind, laboratory experiments were conducted to study the effects of temperature, pH and rate of application of nitrogen on the transformation of band-applied urea, ammonium hydro-

xide and ammonium sulfate with time and distance from the fertilizer zone. The recovery of banded nitrogen was also studied. Subsequently a series of experiments using nitrogen-15 tracer to allow for detailed study of N-loss, whether by immobilization and/or volatilization, were conducted.

LITERATURE REVIEW

FORMS OF NITROGEN IN SOIL

Organic nitrogen

Over 90% of the total nitrogen in the surface horizon of most soils is organically combined. However, in certain subsoils about 30-40% of the total nitrogen may be present as fixed ammonium (Bremner,1965). The nature of the organically combined nitrogen occurring in soils is not well understood. Information concerning these compounds is based largely on studies involving identification and estimation of the forms of nitrogen released by treatments with hot acids (Kojima,1947; Bremner,1965; Stevenson,1957; Cheng and Kurtz,1963). The organic forms isolated generally include amino acids, amino sugars (hexosamines), purine and pyrimidine derivatives, urea and many others (Bremner,1965). About 20-40% of the total soil nitrogen in most surface soils is represented by amino acid-N (Kojima,1947; Bremner, 1949; Stevenson,1954; Keeney and Bremner,1964), and about 5-10% of surface soil nitrogen is in the form of hexosamines (Bremner and Shaw, 1954).

The soil organic nitrogen is highly resistant to microbial attack and is converted to inorganic forms very slowly. The stability of the organic nitrogen may be related to its chemical, physical and physicochemical nature. One theory, which has been discussed by Swaby and Ladd (1962) states that polyphenols, amino acids and other nitrogenous substances are condensed into large molecules which resist decomposition by confining the actions of enzymes only to the outer surface of the three dimensional organic structure. Other

workers (Ensminger and Giesecking, 1942; Lynch and Contnoir, 1956; Pinck and Allison, 1951) postulated that clay minerals are involved in stabilizing the nitrogen by either adsorbing the enzymes or adsorbing proteins in a way that makes them inaccessible to enzymatic attack. They found that the degree of inhibition varied directly with the base exchange capacity of clay. Another group of investigators (Harmsen and van Schreven, 1953; Bartholomew and Clark, 1950) theorized that a low microbial population leads to stability of organic nitrogen. They reported that cropped land with its dense microbial population around the roots exhibits a more rapid breakdown of stable humus than uncropped soil.

Probably there is no single theory that adequately accounts for the stability of organic nitrogen in soil, and the stability is the result of a combination of several factors acting simultaneously.

Inorganic nitrogen

Only a small fraction of the total nitrogen in soil appears as inorganic forms. It is represented chiefly by ammonium (exchangeable ammonium and ammonium in solution), nitrite and nitrate. The amount of nitrite is generally negligible in normal soils. Conditions under which nitrite accumulates will be discussed later. Ammonium and nitrate are the immediate sources of nitrogen for plants. Nitrite can be utilized by crops. However, relatively low concentrations, for example, greater than 15 ppm NO_2^- -N may result in phytotoxic effects (Paul and Polle, 1965).

SOURCES OF SOIL INORGANIC NITROGEN

Inorganic forms of nitrogen are mainly derived from,

- 1) mineralization of organic nitrogen
- 2) nitrification of ammonium and ammonium containing compounds
- 3) addition of nitrogen fertilizers
- 4) addition of minute quantity of ammonium and/or ammonia and nitrate from rainwater.

1) Mineralization

The mineralization process results in the transformation of organic nitrogen to inorganic nitrogen, ammonium and/or ammonia, by non-specific heterotrophs as they decompose the organic matter. The organic carbon supplies the major energy source for the heterotrophs and nitrogen is assimilated as protein, nucleic acid and other nitrogenous constituents in the microbial tissues as a function of the growth of the organisms. The excess nitrogen is thus released as ammonia and/or ammonium into the soil. The amount of inorganic nitrogen released and in turn metabolised by the microorganisms depends on the C:N ratio of the organic material and the metabolizing microorganisms (Bartholomew, 1965). Plant residues have a gross C:N ratio of approximately 30:1 as compared with 6:1 characteristic of protein or 5:1 characteristic of most microbial populations (Campbell and Lees, 1967). Net nitrogen mineralization generally results from the microbial degradation of residues with C:N ratio of less than 20:1. Between C:N ratio of 20:1 and 30:1, nitrogen mineralization may or may not occur depending on the environmental conditions and type of substrate. Residues with C:N ratio greater than 30:1 are generally considered to induce immobilization of nitrogen (Harmsen and van Schreven, 1955; Alexander, 1965; Bartholomew, 1965; Clark, 1966). Ferguson and Gorby (1964) showed that incorporation

of straw into Southern Manitoba soils did not generally induce a nitrogen deficiency in the succeeding crops. However, there was marked reduction in mineralization when soil samples with straw was incubated in laboratory. The reason why there was no nitrogen deficiency in the succeeding crop when straw was applied was explained by the authors (Ferguson and Gorby, 1964) that a low microbial activity was induced by cold temperature and lack of moisture in fall and spring thereby reducing the immobilization of native inorganic nitrogen.

2) Nitrification

The inorganic nitrogen resulting from net mineralization or from applied ammonium fertilizers undergoes nitrification. Nitrification is a biological process in which ammonium is oxidized to nitrate. The oxidation process consists of two steps and is carried out most commonly by two specific obligate aerobic autotrophs. The ammonium is first oxidized to nitrite by Nitrosomonas spp., then nitrite is subsequently oxidized to nitrate by Nitrobacter spp.. The two steps of nitrification are exothermic reactions. Several other forms of inorganic nitrogen such as hydroxylamine (NH_2OH), hyponitrous acid ($\text{HON}=\text{NOH}$), and nitroxyl ($\text{HN}=\text{O}$) may occur as intermediates of autotrophic nitrification, but these compounds are chemically unstable and therefore are not easily detected (Alexander, 1965). Five other genera of autotrophic nitrifiers have been recognized. These are the ammonium-oxidizing Nitrosococcus, Nitrosospira, Nitrosogloea and Nitrosocystis, and the nitrite oxidizer, Nitrocystis (Breed et al., 1957). These autotrophs are less common than Nitrosomonas spp. and Nitrobacter spp.. These autotrophic

nitrifiers do not metabolize organic compounds, but derive all their energy from the oxidation of inorganic nitrogenous compounds and utilize CO_2 as the main source of carbon.

Nitrite accumulation is generally negligible but it may accumulate in neutral and alkaline soils fertilized with a high rate of aqua or anhydrous ammonia or an ammonium-yielding compound, such as urea. Nitrite accumulation can also occur in acid soils whose pH has risen as result of the addition of ammonium and ammonium-yielding compounds (Chapman and Liebig, 1952; Colliver and Welch, 1970; Stojanovic and Alexander, 1958; Broadbent et al., 1958; Stephen and Waid, 1963; Jones and Hedlin, 1970a) when soil and fertilizer are uniformly mixed. Nitrite accumulation was observed near fertilizer granules (Hauck and Stephenson, 1965; Bezdicek et al., 1971) and band-applied fertilizer (Isensee and Walsh, 1971; Wetselaar et al., 1972; Passioura and Wetselaar, 1972). Accumulation of nitrite and the loss of nitrogen due probably to denitrification or volatilization was found to be related (Clark et al., 1960; Hauck and Stephenson, 1965; Meek and MacKenzie, 1965).

Under favorable conditions, nitrite is readily oxidized to nitrate. Nitrate, an end product of nitrification, is the form of nitrogen most readily utilized by plants and microorganisms. On the other hand, nitrate, being very mobile, is subject to loss by leaching. It is also subject to gaseous loss by denitrification when aeration is limited.

Heterotrophs, including bacteria, actinomycetes and fungi, are able to oxidize mineral nitrogen. The intermediate products of the nitrification and the biochemical processes due to the above

microbes differ from those of the autotrophs (Alexander, 1965). The heterotrophs need organic carbon compounds for both energy and carbon supply. They oxidize not only inorganic nitrogenous compounds but also a variety of organic nitrogenous substances including amides, amines, oximes, hydroxamates, and nitrocompounds. Although heterotrophic nitrification occurs, the contribution to inorganic nitrogen economy in soil is of doubtful significance. A list of the heterotrophic nitrifiers and their possible intermediates and products has been summarized by Alexander (1965).

FATE OF INORGANIC SOIL NITROGEN

The available soil nitrogen can become unavailable to plants, at least for a period of time, through the following processes:

- 1) immobilization
- 2) ammonium fixation
- 3) ammonia volatilization
- 4) denitrification by biological or chemical processes
- 5) leaching.

1) Immobilization

Inorganic soil nitrogen and fertilizer nitrogen may be immobilized during the decomposition of carbonaceous residues with a wide C:N ratio. This has been reported by numerous workers who incubated soil with added organic matter, such as straw (Allison and Klein, 1962; Broadbent and Tyler, 1964), sawdust (Bollen and Lu, 1957; Allison and Cover, 1960) and other readily available energy sources, such as sucrose (Windsor and Pollard, 1956; Agarwal et al., 1972). The immobilization of inorganic nitrogen proceeds very rapidly

during the early stages of incubation and is related to the C:N ratio of material added. The rate of nitrogen immobilization is directly proportional to the rate of carbon dioxide evolution. Jansson et al. (1955) demonstrated that immobilization of NH_4^+ -N is greater than NO_3^- -N at the same nitrogen concentration. This was confirmed by Winsor and Pollard (1956a). Agarwal et al. (1972) showed that the addition of sucrose increases immobilization of added nitrogen. However, in absence of added sucrose the immobilization of added N was related to the C:N ratio. These data showed that the rate of decomposition of a carbonaceous material is important in determining the maximum immobilization and also the rate at which it occurs. If only a portion of the added material is readily attacked by microorganisms, for example, straw and sawdust, the immobilization of nitrogen is much less than where a readily available carbon source, for example, sucrose has been added. The microflora is also a factor in N immobilization since fungi, which are more tolerant of acidity than are bacteria, usually contain less nitrogen and liberate a smaller percentage of the added carbon as carbon dioxide.

Allison and Klein (1962) reported that maximum immobilization of N occurs in 20 days and averaged 1.7% of the original weight of straw, while Winsor and Pollard (1956abc) showed that sucrose addition brought about an immobilization of approximately 3.7% of nitrogen based on the weight of added sugar in the presence of abundant mineral nitrogen. Other than the carbon source and C:N ratio, soil environment is also an important factor. Agarwal et al. (1972) showed that N immobilization varies with different soils, being the fastest in alkaline soil in presence of sucrose. The alkaline soil pH favors

the increase of microbial activity which in turn induces N immobilization in soil. Irrespective of the soil, nitrogen immobilization occurs more rapidly with addition of sucrose than finely ground sugar cane bagasse.

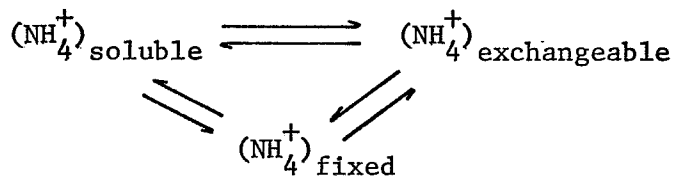
The inorganic nitrogen which becomes immobilized is temporarily unavailable to plants. After the microorganisms die, decomposition and mineralization of the organic substance takes place providing inorganic nitrogen (Winsor and Pollard, 1956c; Allison and Klein, 1962; Agarwal et al., 1972). In general, immobilization is always accompanied by mineralization, and vice versa.

2) Ammonium fixation

Nommik (1965) reported that fixation of ammonium by clay minerals and fixation of ammonia by organic matter is quite extensive. The ammonium and/or ammonia fixed is only very slowly available to plants and other organisms.

Fixation of ammonium by soils

There is an equilibrium between the different forms of ammonium in soils:



According to this equation, a change in one form of ammonium will affect the other forms. When the concentration of soluble NH_4^+ is increased, for example, after addition of NH_4^+ -containing fertilizers, the equilibrium is disturbed and fixation of some of the added NH_4^+ -N results. However, the fixed NH_4^+ will be released at an extremely

slow rate when exchangeable ammonium is removed.

Nommik (1957), Walsh and Murdock (1960) and Hinman (1966) showed that addition of KCl to soil increases the exchangeable K^+ and decreases the amount of NH_4^+ being fixed. The decrease in ammonium fixing capacity is proportional to the amount of KCl added, indicating that K^+ inhibited fixation of NH_4^+ . Potassium and ammonium ions have approximately the same ionic size which can be fixed snugly into the latticeholes of 2:1 type minerals, especially that of micaeous type, and thus become non-exchangeable with other cations (Nommik,1965).

Fixation of NH_3 by organic matter

Fixed ammonia was defined as that fraction of added ammonia which is retained by soil organic matter after intensive leaching with acid (Broadbent et al.,1961; Nommik and Nilsson,1963) and neutral salt solution (Nommik and Nilsson,1963). The organic matter reacts with ammonia and binds it in a difficultly hydrolyzable form which is only very slowly available to plants and soil microflora.

There are two different explanations concerning ammonia fixation by soil organic matter. One theory assumes that the uncharged ammonia is bound by the organic matter while the other feels that charged ammonium ion is bound to soil organic matter.

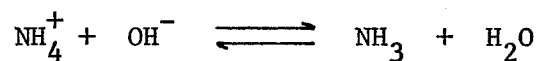
Since the formation of uncharged ammonia is controlled by soil acidity, it is evident that the pH of the soil may significantly affect ammonia fixation. This effect has been studied by Broadbent et al. (1961) and Nommik and Nilsson (1963). Their investigations demonstrated that ammonia fixation occurs to a large extent only

above neutrality. In addition, Broadbent et al. (1961) showed that there is a linear relationship between pH and ammonia fixation above pH 7.0.

On the other hand Sohn and Peech (1958) demonstrated that the greatest ammonia fixing capacity in soils occurs in acid soils containing a large amount of organic matter. The higher the carbon content or organic matter, the more ammonia fixed by the soil (Sohn and Peech, 1958; Burge and Broadbent, 1961; Nommik and Nilsson, 1963). Sohn and Peech (1958) and Mortland (1963) showed that ammonia fixing capacity of soils is reduced to approximately one half when organic matter is oxidized by hydrogen peroxide. In contrast to the above findings, Hinman (1966) demonstrated that destruction of organic matter increases the fixing capacity of soil, and the increase was greater when the organic matter content of the sample was higher. He suggested that the organic matter had either blocked the entry of NH_4^+ to the clay minerals. He speculated that although organic matter itself may have fixed some ammonium, its dominant effect was to prevent fixation of ammonium by clay.

3) Ammonia volatilization

Ammonia can be volatilized from surface-applied urea, ammonium salts and aqua and anhydrous ammonia, especially if the soil is neutral or alkaline in reaction (Volk, 1959; Volk, 1961; Simpson and Melsted, 1962; Gasser, 1964). The loss of N by ammonia volatilization is due to the reaction:



Ammonia volatilization usually does not occur when pH is below

neutrality (Martin and Chapman,1951). The quantity of ammonia volatilized is reduced when fertilizer is incorporated into the soil (Ernst and Massey,1960; Volk,1961). Volatilization loss as ammonia also occurs in slightly acidic soils, especially after application of high rate of urea (Ernst and Massey,1960; Wahhab et al.,1960). Hydrolysis of urea to ammonium carbonate results in an increase of soil pH near or above neutrality, a condition which favours ammonia volatilization. On the other hand, application of acidic ammonium salts to acid soils is less prone to induce ammonia volatilization (Martin and Chapman,1951; Volk,1961) since there is no increase in soil pH upon their addition. Therefore, soil pH,type of fertilizer and methods of fertilizer application have a great effect on the amount and rate of ammonia volatilization from soil.

Other factors, for example, high temperature and drying of soil also promote ammonia volatilization (Volk,1959; Chin and Kroontje, 1963). High clay content and a corresponding high cation exchange capacity favor the sorption of ammonium and a decrease in ammonia volatilization. Gasser (1964) reported that over 20% of the nitrogen was lost as ammonia from a soil with a cation exchange capacity of less than 10 meq. per 100g soil when 100 pound $(\text{NH}_2)_2\text{CO-N}$ per acre was broadcast on the soil surface. Ammonia volatilization losses amounted to about 10% from soils with cation exchange capacities greater than 20 meq/100g.

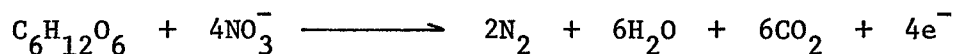
4) Denitrification

Gaseous loss of nitrogen may come about as a result of denitrification as well as ammonia volatilization. The term denitrification is commonly used to refer to the biological reduction of

nitrate or nitrite to volatile gases, usually nitrous oxide (N_2O) and/or molecular nitrogen. There is also evidence that nitrite formed during nitrification or added to soil may decompose or react with soil organic matter to yield gaseous nitrogen. These reactions are called chemodenitrification because they are not biological reactions.

Biological denitrification

The general equation of biological denitrification may be described by the following equation:



An anaerobic or partially aerobic soil atmosphere, a readily available energy source and the presence of nitrate or nitrite will lead to biological denitrification by non-specific anaerobic and facultative anaerobic heterotrophs. The denitrifier population is very abundant in most soils. In the absence of oxygen, but in the presence of nitrate, the denitrifiers use nitrate as an electron acceptor for the respiration process. Nitrate is reduced to nitrite and then simultaneously converted to gaseous products, N_2O and/or N_2 (Wijler and Delwiche, 1954; Nommik, 1956; Schwarzbeck et al., 1961; Bollag et al., 1973). Gaseous loss via denitrification appears to be pronounced in neutral and alkaline soils, and in soils in which nitrite has been observed to accumulate following the addition of ammonia and ammonium-yielding materials.

Denitrification has been reported to occur in apparently well aerated soils (Allison et al., 1960; Broadbent and Stojanovic, 1952; Carter and Allison, 1960; Cady and Bartholomew, 1960.1961; Myers and McGarity, 1972).

Two hypotheses are commonly given:

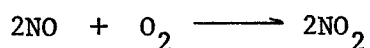
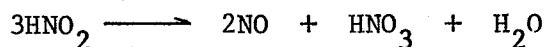
- (a) Slow diffusion of oxygen into the center of large aggregates, resulting a certain degree of anaerobiosis even though the soil is evidently aerated.
- (b) In presence of easily decomposable organic materials, there is a rapid uptake of oxygen. If the rate of oxygen demand is greater than oxygen supply, a demand for an alternative electron acceptor to oxygen occurs.

Chemodenitrification

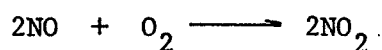
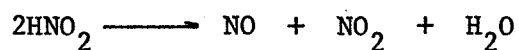
There is strong evidence that a significant loss of fertilizer nitrogen can occur through chemical decomposition of nitrite formed by nitrification of ammonium and ammonium-yielding fertilizers in soil (Broadbent and Clark, 1965; Allison, 1966). Numerous investigators (Smith and Clark, 1960; Tyler and Broadbent, 1960; Reuss and Smith, 1965; Jones and Hedlin, 1970b; Nelson and Bremner, 1970; Bollag et al., 1973) have demonstrated that chemodenitrification occurs in nitrite supplemented soils of acidic or slightly acidic nature.

A review by Broadbent and Clark (1965) summarized the possible channels for chemical denitrification to occur:

- (a) The chemical decomposition of nitrous acid at pH values below pH 5

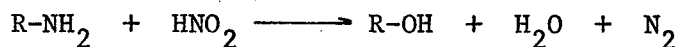


Some of the nitrogen dioxide (NO_2) may react with water to form nitrous acid and nitric acid. Recent work by Nelson and Bremner (1969) suggested that self decomposition of nitrous acid is better represented by

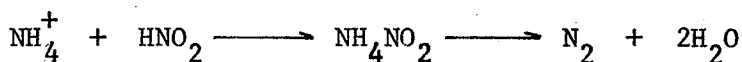
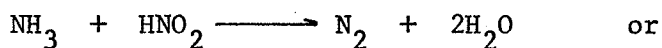


The nitrogen dioxide (NO_2) produced in the process can be converted to nitrate. In absence of oxygen, nitric oxide (NO) and NO_2 are the gaseous products of chemodenitrification. They suggested the failure of some investigators to detect NO_2 can be attributed to the sorption of NO_2 by moist soil and conversion of NO_2 to nitrate within closed experimental systems.

(b) Reactions of nitrous acid with α -amino acids at pH5 or lower (a van Slyke reaction).



(c) Reaction of nitrous acid with ammonia



(d) Reactions of nitrous acid with other soil constituents such as organic matter, organic reducing compounds and even soil minerals. Recent work by Nelson and Bremner (1969, 1970ab) demonstrated the loss in nitrite-treated soil is related to organic matter, and the role of soil minerals in nitrite decomposition was not significant. They reported that phenolic substances react readily with nitrite under soil conditions to form nitrosophenols that are decomposed by nitrous acid with formation of N_2 and N_2O . Stevenson et al., (1970) showed that molecular nitrogen, N_2O and NO are identified in gases formed by reacting NaNO_2 with soils, humic and fulvic acids, lignins, lignin-building units and polyphenols in aqueous buffer solutions of pH 6 and 7 in the absence of oxygen.

Bollag et al. (1973), working with sterile and non-sterile soil with pH adjusted to values ranging from pH 5 to 8.3, found that the gaseous products of biological denitrification are N_2O and/or N_2 , and that the products of chemodenitrification are NO and/or NO_2 . After 4 days incubation at 30C, Bollag et al. (1973) showed that approximately 80% of 250ppm NO_2^- -N added was lost from non-sterile soil and approximately 70% from sterile soil. The losses resulted from chemical denitrification and at pH 5 occurred under either aerobic or anaerobic conditions.

5) Leaching

The most reduced form of nitrogen, NH_4^+ , is relatively immobile in soil. As mentioned previously, it can either be fixed by clay minerals and bound in organic matter or exchange with cations on the soil colloids. On the other hand, the most oxidized form, NO_3^- , is very mobile. Nitrite, an intermediate of nitrification, is also very mobile, but it is very unstable. Nitrite can be converted to nitrate very quickly under aerobic conditions, but under anaerobic conditions it is quickly reduced to gaseous products and lost to the atmosphere.

When rainfall is sufficient to cause water to percolate through the soil profile, any nitrate present is gradually diluted out of the top soil but is not completely displaced (Wetselaar, 1961, 1962). In a fertilized field, the intensity of leaching losses depends on the cation exchange capacity of the soil and type of fertilizers. In a laboratory experiment, McGill (1971) observed that when fertilizer nitrogen was surface applied on two soils and then leached with ten inches of water, the greatest amount of nitrogen leached occurred with

KNO_3 , followed by NH_4NO_3 , $(\text{NH}_2)_2\text{CO}$ and $(\text{NH}_4)_2\text{SO}_4$. In both soils, most of the nitrogen was recovered as ammonium and little as nitrate.

Nitrate loss is much less from cropped land than fallow land because nitrate is assimilated by the crop and also the high evapotranspiration reduces the amount of water in the soil preventing accumulation and downward movement of nitrate (Wetselaar, 1961). Michalyzna and Hedlin (1961) have shown that large amount of nitrate may be located below the rooting zone when summerfallow is included in crop rotations. Hedlin (1971) also reported toxic concentrations of nitrate in water in shallow wells located in farmsteads. The nitrate presumably resulted from mineralization and nitrification of urea and other animal and human wastes.

In order to reduce loss of nitrogen through leaching, nitrification inhibitors and slow-release nitrogen fertilizers have been developed (Gasser, 1970; Prasad et al., 1971). Perhaps the best example of a nitrification inhibitor is 2-chloro-6(trichloromethyl)pyridine, N-serve, which was found to retard the nitrification process (Goring, 1962ab; Zowakowski and Gasser, 1967; Campbell and Aleem, 1965ab; Spratt and Gasser, 1970). N-serve has been used commercially with nitrogen fertilizers for this purpose.

The foregoing paragraphs summarize some of the features of nitrogen gains and losses in soil. The complete scheme for nitrogen turnover in soil can be summarized diagrammatically as shown in figure 1.

FACTORS AFFECTING NITRIFICATION

The above mentioned processes of gains and losses of soil nitrogen, except ammonium fixation, ammonia volatilization, chemo-

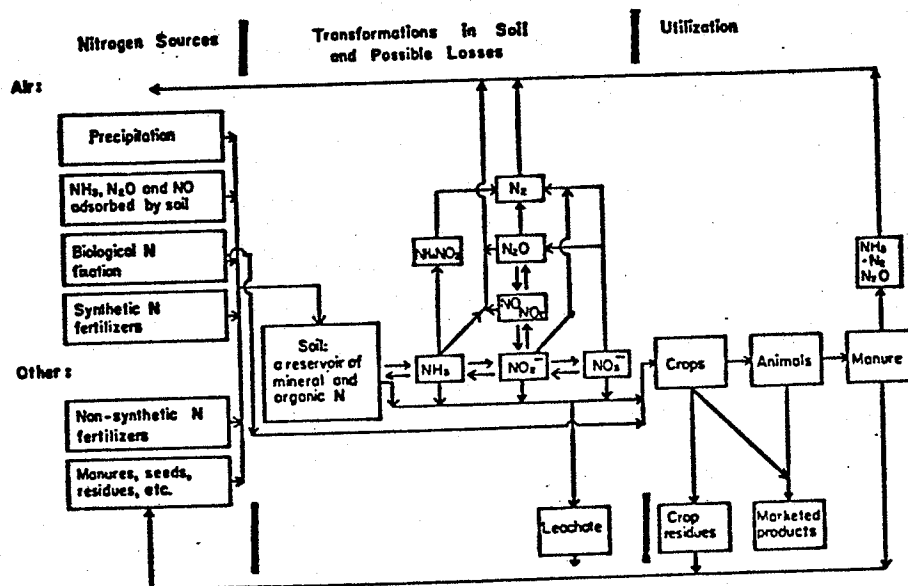


FIG. 1. DIAGRAMATIC SCHEME OF SOIL NITROGEN SOURCES AND TRANSFORMATIONS AND FATE OF THE END PRODUCTS (ALLISON, 1965).

denitrification and leaching, are of biological origin. Therefore factors such as pH, temperature, moisture and aeration which affect the growth of microorganisms also affect nitrogen transformations in soils. The factors which affect the nitrification of nitrogen in soil will be reviewed in detail in the following sections.

pH

Autotrophic nitrifiers are very sensitive to acidity. Successful isolation and maintenance of these microorganisms in the laboratory involves use of buffered, mildly alkaline media containing carbonate or bicarbonate (Alexander and Clark, 1965). In the field, oxidation of ammonium and nitrite occur over a wide range of soil pH values of from 5-10 (Alexander, 1965). Generally nitrification is favored by neutral and slightly alkaline pH. The capability of a soil to nitrify mineral nitrogen is also related to the nitrifier population. Morrill and Dawson (1962) demonstrated that the population of ammonium oxidizing microorganisms is greater in neutral and alkaline soils than in acid soils. They also showed that the most favorable pH for growth of Nitrobacter spp. is 6.2-7.0 while that for Nitrosomonas spp. is above pH 7.6. Later, Morrill and Dawson (1967) observed four general nitrification patterns of 116 soils as related to the measured soil pH, the number of Nitrosomonas spp. and Nitrobacter spp. and the rate of ammonium oxidation after percolating soils with ammonium sulfate solutions. These patterns are as follows: I. pH greater than 7.3 --- ammonium was rapidly oxidized to nitrite which accumulated over a long period of time before being oxidized to nitrate. The accumulation of large amounts of nitrite was related to the high initial activity of Nitrosomonas

spp. accompanied by a long lag period in proliferation of Nitrobacter spp.; II. pH 6-7.3 --- ammonium and the accumulated nitrite were rapidly oxidized to nitrate. The lower Nitrosomonas spp. activity and the very short lag period for Nitrobacter spp. was responsible for the very small quantity of nitrite being accumulated; III. pH 5.5-5.9 --- ammonium was oxidized to nitrate without any detectable nitrite being produced; IV. pH less than 5.4 --- ammonium oxidation was not detectable by either nitrite and nitrate formation. Subsequent nitrifer count failed to find significant numbers of Nitrosomonas spp. and Nitrobacter spp. These workers found that a type III pattern is converted to type II nitrification pattern after it was limed to contain a free phase of CaCO_3 . This conversion is due to the increase in the number of nitrifying organisms in the limed soil. The results of Morrill and Dawson (1967) are in agreement with those of other workers in that nitrite seldom accumulates in acid soils (Cornfield, 1952; Broadbent et al., 1958) although it tends to accumulate in neutral or alkaline soils (Chapman and Liebig, 1952; Stojanovic and Alexander, 1958; Court et al., 1964ab; Hauck and Stephenson, 1965). This suggests that Nitrobacter spp. is more active in acid soils while Nitrosomonas spp. is more active in alkaline soils. Dancer et al. (1973) observed a significant increase in nitrification of applied ammonium sulfate with soils whose pH ranged from 5.7-6.6 as a result of applications of lime over a period of years. They suggested that soil pH appears to be an excellent indicator of soil nitrification capabilities. Ishaque and Cornfield (1972) found that one of their experimental soils with initial soil pH 4.2 accumulated NH_4^+ when limed with CaCO_3 to pH greater than 5. They

attributed this to the incapability of fungi, a heterotrophic nitrifier, to nitrify applied nitrogen at higher pH values.

A combination of high pH and high ammonium concentration inhibit nitrite oxidation and an accumulation of nitrite results (Stojanovic and Alexander, 1958; Aleem and Alexander, 1960; Paul and Polle, 1965; Morrill and Dawson, 1967; Jones and Hedlin, 1970a). Aleem and Alexander (1960) suggested that non-ionized ammonia is the causative agent which interferes with the respiratory process of Nitrobacter agilis at the alkaline pH. They believed that the ammonium ion does not inhibit the nitrite oxidizing enzyme itself because extracts of Nitrobacter agilis oxidizes nitrite in the presence of ammonium ion. Activity of Nitrosomonas spp. is also reduced by a high concentration of ammonium, but these organisms are much less sensitive than Nitrobacter spp. Recent studies by Nako and Wolcott (1972) with liquid culture in a stationary condition buffered at pH 8-8.3 suggested nitrite accumulation in the presence of high ammonium concentrations is due to an imbalance in numbers of Nitrosomonas spp. relative to Nitrobacter spp. This indicates a bacteriostatic effect of ammonium or free ammonia on growth of Nitrobacter spp. rather than an inhibitory effect on the oxidation as suggested by Aleem and Alexander (1960). In fact, nitrite did not accumulate in their experimental system with ammonium levels ranging up to 750 ppm $\text{NH}_4^+\text{-N}$.

Nitrite accumulation is considered to be negligible under field conditions, but is frequently observed with application of anhydrous and aqua ammonia, and in soils of high pH or in soils which have become alkaline as the result of added nitrogen fertilizer. Under

alkaline conditions, the ammonia-ammonium equilibrium shifts towards ammonia which is believed to have an inhibitory effect on the activities of Nitrobacter spp. (Aleem and Alexander, 1960; Nako and Wolcott, 1972; Larsen, 1970). This results in nitrite accumulation.

Temperature

An ordinary chemical reaction is temperature dependent and the rate constant (K) of the reaction follows closely the Arrhenius equation, $K = Ae^{-E/RT}$, which expresses the functional relationship between a rate constant (K), temperature in degree Kelvin (T) and the activation energy of the reaction (E) (Bull, 1951). The notations A and R are constants. However, in biological processes, this relationship applies over a very limited temperature range since the enzymes involved in biological reactions are adversely affected by high temperatures. The rate of a biological reaction doubles or sometimes more than doubles for a 10°C rise in temperature. This variation due to 10°C rise in temperature is known as Q_{10} which is defined as the ratio of the speed of reaction at one temperature to that at a temperature 10°C lower. In the case of nitrogen transformations, the rate constant, K, is different for each step.

Nitrification in the field and laboratory occurs over the temperature range 2-40°C (Frederick, 1956; Sabey et al., 1959; Parker and Larson, 1962; Justice and Smith, 1962; Anderson et al., 1971; Thiagalingam and Kanehiro, 1973). They indicated that the rate of ammonium and nitrite oxidation increases with increased temperature when other conditions are favorable. Tyler et al. (1959) and Justice and Smith (1962) reported that nitrite tends to accumulate at lower temperatures. This suggests that Nitrobacter spp. are more sensitive

to low temperature than Nitrosomonas spp.. The optimum temperature for nitrification in soil varies but is usually between 25 and 35C. Frederick (1957) attributed the differences between soils in regard to the temperature range over which nitrification occurs to differences in the initial population of nitrifiers. Recent work by Anderson et al. (1971) confirmed the findings of Frederick (1957). By inoculation of sterilized synthetic soil with a fertile acid soil, Anderson et al. (1971) obtained similar nitrification rates for all soils. These workers used a synthetic soil to eliminate toxic factors that might contribute to slow nitrification in some acid soils (Theron, 1951; Brar and Giddens, 1969).

The above studies were carried out under a constant temperature regime. Frederick (1956), Campbell et al. (1971) and Campbell and Biederbeck (1972) studied the influence of fluctuating temperatures on the rate of nitrification. Early work by Frederick (1956) showed an increased rate of nitrification when the fluctuation of temperatures involved a combination of temperatures below 15C as compared to soil incubated at constant temperature of 15C. At temperatures above 15C, the nitrification rate at a constant temperature was usually as high or higher than the rate with fluctuating temperatures in the $(\text{NH}_4)_2\text{SO}_4$ amended soil. Campbell et al. (1971) reported there was no difference in nitrification when frozen $(\text{NH}_4)_2\text{SO}_4$ -amended soil was reincubated at fluctuating temperature (12 hours at 14C and 12 hours at 3C) and at constant temperature (8.5C).

At low temperatures, soils usually have lower microbial activities and thus biological processes such as ammonium and nitrite oxidation are hindered. Temperature range at which nitrification by

autotrophs is optimum is also optimal for other biological processes such as mineralization, immobilization, nitrogen fixation and biological denitrification.

Moisture

Biological processes including nitrification are essentially non-existent when the soil is air dry. The microorganisms in soil are dormant when soil is dry, but their activities recover gradually when soil is remoistened. Justice and Smith (1963) reported that when soil was incubated at 10 bar tension at 10C there was a delay of the initiation of the nitrification while nitrification was completely inhibited at 415 and 115 bar moisture tensions. At a higher temperature, 25C, the rate of nitrification increased with decreasing moisture tension from 15 bar to 1 bar. Justice and Smith (1963) also demonstrated that at 15 bar tension half of the added ammonium sulfate (150 ppmN) was oxidized to NO_3^- -N in 28 days while at 10, 7, and 1 bars nitrification was completed in 28, 20 and 12 days, respectively. They further concluded that temperature (both high and low) and lowered moisture content had greater inhibiting effect on nitrite oxidizers than ammonium oxidizers. Reichman et al. (1966) demonstrated that nitrification of soil inorganic nitrogen is almost directly proportional to soil water content at suctions between 0.2 and 15 bars.

High moisture content in soil is also known to retard the nitrification process. Parker and Larson (1962) showed that nitrate production was retarded at soil moisture tension below 50cm of water (≈ 0.05 bar). At a higher moisture levels, considerable mineral nitrogen was lost from soil. The high moisture content inhibits respiration of autotrophic nitrifiers which require oxygen to carry out

the nitrification process. On the other hand, this condition promotes the proliferation of non-specific heterotrophic denitrifiers resulting in loss of mineral nitrogen by denitrification.

Aeration

A soil matrix is made up of three phases, solid, liquid and gaseous. Since solid space cannot be taken over by liquid and gases, the gaseous space and liquid space have an inverse relationships.

Since nitrification of mineral nitrogen in soil is mainly carried out by obligate aerobic autotrophs, oxygen is essential for the process. In an oxygen deficient soil, ammonium tends to accumulate (Waring and Bremner, 1964; Patrick and Wyatt, 1964) and nitrate, if present in soil, will be reduced to nitrite by non-specific heterotrophs and then to one or more of the gaseous nitrogen compounds, N_2 , NO , N_2O and NO_2 (Wijler and Delwiche, 1954; Cady and Bartholomew, 1960; Patrick and Wyatt, 1964; Schwarzbeck et al., 1961; Bailey and Beauchamp, 1973; Bollag et al., 1973). The oxygen demand for the Nitrosomonas spp. and Nitrobacter spp. is quite high. Nitrosomonas spp. requires approximately 1.5 mole oxygen for the oxidation of one mole of NH_4^+ (the energy substrate), while Nitrobacter spp. requires 0.5 mole oxygen for the oxidation of nitrite (Alexander, 1965). The optimum percentage of oxygen for rapid nitrate production is similar to that found in air.

NITROGEN FERTILIZER

Methods of nitrogen fertilizer application

Nitrogen fertilizers are generally applied broadcast, with the seed, uniformly incorporated into the soil or as a band appli-

cation. In order to reduce the loss of ammonium and ammonium-yielding fertilizers through ammonia volatilization, incorporation of these fertilizers in soils is preferred over the broadcast method where the fertilizer is left on the surface (Ernst and Massey, 1960; Vold, 1961; Stephen and Waid, 1964). When ammonium and ammonium-yielding fertilizers are applied with the seed, there may be phytotoxic effects. This may result simply from a high osmotic pressure or from ammonia and/or nitrite accumulation (Brage et al., 1960; Stephen and Waid, 1964; Paul and Polle, 1965; Bennett and Adams, 1970; Carefoot, 1971).

During fertilizer application, the rate of 50 lbsN/acre, for example, which results in 25 ppm N if uniformly mixed with top 6 inches of soil, can never be attained in the field. Thus, the concentration of nitrogen near the fertilizer pellets and granules is frequently several hundred ppm.

Chemistry of nitrogen fertilizers

Most nitrogen fertilizers are water soluble. Upon contact with moist soil, the nitrogen fertilizer dissolves and immediately forms a local environment which is either acid, neutral or alkaline in reaction depending on the form and amount of nitrogen fertilizer employed.

Ammonium sulfate, ammonium nitrate and ammonium chloride produce an acid environment in the immediate vicinity of the fertilizer zone when dissolved in the soil solution. On the other hand, ammonium carbonate and diammonium phosphate produce a locally alkaline environment. Urea produces a similar effect since it hydrolyzes rapidly to form ammonium carbonate which is an alkaline salt. However

potassium nitrate, sodium nitrate and calcium nitrate are essentially neutral (Fuller,1963; Alexander,1965). The effect of fertilizer additions on the soil reaction near the fertilizer zone is usually temporary. This is particularly true where an ammonium salt, which is subject to nitrification, has been applied.

Nitrification near the fertilizer zone

A nitrogen concentration gradient usually exists at the proximity of the fertilizer pellets and granules. When anhydrous and aqua ammonia is injected to soil, both ammonia and ammonium are adsorbed readily by soil materials. The distribution of these ions near the fertilizer zone depends on the rate of application, clay and organic matter content and moisture (Jackson and Chang,1947; Parr and Papendick,1966; Papendick and Parr,1966; Parr,1969). The high local concentration of ammonium and ammonia has a partial sterilizing effect (Eno and Blue,1954,1957; Parr,1969). The sterilizing effect on fungi is the greatest. However, bacteria and actinomycetes decrease in numbers initially but increase in numbers as the local ammonium and/or ammonia concentration and soil pH begin to decrease. Subsequently, oxidation of reduced nitrogen proceeds at the periphery of the ammonia and/or ammonium retention zone where the nitrifiers are more active.

The above processes that occur with aqua and anhydrous ammonia may also be applicable when granule or pelleted solid fertilizers are placed as bands in the soil drilled with the seed or are concentrated in localized areas after broadcast application.

There have been relatively few studies on the transformation of band-applied nitrogenous fertilizers. Hauck and Stephenson (1965)

have shown that nitrogen granules can either stimulate or retard nitrification depending on the granule size, pH of the granules site and the ammonium concentration. They reported that large granules, high rates of nitrogen application, low soil buffer capacity, as well as an alkaline pH of the immediate granule environment, may lead to nitrite accumulation even in acid soils. Similar observations were obtained by Isensee and Walsh (1971). They also observed the pH and ionic composition within and near a fertilizer zone are totally different from that of the surrounding soil. The availability of certain elements, K, Ca and Mg, and the microbial population are altered at and near the fertilizer zone (Isensee and Walsh, 1971, 1972). Nitrite accumulation was observed in alkaline soil (pH 7.1) with various band-applied fertilizer combinations of monocalcium phosphate, potassium chloride, ammonium nitrate, diammonium phosphate and urea. However, high to toxic levels of nitrite only accumulated near the fertilizer band when urea was present. Bezdicek et al. (1971), using an alkaline soil (pH 8.2) and three nitrogen carriers, diammonium phosphate, ammonium sulfate and urea, found that the geometry of the fertilizer granule spacing in the soil influences the rate of NH_4^+ -N disappearance and appearance of NO_2^- -N. They showed that NH_4^+ from diammonium phosphate was confined largely to the granule site while diffusion of NH_4^+ from $(\text{NH}_4)_2\text{SO}_4$ and urea was more intense. Although NO_2^- accumulated with the three carriers, urea application resulted in the greatest amount of nitrite and free ammonia which was correlated with the highest soil pH (Bezdicek et al., 1971). Wetselaar et al. (1972) investigated the distribution of NH_4^+ , NO_2^- and NO_3^- at various times within a soil column (pH 7.8) when ammonium sulfate and

urea were band-applied. Their results indicated that the nitrogen-containing ionic species produced and their distribution varied with time and also depended on soil properties and the nitrogen carrier used. A large quantity of nitrite was accumulated following urea application while only a very small quantity was produced from ammonium sulfate. They attributed this to the effect of urea on soil pH. In a later study, Passioura and Wetselaar (1972) presented data on pH changes near the fertilizer band during incubation.

A mathematical analysis of the NH_4^+ , NO_2^- and NO_3^- distribution within a soil during constant addition of NH_4^+ to the soil surface under convective systems was presented by Cho (1971). His analysis indicates that the distribution of various forms of nitrogen compounds within a soil are functions of many variables such as nitrifying activities, cation exchange capacity of the soil, diffusion coefficient and solution velocity.

MATERIALS AND METHODS

Soil samples

Four soils, acid Keld, acid Wellwood (I), neutral Wellwood (II) and alkaline Morton, were collected in early fall, 1971 from 0-15 cm depths of cultivated fields. Immediately after collection, the soils were air-dried, ground to pass a 2 mm sieve and stored at 5C. The locations of the soils and some of their physical and chemical properties are shown in Table 1.

Incubation column for band-applied fertilizers

An incubation column was constructed using plexiglass sections with an internal diameter of 3.85 cm and a length of 1-cm. These 1-cm sections were fastened by taping with black adhesive tape to form a column of 12-cm in length (Fig. 2). One end of the column was closed with an acrylic disc.

Experiment I. Transformation and movement of band-applied urea, ammonium hydroxide and ammonium sulfate in soils

The incubation column described in the previous section was packed loosely to a depth of 6 cm with soil moistened to field capacity. The desired quantity of fertilizer was applied uniformly on the soil surface where urea and ammonium sulfate were used, and 6 cm of moistened soil was packed on top of the fertilizer. In experiments involving ammonium hydroxide, the solution was injected through two small openings (≈ 0.10 mm diam.) in the mid-section of the 12-cm column after the column was filled with soil. Ammonium hydroxide was used to represent anhydrous ammonia. The nitrogen application was equivalent to 800 kg N/ha on area basis and corresponded to 92 mg N per

TABLE 1. SUBGROUP DESIGNATION, LEGAL LOCATION, AND SOME PHYSICAL AND CHEMICAL PROPERTIES OF THE SOILS

<u>Soil Name</u>	<u>Keld</u>	<u>Wellwood I</u>	<u>Wellwood II</u>	<u>Morton</u>
Subgroup	Gleyed Black	Chernozemic Black	Chernozemic Black	Orthic Grey Wooded
Legal Location	NE4-25-20W	NE19-12-14W	NE19-12-14W	SW26-16-2E
Texture	SiCl	CL	CL	VFSL
pH	5.4	6.0	6.6	8.2
C.E.C. (meq./100 g)	37.0	20.0	27.0	13.0
Conductivity (mmho)	0.8	0.7	0.3	0.3
% Organic Matter	8.7	6.5	7.1	2.6
% CaCO ₃	0	0	0	17.6
% H ₂ O (1/3 atm.)	42.0	32.0	32.0	17.0

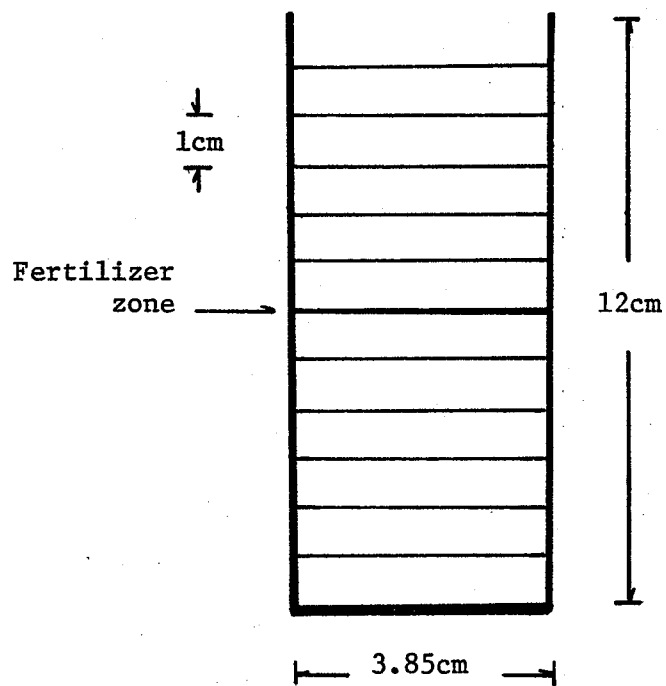


FIG. 2. INCUBATION COLUMN

column. The quantities of individual compounds used were 0.2 g $(\text{NH}_2)_2\text{CO}$, 0.44 ml of 14.8N NH_4OH and 0.44 g of $(\text{NH}_4)_2\text{SO}_4$ per column. The weights of the dry soil required to fill a column were 95 g, 110 g and 140 g for Keld, Wellwood II and Morton soils, respectively. The rate of 800 kg/ha banded in this experiment stimulates the fertilizer band obtained when approximately 50 lbs of N per acre is placed in a band 1" wide and fertilizer bands are spaced 16" apart.

The columns were placed vertically in a large desiccator having a small opening (0.1 cm diam.) to allow air exchange. Water was placed at the bottom of the desiccator to maintain a high relative humidity so that the soil samples would not lose moisture during incubation. Control soils without added nitrogenous fertilizers were incubated simultaneously in order to estimate the amount of NH_4^+ , NO_2^- and NO_3^- -N derived from soils. The soil columns were incubated at 20C for periods of 2, 4, 6 and 8 weeks.

After incubation the columns were segmented into 1-cm sections. The soil in each section was analyzed for NH_4^+ , NO_2^- and NO_3^- -N. The soil pH and moisture were also determined (details of the analyses are described in a later section).

Experiment II. Effect of rate of nitrogen application on transformation of band-applied fertilizers in neutral Wellwood II soil

Only neutral Wellwood II soil was chosen for this experiment because the results obtained from Experiment I indicated that this soil differed from the other soils in nitrifying properties and further investigations seemed desirable.

Three rates of nitrogen, 800, 200 and 100 kg/ha on an area basis, were used which corresponded to 92, 23 and 11.5 mg N per column

of Wellwood II soil (pH 6.6), respectively. The three fertilizers, $(\text{NH}_2)_2\text{CO}$, NH_4OH and $(\text{NH}_4)_2\text{SO}_4$, were applied in a band. The soils were incubated at 20C for 2, 4, 6 and 8 weeks, and samples of the soil were analyzed as described in Experiment I.

Experiment III. Effect of temperature on transformation and movement of band-applied nitrogenous fertilizers in neutral Wellwood II soil

After band-application of 0.2g of $(\text{NH}_2)_2\text{CO}$, 0.44ml of 14:8N NH_4OH and 0.44g of $(\text{NH}_4)_2\text{SO}_4$ in Wellwood soil (pH 6.6), the soil columns were incubated at 10, 15 and 20C for 2, 4, 6 and 8 weeks. After incubation, soil in each section was analyzed for NH_4^+ , NO_2^- and NO_3^- -N. Soil pH and moisture were also determined.

Experiment IV. Effect of pH on transformation of band-applied urea in Keld and Wellwood II soils

Wellwood II (pH 6.6) and Keld (pH 5.4) soils were used for this investigation in order to ascertain whether differences in the nitrifying capacity of these soils could be explained on the basis of pH. Wellwood II soil was acidified to pH 5.4 with 20ml of 0.1N H_2SO_4 per 100g dry soil. This was equivalent to 2 meq H_2SO_4 /100g soil. The Keld soil was limed with 0.5g and 1.0g $\text{Ca}(\text{OH})_2$ per 100g of dry soil to increase the pH to 6.5 and 7.3, respectively. These amounts were equivalent to 13.5 and 27.0 meq of $\text{Ca}(\text{OH})_2$ /100g soil. The soils were preincubated for 1 week at 20C before 0.2g of $(\text{NH}_2)_2\text{CO}$, equivalent to 92mg N, was applied in band as previously described. The columns were then incubated for 2, 4, 6 and 8 weeks at 20C. Soil pH, moisture, NH_4^+ , NO_2^- and NO_3^- -N were determined in the samples after each incubation period.

Experiment V. The recovery of banded KNO_3 and KNO_2 in presence of added urea with Wellwood I soil

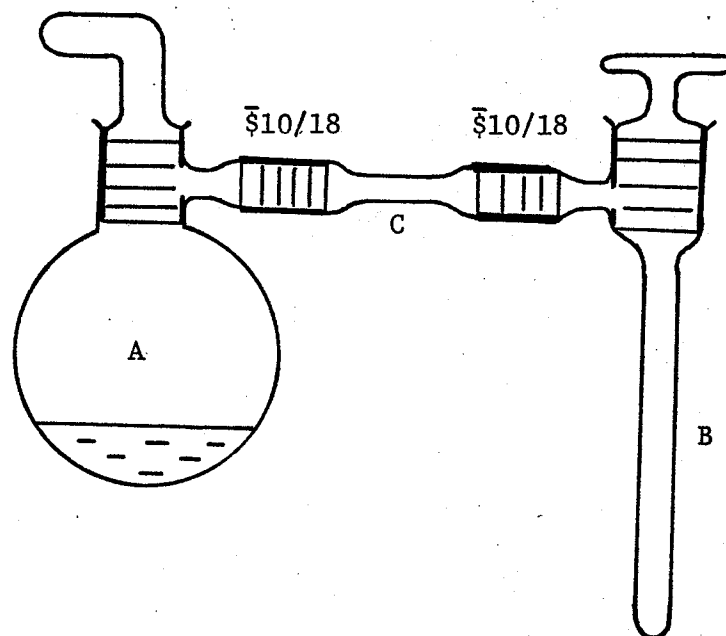
An acidic Wellwood (I) of pH 6.0 was used in this investigation in order to simulate the soil acidity near the fertilizer zone of urea-band-applied Wellwood II as observed in Experiment I. The Wellwood I (pH 6.0) in this investigation was treated with KNO_3 , $(\text{NH}_2)_2\text{CO} + \text{KNO}_3$, KNO_2 and $(\text{NH}_2)_2\text{CO} + \text{KNO}_2$ in a band and incubated at 20C for 1 and 2 weeks as described in previous experiments. The samples were then analyzed for NH_4^+ , NO_2^- and NO_3^- -N. The fertilizer N treatments are shown in Table 2.

TABLE 2. NITROGEN TREATMENTS IN ACID WELLWOOD I SOIL

	Treatments	Kg N/ha (on area basis)
1	11.5 mg KNO_3 -N	100
2	11.5mg KNO_3 -N + 11.5mg $(\text{NH}_2)_2\text{CO}$ -N	200
3	11.5mg KNO_2 -N	100
4	11.5mg KNO_2 -N + 11.5mg $(\text{NH}_2)_2\text{CO}$ -N	200

Experiment VI. A mass spectrometric investigation on denitrification from nitrite-treated soil

Twenty-gram samples of acidic Wellwood soil (pH 6.0) were treated with 0.0985g of ^{15}N -labelled and non-labelled NaNO_2 . The soil and NaNO_2 were carefully mixed resulting in a uniform concentration of 1,000 ppm of NO_2^- -N. The samples were incubated for 1 week in a closed container (Fig.3), gas collected from the vessel containing the soil was analyzed mass-spectrometrically by scanning for mass to



- A Soil incubation vessel (150 ml)
B Gas collecting vessel (20 ml)
C Inner adaptor $\bar{\text{S}}10/18$

FIG. 3. INCUBATION APPARATUS FOR MASS-SPECTROMATRIC ANALYSIS OF GASEOUS PRODUCTS DUE TO DENITRIFICATION.

charge ratio (m/e) of 28 through 48. An air sample was also analyzed in order to estimate the m/e of 28 to 48 present in atmosphere. It was necessary to run the air sample simultaneously with the gas samples since some nitrogen combined with oxygen to form oxides within the analytical tube of the mass spectrometer.

During the assembling of the incubation apparatus (Fig. 3), the gas collecting vessels were evacuated under high vacuum. After the incubation flask was in position, the stopcocks on the gas collecting vessels were opened. This resulted in an atmospheric pressure within the incubation apparatus of approximately 68 cm Hg. This step was taken in order to avoid an atmosphere greater than 76 cm of Hg within the incubation apparatus in the event that gases were produced during incubation.

Experiment VII. Recovery of band-applied $(^{15}\text{NH}_2)_2\text{CO}$, $^{15}\text{NH}_4\text{OH}$ and $(^{15}\text{NH}_4)_2\text{SO}_4$ from Wellwood II soil by ^{15}N tracer technique

Five hundredth (0.05) gram of $(^{15}\text{NH}_2)_2\text{CO}$ (29.7 atom % ^{15}N excess), 0.11 ml of 14.6N $^{15}\text{NH}_4\text{OH}$ (52.2 atom % ^{15}N excess) and 0.11 g of $(^{15}\text{NH}_4)_2\text{SO}_4$ (52.2 atom % ^{15}N excess) was banded at the mid-section of a 12-cm neutral Wellwood II soil (pH 6.6) column as described previously and incubated at 20C for 1 and 4 weeks. Nitrogen-15 labelled urea and ammonium sulfate were obtained commercially from Germany. Nitrogen-15 labelled ammonium hydroxide was not available commercially and thus $^{15}\text{NH}_4\text{OH}$ was prepared from $(^{15}\text{NH}_4)_2\text{SO}_4$ as described in next section.

After incubation the soil was mixed uniformly. Two 5-g samples of moist soil were weighed out for total nitrogen and ^{15}N abundance determinations. Two 10-g samples of moist soil were weighed out and

extracted with 100 ml 2N KCl for total inorganic nitrogen and ^{15}N determination.

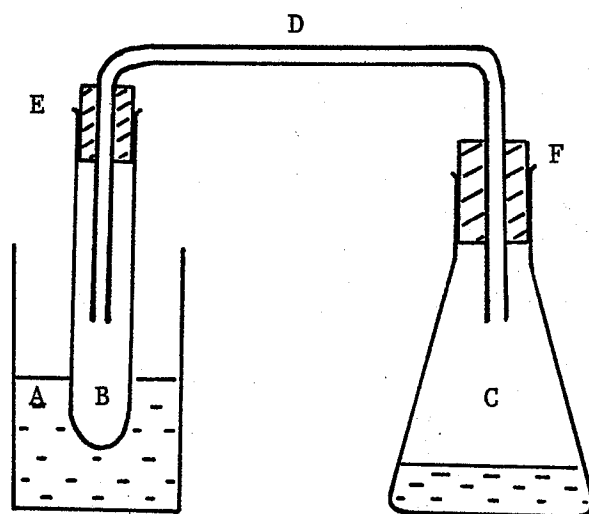
Preparation of 14.8N $^{15}\text{NH}_4\text{OH}$ (52.2 atom % ^{15}N)

Two grams of NaOH was dissolved in 3 ml of distilled water in a 25 ml Erlenmyer flask with the attachment as shown in Figure 4. Three grams of $(^{15}\text{NH}_4)_2\text{SO}_4$ (52.2 atom % ^{15}N) was added to the flask. Then it was closed immediately with a rubber stopper to avoid a large loss of ammonia in the initial reaction. The test tube (10 ml) was immersed in liquid nitrogen in order to freeze the volatilized ammonia onto the test tube. When the reaction in the Erlenmyer flask stopped, another portion of NaOH (2 g) was added to the flask. More NaOH was added until no reaction occurred upon addition of NaOH. Three millilitres of distilled water was pipetted into the test tube containing ammonia. The test tube was then sealed tightly and removed from the flask containing liquid nitrogen. The ammonium hydroxide concentration was determined by titration of 1 ml of the prepared solution with 1N H_2SO_4 , and the ^{15}N abundance was determined mass spectrometrically. The $^{15}\text{NH}_4\text{OH}$ was then stored in the refrigerator.

Experiment VIII. Nitrification of urea in soil suspension and the nitrifier population

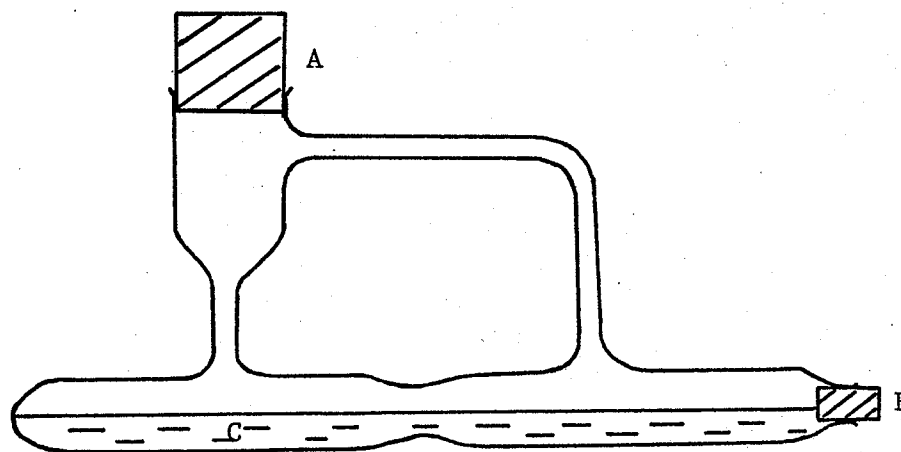
An incubation vessel described by Pang (1970) (Fig. 5) was used for this investigation. Half a gram (0.5 g) of acidic Keld, neutral Wellwood II and alkaline Morton soils was shaken in 25 ml of 0, 25, 50, 75 and 100 ppm N of urea solution in the incubation vessel (Fig. 5) for 1, 2, 3 and 4 weeks at 20C on a rocking apparatus.





- A Dewar flask containing liquid nitrogen
- B 10 ml test tube to collect ammonia gas
- C 25 ml Erlenmeyer flask containing $(\text{NH}_4)_2\text{SO}_4$, NaOH and distilled water
- D Glass tubing, 0.25 cm I.D. diam.
- E Rubber stoppers
- F Rubber stoppers

FIG. 4. APPARATUS FOR PREPARATION OF $^{15}\text{NH}_4\text{OH}$ (52.20 atom % ^{15}N)



- A Size #3 rubber stopper
- B Size #1 rubber stopper
- C 0.5 g soil + 25 ml urea solution

FIG. 5. INCUBATION VESSEL FOR THE STUDY OF NITRIFICATION IN SOIL SUSPENSION.

The stopper (size #3) on the incubation vessel was removed daily for 5 to 10 minutes to allow air exchange. After incubation, the pH of the soil suspension was measured before extraction of the inorganic nitrogen. The nitrogen was extracted by adding 3.75 g KCl to provide 2N KCl solution and the solution filtered.

Estimation of nitrifier numbers

Enumeration of Nitrosomonas spp. and Nitrobacter spp. in soils was obtained by the most-probable-number (MPN) method described by Alexander and Clark (1965).

Ten grams of moist soil at field capacity was transferred to a water blank containing 95 ml of sterile distilled water. The tightly capped bottle was shaken vigorously by hand for 5 minutes. A 10^{-2} dilution was established by transferring a 10-ml aliquot from the soil suspension to a 90-ml water blank. Transferring 10 ml quantities to further sterile water blanks of 90 ml provided dilution series through 10^{-5} for Keld soil and 10^{-7} for Wellwood II soil and Morton soils. From the highest dilution and the four next lower and consecutive serial dilutions prepared, 1 ml aliquots were transferred to each of 5 tubes containing ammonium-calcium carbonate medium for Nitrosomonas and 5 tubes containing nitrite-calcium carbonate for Nitrobacter. The inoculated tubes were incubated for 3 weeks at 28C. After incubation a qualitative test for the presence of nitrite was made using the Griess-Ilosvoy reagent and for nitrate using the Zn-Cu-MnO₂ mixture as described by Alexander and Clark (1965).

Chemical Analysis

pH

The soil pH was measured with a digital pH meter equipped with

glass and calomel electrodes (Model 801, Ionalyzer Orion Research) using a 1:4 ratio of soil and water. Samples were shaken for 30 minutes before the soil pH was determined.

Exchangeable potassium

Two and a half grams of soil was shaken for 1 hour with 50 ml 1N NH_4OAc . The potassium in the soil extract was determined by flame photometer via a Technicon autoanalyzer.

Available phosphorous (NaHCO_3 extractable)

Two and a half grams of soil was shaken with 50 ml of 0.5M NaHCO_3 solution for 1 hour. The NaHCO_3 extractable phosphorous was determined colorimetrically using a Technicon autoanalyzer.

Calcium carbonate

The CaCO_3 content was determined manometrically by the method described by Skinner et al. (1959).

Organic matter

The potassium dichromate-conc. H_2SO_4 oxidation method described by Peech et al. (1947) was employed to determine the soil organic matter. Samples of 0.5 g were used and after oxidation the excess chromic acid in the solution was back titrated with 0.5N FeSO_4 using an automatic titrator.

Electrical conductivity

The conductivity was measured on a soil-water (1:4 ratio) suspension with a Radiometer direct reading conductivity meter (Type CDM 2, Bach-Simpson Ltd.).

Cation exchange capacity

The cation exchange capacity was determined by the ammonium saturation method of Chapman (1965) in which a 25-g soil sample was shaken with 250 ml 1N NH_4OAc (pH 7.0). After filtering the excess NH_4OAc was washed from the soil with 200 ml of 95% ethyl alcohol. Then the soil was leached with 250 ml 1N NaCl solution to remove ammonium from the exchange sites. The leachate was distilled into 50 ml boric acid (4%) solution. The ammonium in the distillate was determined by titration with 0.1N H_2SO_4 .

Total nitrogen

Total nitrogen including nitrite and nitrate, was determined as described by Bremner (1965). The method involved a pretreatment of 5-g of moist soil with 5% potassium permanganate to oxidize nitrite to nitrate. This was followed by reduction of nitrate to ammonium by iron powder in a slightly acid solution. The pretreatment usually required 15-20 minutes after addition of the finely ground iron powder. It was followed by the conversion of organic nitrogen to ammonium by digesting for 3 hours in a mixture of 25 ml conc. H_2SO_4 and a catalyst (Kel-pak) containing 0.3 g CuSO_4 and 10 g K_2SO_4 . The ammonium in solution was converted to ammonia with 60 ml conc. NaOH by distillation. The ammonia was collected in 25 ml 0.1N H_2SO_4 containing methyl red indicator. The total nitrogen was calculated from the titration of the distillate in 0.1N H_2SO_4 with 0.1N NaOH.

When ^{15}N analysis was required, the titrated solution was made acid with 1 drop of conc. H_2SO_4 . Then the acidified solution was reduced to a volume of 10 ml by evaporation, and stored in test tubes. This condensed solution was then converted to nitrogen gas

in vacuum by NaOBr. The nitrogen gas was analyzed for mass to charge ratio (m/e) 28 and 29 mass spectrometrically as described by Pang (1970).

Total inorganic nitrogen by the distillation method

Ten grams of wet soil was shaken for 1 hour in 100 ml 2N KCl solution to extract the nitrogen. Eighty millilitres of soil extract was pipetted into an 800 ml Kjeldahl flask, and 100 ml distilled water was added making a total volume of 180 ml. Then 50 ml conc. NaOH was added slowly, and it was followed by addition of 1.5 g of Devarda's alloy (British Drug House, England). The ammonia released was distilled into 30 ml 0.01N H_2SO_4 containing methyl red indicator. The distillate was back titrated with 0.01N NaOH. The abundance of ^{15}N was determined as described in previous section.

Ammonium nitrogen

The inorganic nitrogen was extracted by shaking 5-g of moist soil in 50 ml of 2N KCl solution for 1 hour. The suspension was filtered through Whatman No. 42 filter paper. The ammonium was determined colorimetrically by Nesslerization as described by Jackson (1958).

Five millilitre of extract was pipetted into a 50 ml volumetric flask, followed by 2 ml of 10% sodium tartrate and a pinch of gum acacia. Approximately 30 ml of distilled water was added and the solution was mixed thoroughly. Then 2.5 ml Nessler reagent was added and the solution diluted to 50 ml with distilled water. The intensity of the yellow color developed was measured spectrophotometrically using the wavelength of 410 nm. A standard curve was

constructed everytime a new set of samples was analyzed. The standard solutions consisted of 0.5, 1.0, 1.5, 2.0, 3.0, 4.0 and 7.0 ppm N of NH_4Cl .

Nitrite and nitrate nitrogen

The nitrite and nitrate nitrogen were determined using the same soil extract prepared for the ammonium determination. From the same aliquot of soil extract, nitrite and the sum of nitrate and nitrite were determined colorimetrically using a Technicon autoanalyzer as outlined by Kamphake et al. (1967). The nitrate content was estimated by difference.

Soil ^{15}N analysis

The determination of ^{15}N abundance in soil involved three steps:

1. Kjeldhal digestion of soil as described in the section for total nitrogen determination.
2. Conversion of ammonia to molecular nitrogen by sodium hypobromide (NaOBr) in a vacuum system as outlined by Pang (1970).
3. The nitrogen gas was analyzed mass spectrometrically for m/e 28 and 29. The abundance of ^{15}N was expressed as atom percent ^{15}N excess which was calculated using the following equation:

$$\text{Atom \% } ^{15}\text{N excess} = \left(\frac{100}{2R + 1} \right) - 0.367$$

in which R is the ratio of the relative peak height of mass to charge (m/e) of 28 and 29 and the value of 0.367 is the atom percent ^{15}N in the atmosphere.

RESULTS AND DISCUSSIONS

Experiment I. Transformation and movement of band-applied urea, ammonium sulfate, and ammonium hydroxide during incubation in three soils

This investigation was undertaken to determine the distribution and persistence of ammonium from three different fertilizer materials, their transformation, and their effects on local pH in three soils incubated at 20C for various times. The nitrogenous materials were band-applied at the rate of 92mg N of $(\text{NH}_2)_2\text{CO}$, NH_4OH and $(\text{NH}_4)_2\text{SO}_4$. This amount of nitrogen is equivalent to 800 kg N/ha on an area basis. The soils were selected on the basis of pH: one being acid, one near neutral, and one alkaline.

Effects of nitrogen fertilizer on soil pH

In the Keld soil (pH 5.4), application of both urea and ammonium hydroxide resulted in a marked increase in pH (Fig. 6A) near the point of application after 2 weeks incubation. There was relatively little change in pH between the 2 and 8 weeks incubation periods, although there was some reduction in pH about 3.5 cm above and below the point of fertilizer application, particularly where urea was applied. Ammonium sulfate resulted in a slight decrease in pH in the Keld soil with the pH remaining quite constant throughout the incubation period.

In the Wellwood II soil (pH 6.6), the pH (Fig. 6B) had increased markedly near the point of application after 2 weeks incubation when urea or ammonium hydroxide were applied. In both instances the pH had decreased after 4 weeks incubation and by the time the soil had been incubated 8 weeks soil columns to which these carriers had

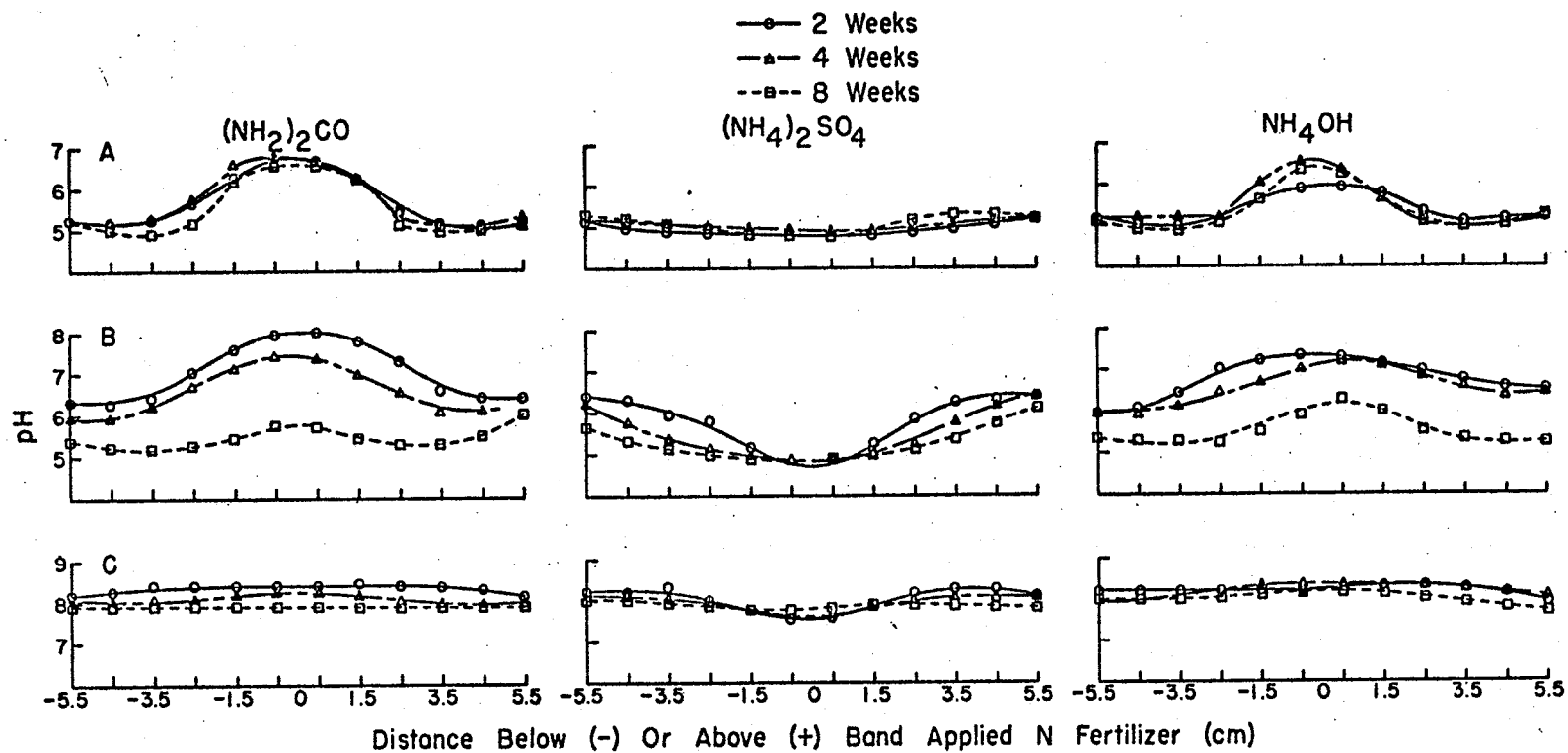


FIG. 6. CHANGES IN pH DUE TO 92 mg N BAND-APPLIED $(\text{NH}_2)_2\text{CO}$, $(\text{NH}_4)_2\text{SO}_4$, AND NH_4OH IN ACID KELD (A), NEUTRAL WELLWOOD II (B), AND ALKALINE MORTON (C) SOILS AT 20C.

been added were below pH 6 throughout, with pH slightly higher near the point of fertilizer application and at each end. Application of ammonium sulfate resulted in a sharp decrease in the pH of the Wellwood II soil. This was most pronounced near the point of fertilizer application, particularly after 2 weeks incubation. There was a substantial lowering of soil pH throughout the column after 8 weeks incubation.

Changes in pH in the calcareous Morton soil (pH 8.2) (Fig. 6C) were relatively minor. This was because it was too alkaline for urea or ammonium hydroxide to increase its pH and contained sufficient calcium carbonate to resist a pH drop due to acid products of nitrification reactions.

Distribution of various forms of nitrogen in acid Keld soil (pH 5.4)

There was virtually no NO_2^- or NO_3^- in the Keld soil after 2 weeks incubation (Figs. 7, 8, 9) irrespective of the fertilizer material applied. The shape of the distribution curve of ammonium form was very similar to that of a normal distribution curve. With increasing time of incubation, the peak height of ammonium form decreased and spread outward. A noticeable production of NO_3^- was observed that amounted to about 15-20% of the fertilizer nitrogen applied after 8 weeks. The positions of maximum NO_3^- concentration were located about 3.5 cm above and below the middle of the column. These positions correspond to the point where the greatest amount of H^+ was produced (Fig. 6A). The NO_3^- found in the middle of the column might have come from outside sections by diffusion because conditions were unfavorable for nitrification in the middle with respect to pH and concentration of ammonium nitrogen (Bartholomew and Clark, 1965;

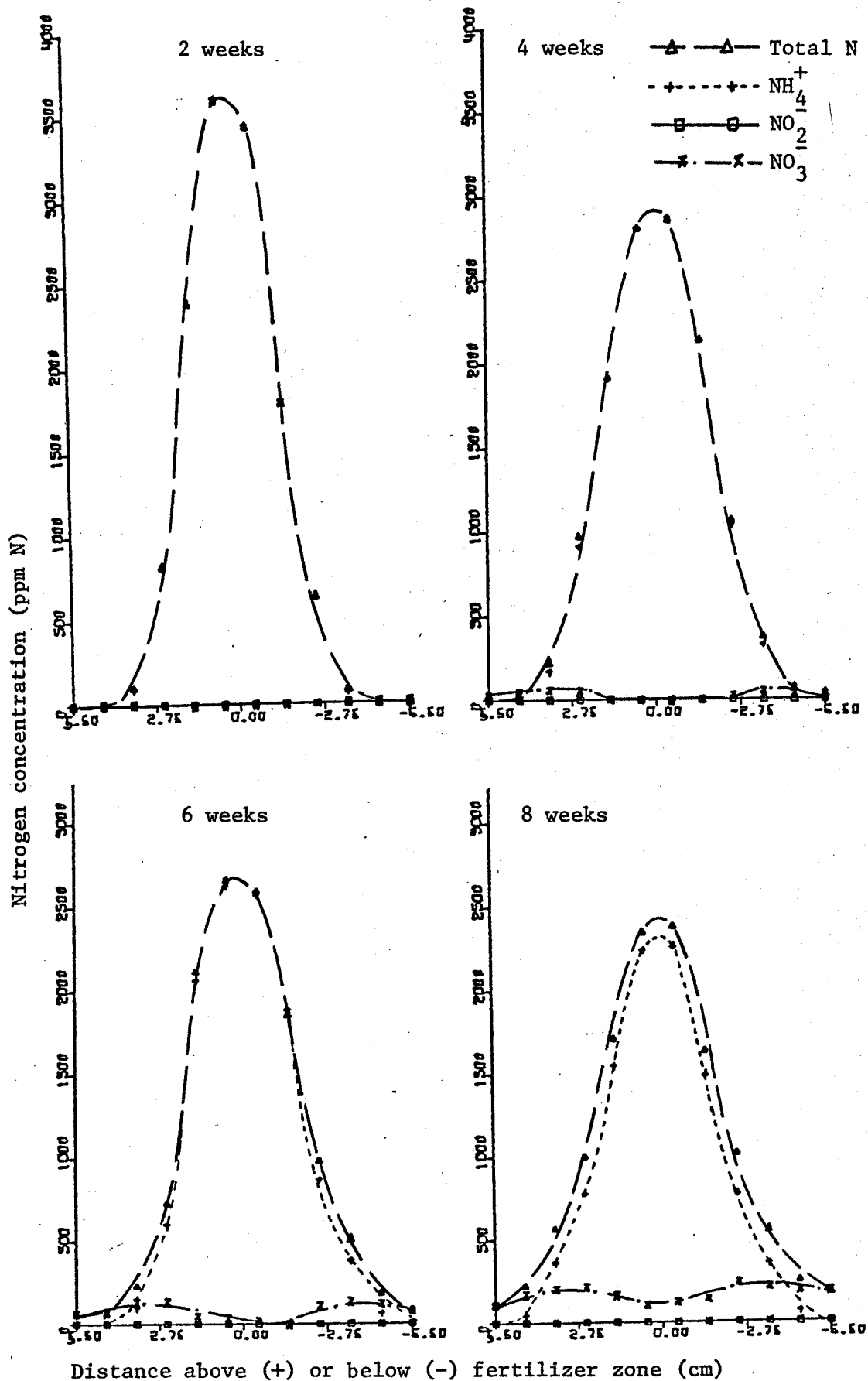


FIG. 7. DISTRIBUTION OF TOTAL INORGANIC N, NH₄⁺-N, NO₂⁻-N AND NO₃⁻-N IN THE ACID KELD SOIL (pH 5.4) FROM 92 mg N BAND-APPLIED UREA AT 20C.

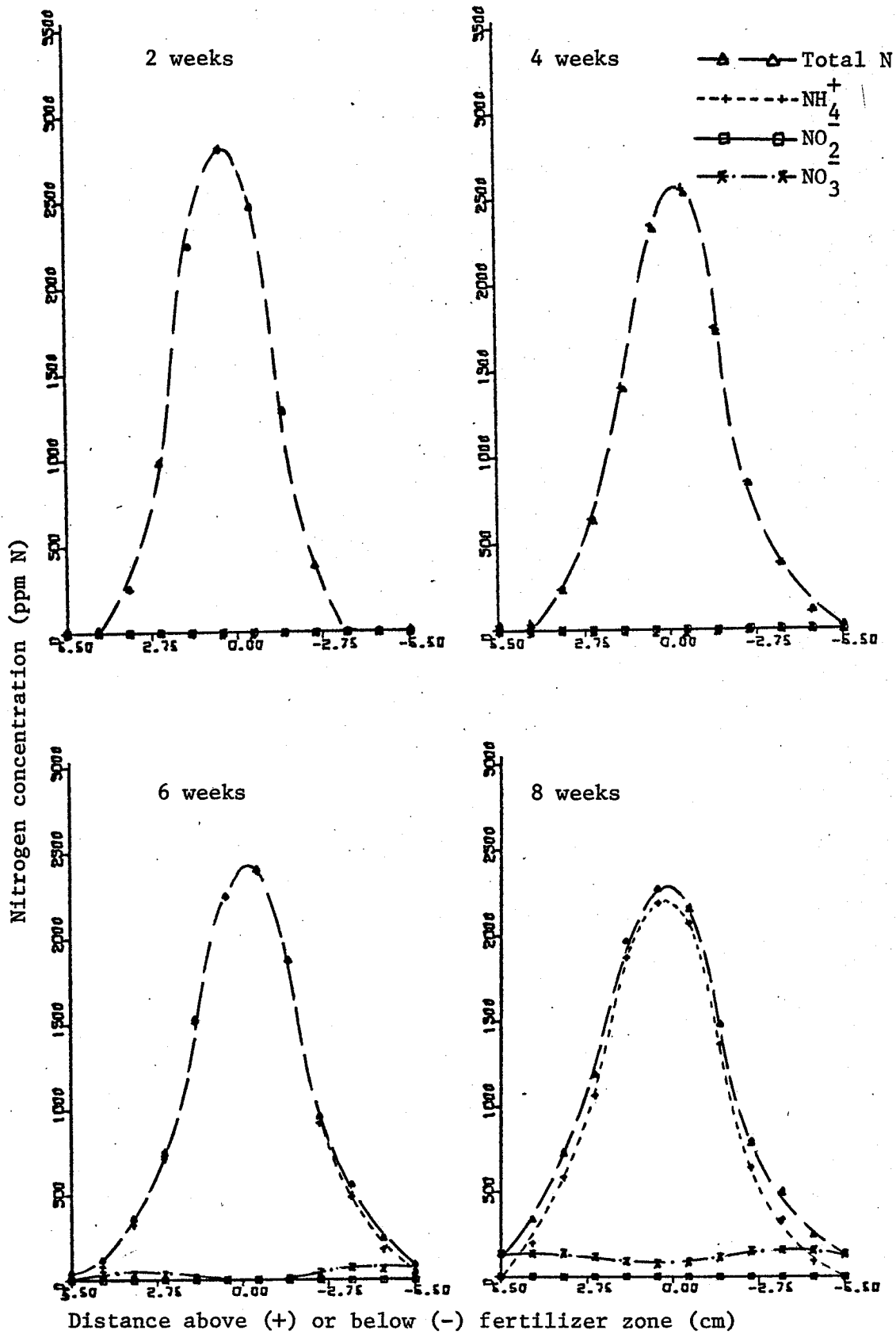


FIG. 8. DISTRIBUTION OF TOTAL INORGANIC N, NH_4^+ -N, NO_2^- -N AND NO_3^- -N IN THE ACID KELD SOIL (pH 5.4) FROM 92 mg N BAND-APPLIED NH_4OH at 20C.

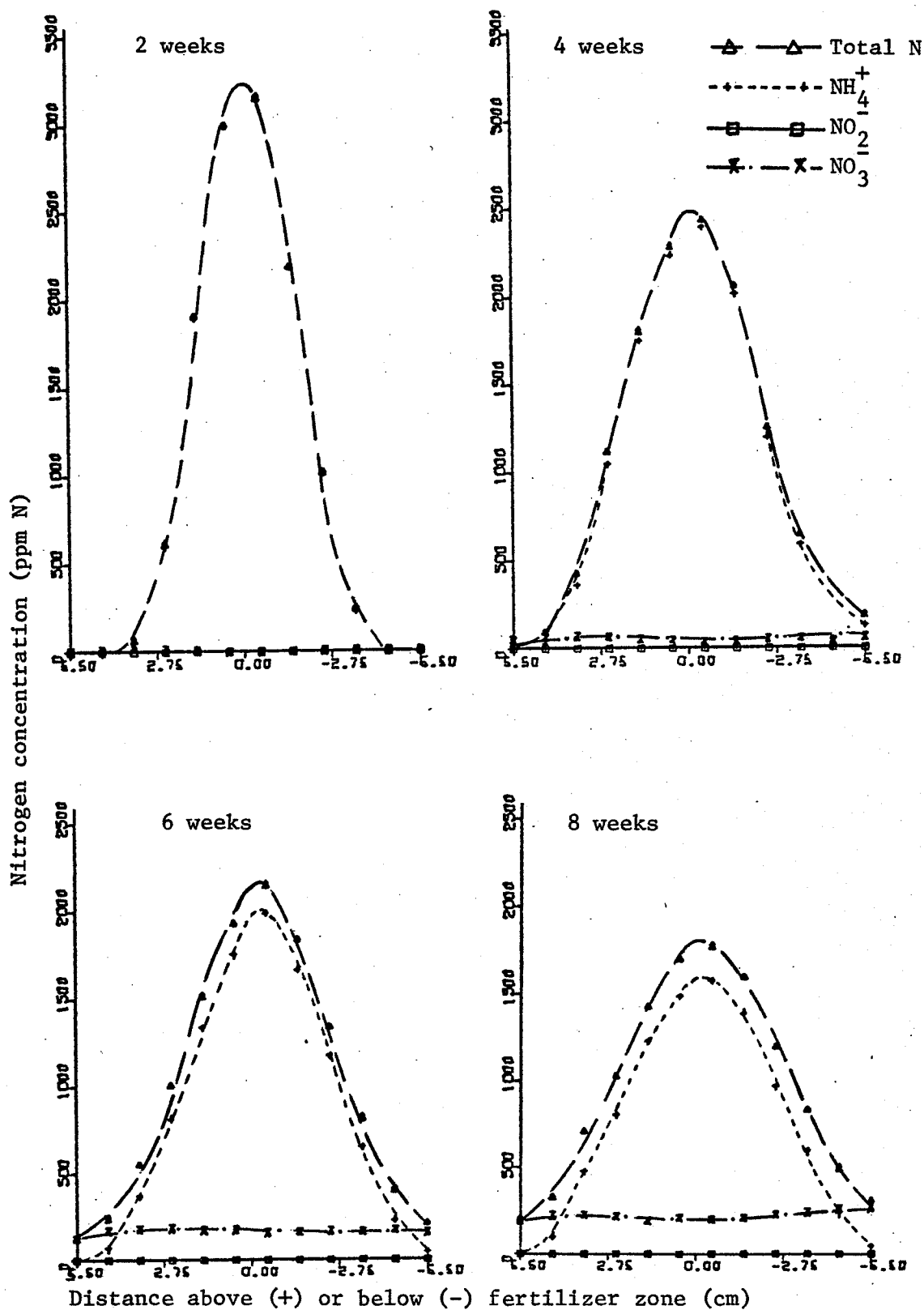


FIG. 9. DISTRIBUTION OF TOTAL INORGANIC N, NH₄⁺-N, NO₂⁻-N AND NO₃⁻-N IN THE ACID KELD SOIL (pH 5.4) FROM 92 mg N BAND-APPLIED (NH₄)₂SO₄ AT 20C.

Passioura and Wetselaar 1972). The majority of the applied nitrogen from all carriers was still in the form of ammonium even after 8 weeks incubation at 20°C as indicated by the comparison of the ammonium and total mineral nitrogen curves (Fig. 7, 8, 9).

Distribution of various forms of nitrogen in the neutral Wellwood II soil (pH 6.6)

After 2 weeks incubation, the majority of applied nitrogen was in the form of ammonium (Fig. 10, 11, 12). Unlike the Keld soil, however, some transformation of ammonium to oxidized forms took place in the first 2 weeks. With urea and NH_4OH the formation of both nitrite and nitrate was evidenced (Figs. 10, 11) whereas only the formation of nitrate was observed from $(\text{NH}_4)_2\text{SO}_4$ (Fig. 12). Both nitrite and nitrate distribution curves are symmetrical with two points of maximum concentration. The position of maximum NO_2^- concentration was found near ± 1.5 cm where the ammonium concentration was above 1,000 ppm whereas the position of maximum NO_3^- concentration was found about ± 3.5 cm away on both urea and NH_4OH -banded Wellwood II soil at 2 weeks. The position of maximum concentration of NO_3^- on $(\text{NH}_4)_2\text{SO}_4$ -banded Wellwood II soil at 2 weeks was around ± 2.5 cm from the zone of application. The pH near this position was approximately 6.0.

With increasing time of incubation the ammonium form decreased and the oxidized form increased. The NO_2^- concentration in both urea and NH_4OH -banded Wellwood II soil at 4 weeks increased as compared with the 2 week period (Figs. 10, 11). Also, the presence of two maximum zones of concentration of NO_2^- disappeared, having apparently merged into one. The presence of a single maximum concentration of

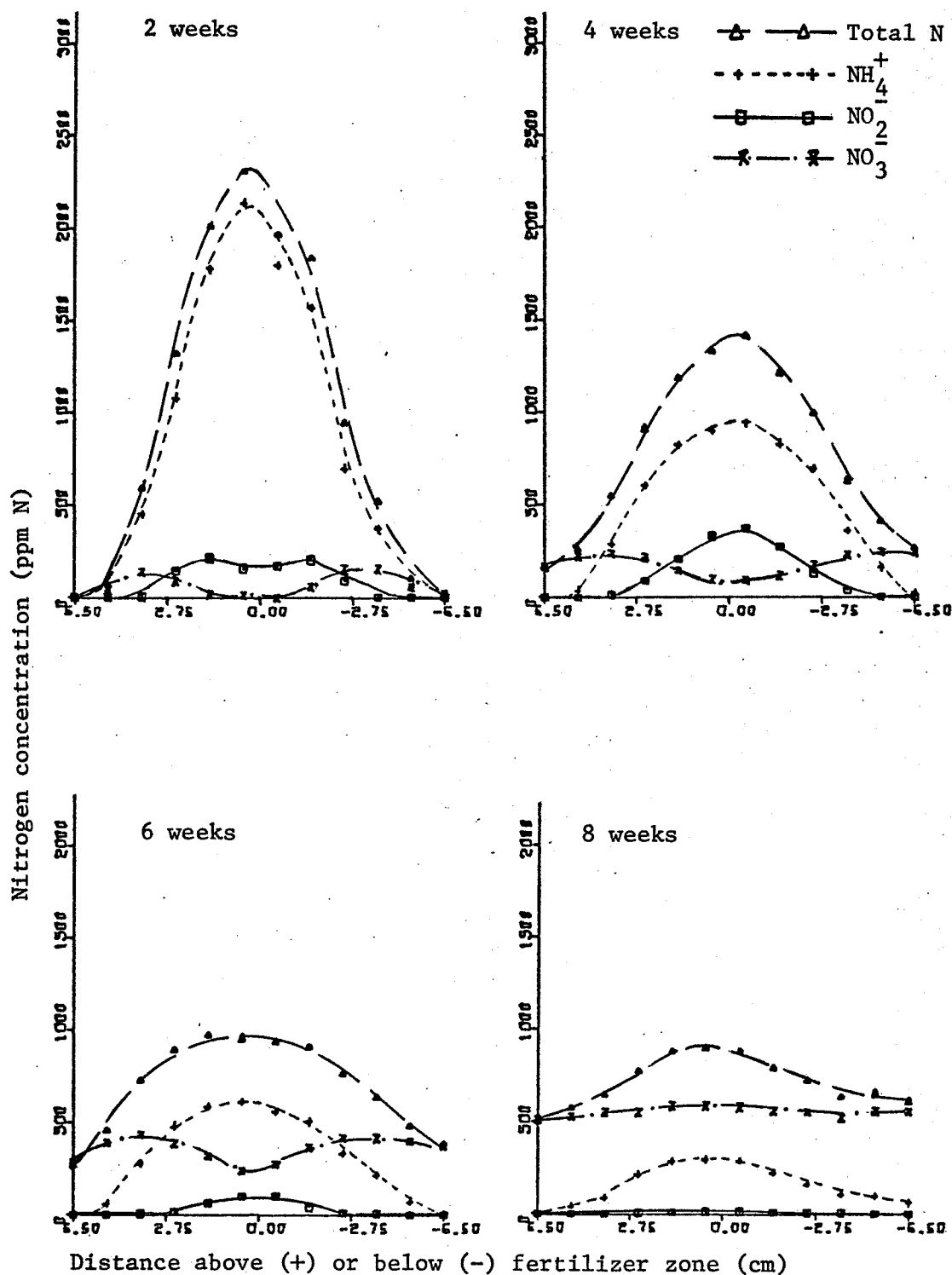


FIG. 10. DISTRIBUTION OF TOTAL INORGANIC N, NH₄⁺-N, NO₂⁻-N AND NO₃⁻-N IN THE NEUTRAL WELLWOOD II SOIL (pH 6.6) FROM 92 mg N BAND-APPLIED (NH₂)₂CO AT 20C.

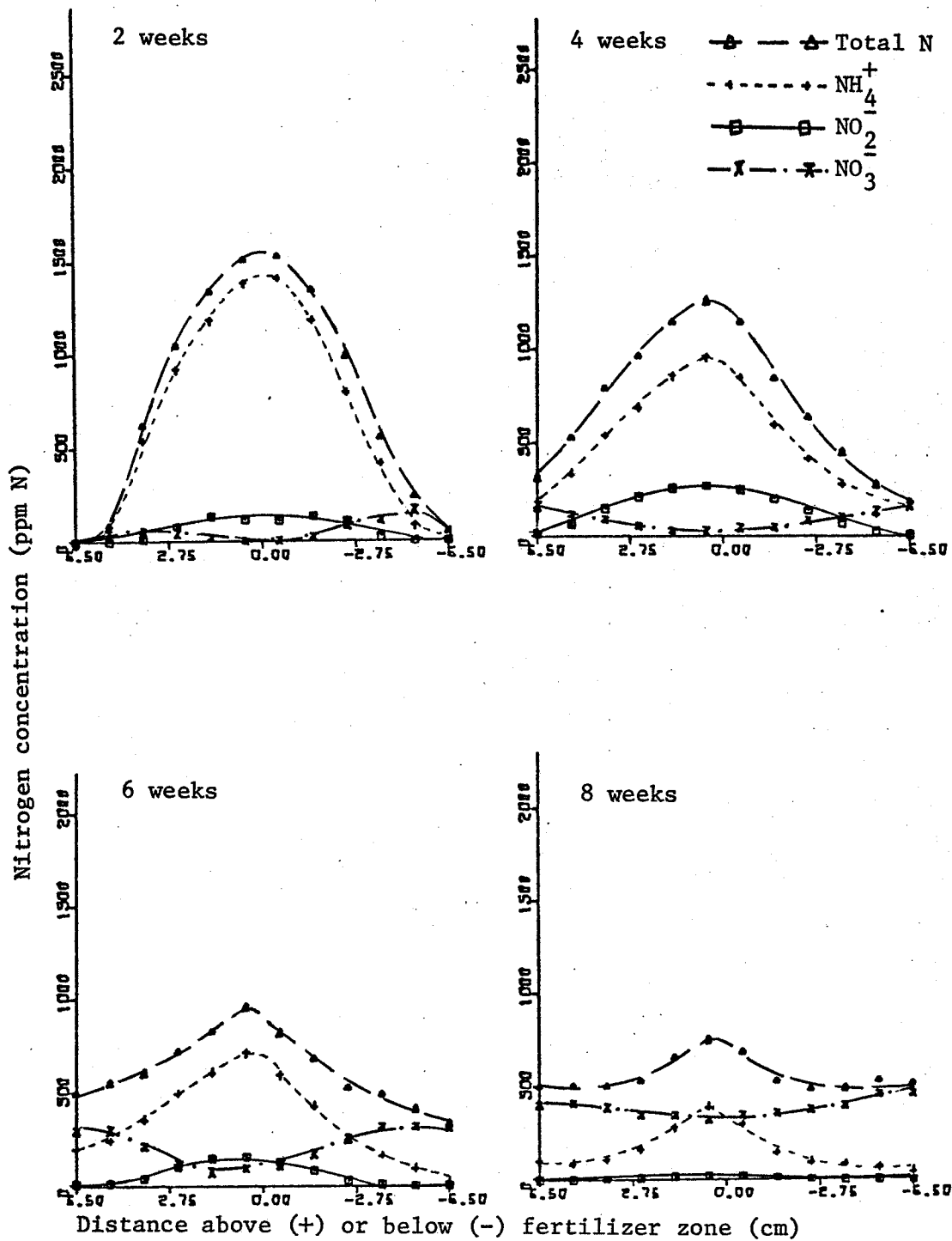


FIG. 11. DISTRIBUTION OF TOTAL INORGANIC N, NH₄⁺-N, NO₂⁻-N AND NO₃⁻-N IN THE NEUTRAL WELLWOOD II SOIL (pH 6.6) FROM 92 mg N BAND-APPLIED NH₄OH AT 20C.

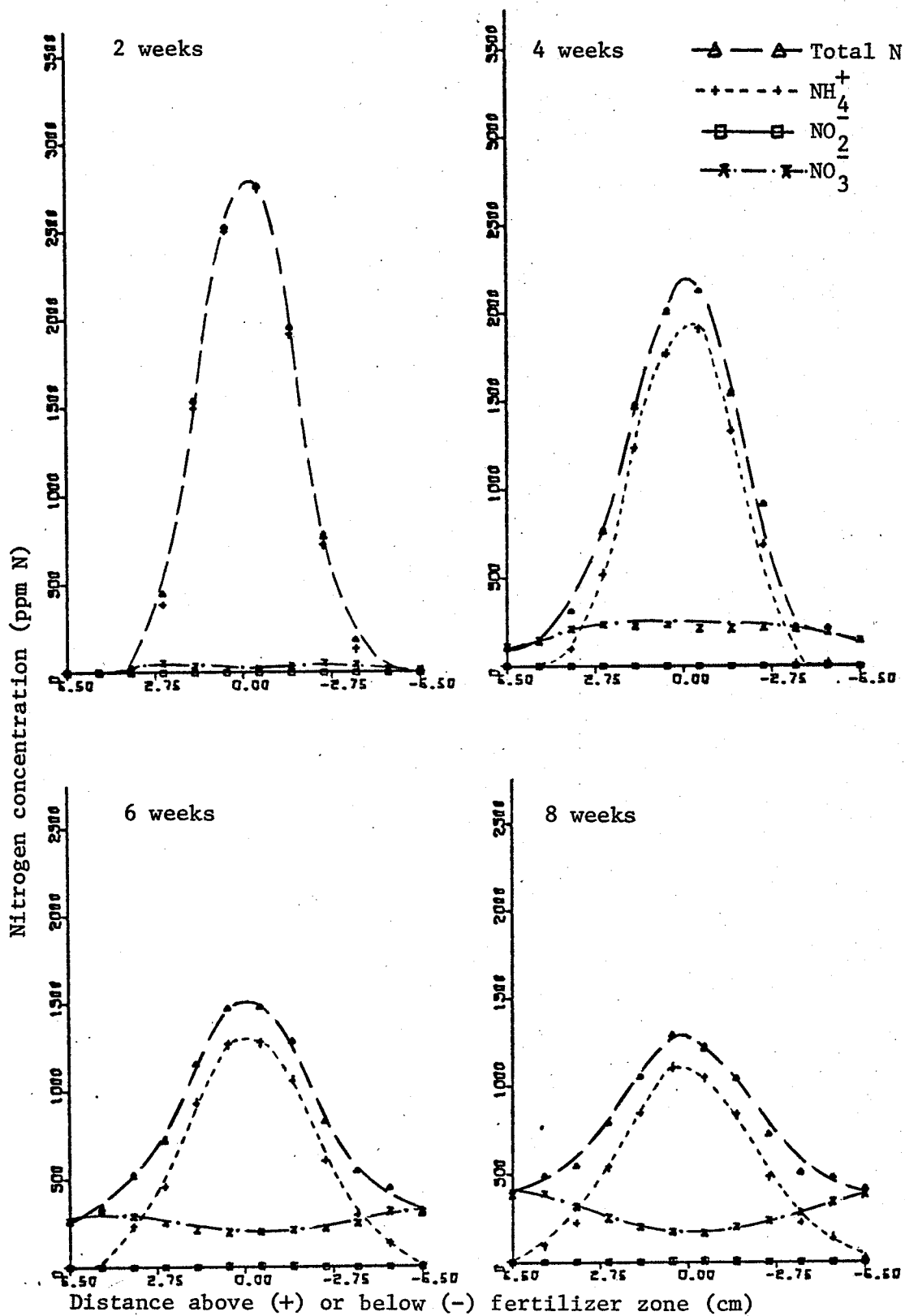


FIG. 12. DISTRIBUTION OF TOTAL INORGANIC N, NH_4^+ -N, NO_2^- -N AND NO_3^- -N IN THE NEUTRAL WELLWOOD II SOIL (pH 6.6) FROM 92 mg²N BAND-APPLIED $(\text{NH}_4)_2\text{SO}_4$ AT 20C.

NO_2^- , the position that corresponds to that of the maximum concentration of the ammonium form and the minimum concentration of NO_3^- indicates the interference of NO_2^- oxidation to NO_3^- by either a high ammonium or a high NO_2^- concentration. With $(\text{NH}_4)_2\text{SO}_4$ -banded Wellwood II soil at 4 weeks there was no detectable NO_2^- as in the 2 week period (Fig. 12). However, the presence of two maximum points of NO_3^- concentration observed at the 2 week period had disappeared at the 4 week period and the amount of NO_3^- greatly increased.

The ammonium form decreased greatly and NO_3^- became the major form of nitrogen for urea and NH_4OH -banded Wellwood II after 8 weeks incubation (Figs. 10, 11). Nitrite had almost disappeared and only a small concentration (approximately 1% of the initially added nitrogen) could be detected, although an appreciable amount of ammonium (approximately 20% of the initially added nitrogen) was present. The concentration of NO_3^- throughout the column was more or less uniform whereas the ammonium form still exhibited a high concentration at the middle. In the $(\text{NH}_4)_2\text{SO}_4$ -banded Wellwood II soil the major form of nitrogen after 8 weeks incubation was still the ammonium form (Fig. 12) even though an appreciable transformation of the ammonium form to NO_3^- had taken place. The presence of a minimum concentration of NO_3^- in the middle of the column indicates some inhibition of nitrification. This was probably due to a high concentration of the ammonium form or the low pH.

Distribution of various forms of nitrogen in the alkaline Morton soil (pH 8.2)

After 2 weeks incubation there were small amounts of NO_2^- and NO_3^- produced in urea and $(\text{NH}_4)_2\text{SO}_4$ -banded Morton soil (Figs. 13, 15).

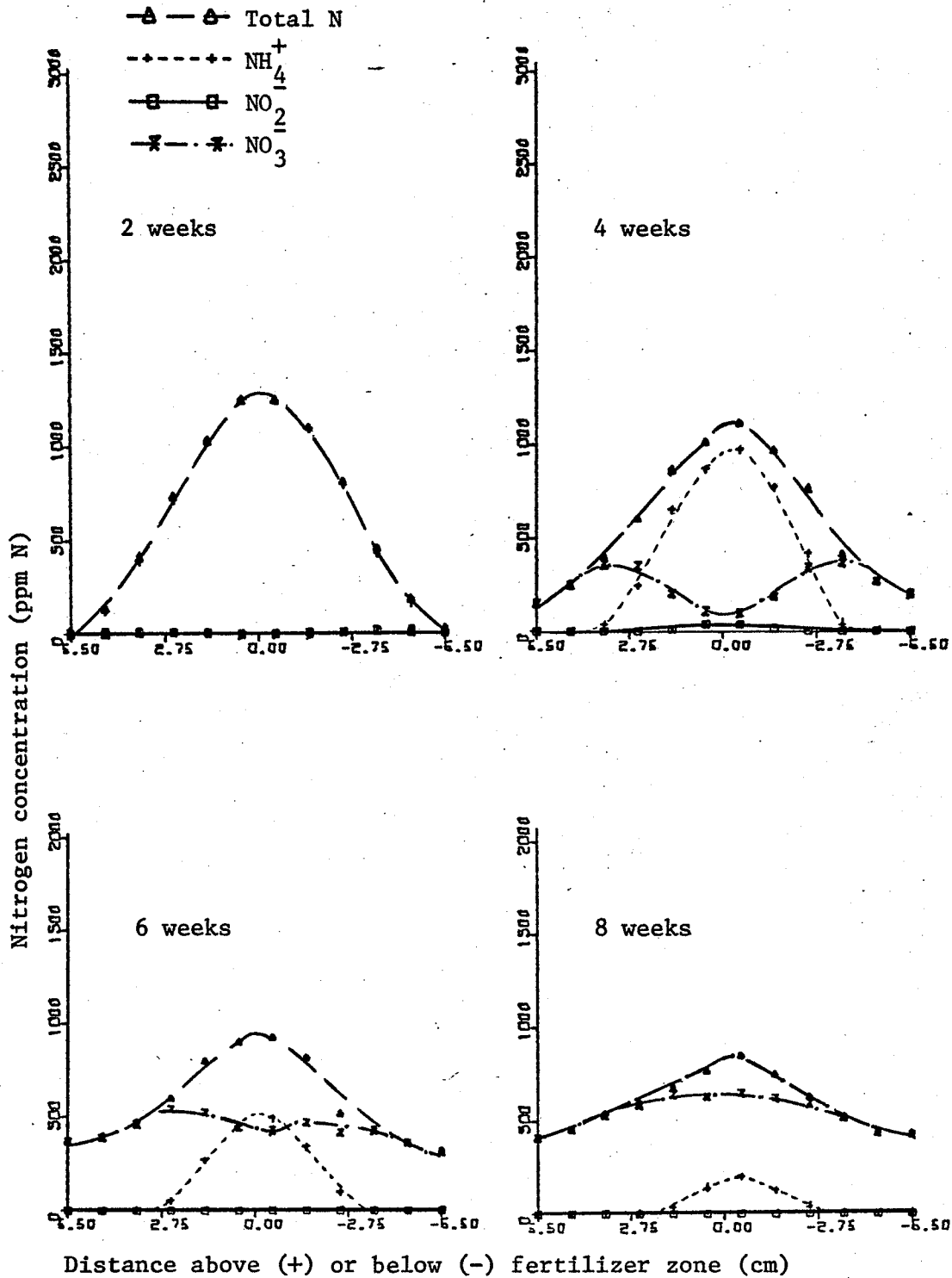


FIG. 13. DISTRIBUTION OF TOTAL INORGANIC N, NH₄⁺-N, NO₂⁻-N AND NO₃⁻-N IN THE ALKALINE MORTON SOIL (pH 8.2) FROM 92 mg N BAND-APPLIED UREA AT 20C.

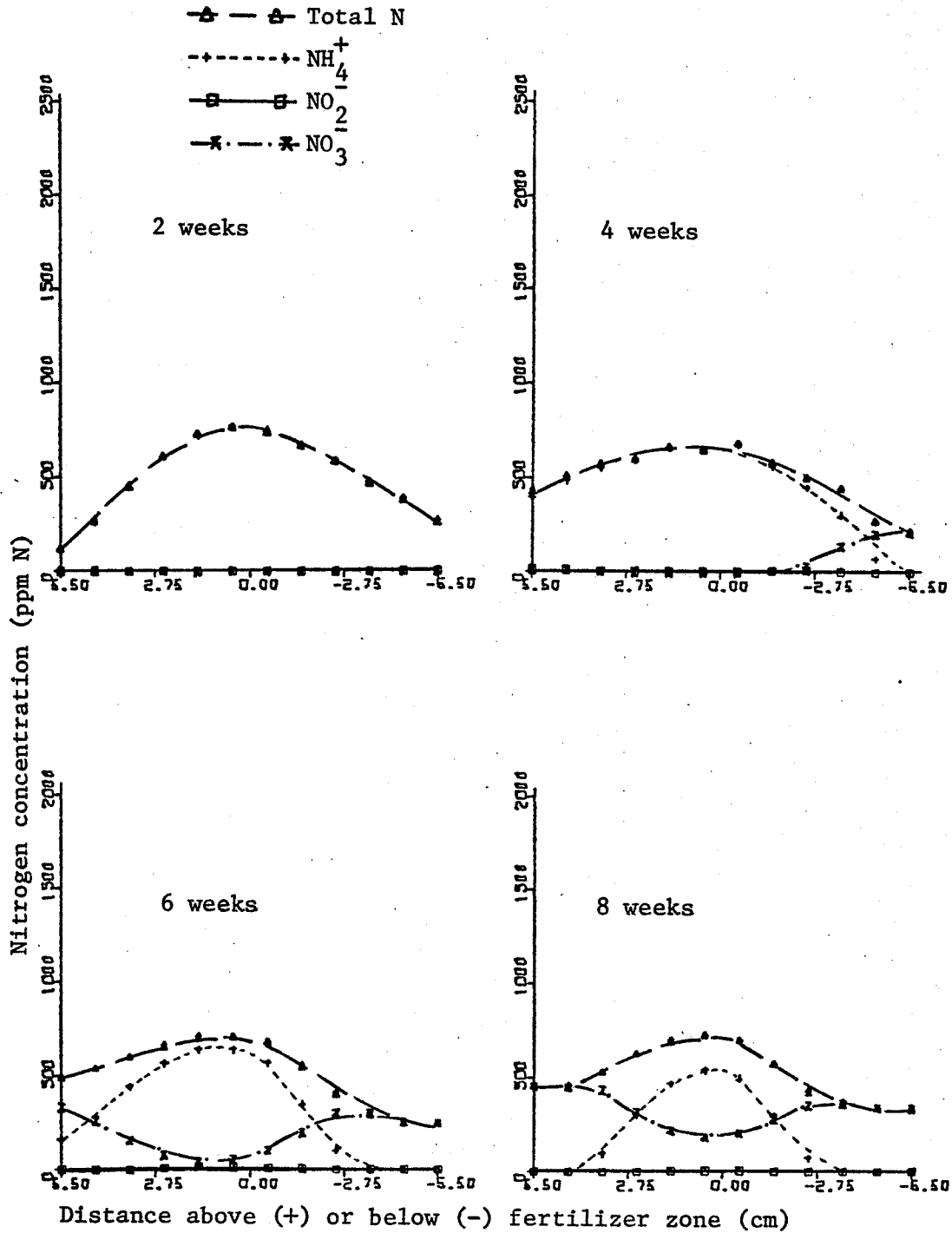


FIG. 14. DISTRIBUTION OF TOTAL INORGANIC N, NH₄⁺-N, NO₂⁻-N AND NO₃⁻-N IN THE ALKALINE MORTON SOIL (pH 8.2) FROM 92 mg N BAND-APPLIED NH₄OH AT 20C.

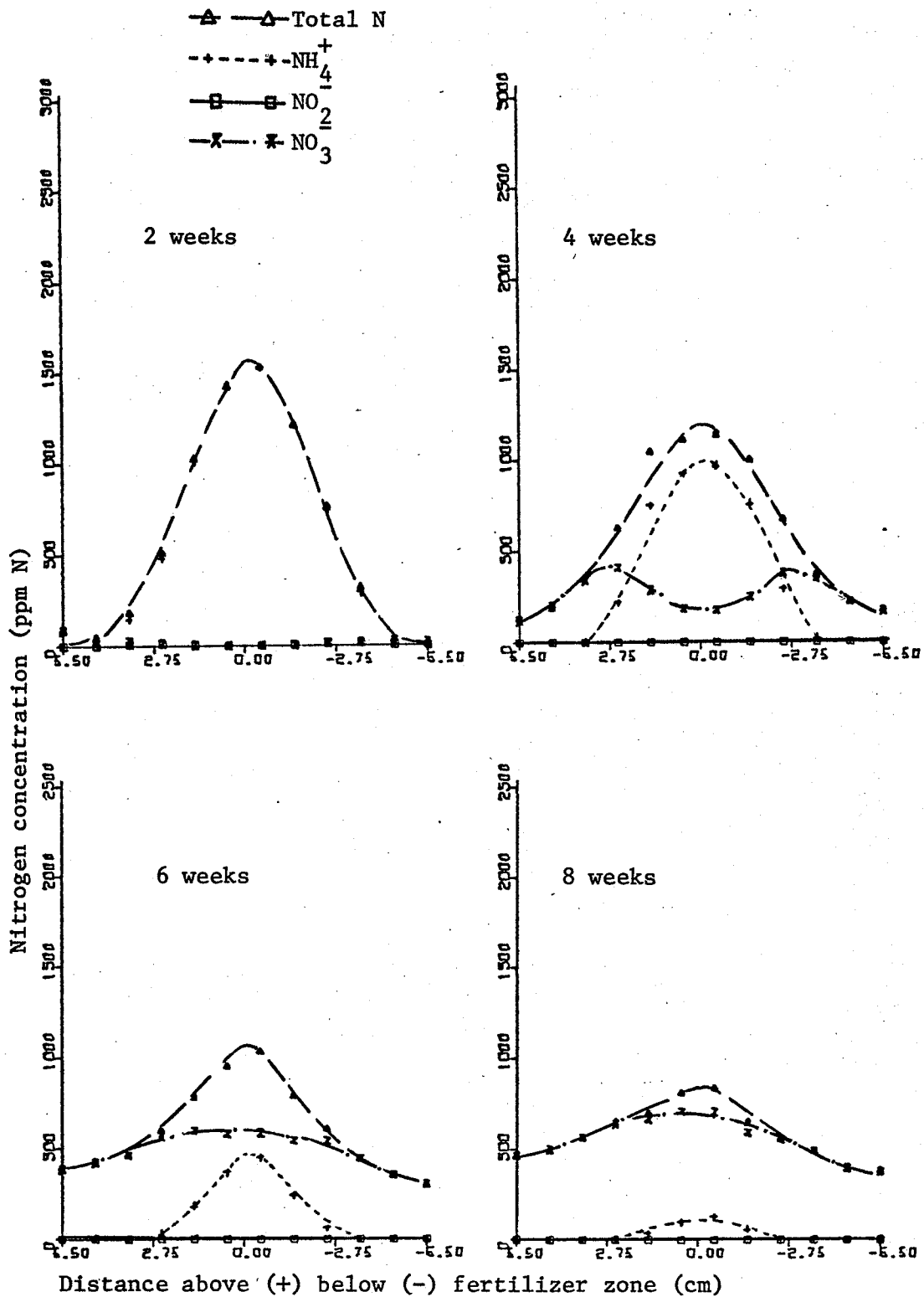


FIG. 15. DISTRIBUTION OF TOTAL INORGANIC N, NH_4^+ -N, NO_2^- AND NO_3^- -N IN THE ALKALINE MORTON SOIL (pH 8.2) FROM 92 mg N BAND-APPLIED $(\text{NH}_4)_2\text{SO}_4$ AT 20C.

However, nitrification of NH_4OH was negligible (Fig. 14). The shape of the ammonium distribution curves at the 2 week period varied with the different fertilizer materials. This will be discussed later in conjunction with the calculated diffusion coefficient.

With increasing time of incubation the transformation of ammonium to nitrate took place very rapidly. The distribution curves obtained from urea and $(\text{NH}_4)_2\text{SO}_4$ applications at the 4 and 8 week periods were almost identical (Figs. 13, 15). There were two points of maximum NO_3^- concentration about 3-3.5 cm from the middle of the column at the 4 week period. There was a small accumulation of NO_2^- in the middle of the column due to urea banding but this was not noticeable with the $(\text{NH}_4)_2\text{SO}_4$ treatment. The shape of the distribution curve obtained from the NH_4OH treatment at the 4 week period was distinctly different from that obtained by other treatments (Fig. 14). It is not known why the difference was obtained, although it is possible that this column had a moisture content in excess of the 1/3 atm tension so that downward movement of water and nitrogen took place.

After 8 weeks incubation, the majority of nitrogen was observed as NO_3^- in urea and $(\text{NH}_4)_2\text{SO}_4$ treatments (Figs. 13, 15). Only a small quantity of ammonium was still remaining near the zone of application. With the NH_4OH treatment, nitrification was less rapid (Fig. 14), so a greater amount of ammonium remained after 8 weeks than with the other carriers.

Recovery of inorganic forms of nitrogen

The total inorganic nitrogen in Figures 7-15 represent the sum of ammonium, NO_2^- , and NO_3^- . The curves were included in these figures to present the distribution of all inorganic forms. The sum

of these inorganic forms was calculated as a percentage of the nitrogen added. The recovery ranged from a high of 115% to a low of 66% (Table 3). The recovery of the original nitrogen as inorganic forms was relatively constant for both the acidic Keld and alkaline Morton during the period from 2 to 8 weeks incubation, irrespective of the fertilizer materials. On the other hand, the recovery from the neutral Wellwood II soil decreased with increasing time of incubation. Comparison of the fertilizer materials, irrespective of the duration of incubation, indicates that the recovery was always lower with NH_4OH than with urea or $(\text{NH}_4)_2\text{SO}_4$. Effects of soil characteristics upon recovery are difficult to explain because of the changing nature of recovery with time. However, up to 2 weeks incubation, the lowest recovery was obtained from alkaline Morton soil irrespective of the fertilizer material. There is, at present, no further explanation for these variations other than that they are functions of soil characteristics, time of incubation and the fertilizer materials.

Disappearance of nitrogen could have been due to volatilization of ammonia, ammonia fixation, denitrification or nitrogen immobilization. Immobilization and ammonia volatilization are means of disappearance that would most likely occur early in the incubation period. Because some disappearance took place between the 4 and 8 week incubation periods it seems probable that some denitrification took place.

Diffusion coefficients of ammonium in three soils

Diffusion coefficients of ammonium in the experimental soils were determined by employing the distribution curves of ammonium at the 2 week period. A method of plotting the normal distribution curve

TABLE 3. PERCENT RECOVERY OF ADDED FERTILIZER N AS TOTAL INORGANIC NITROGEN FROM SOILS
INCUBATED AT 20C FOR VARIOUS TIMES

Soil	$(\text{NH}_2)_2\text{CO}$			$(\text{NH}_4)_2\text{SO}_4$			NH_4OH		
	2 wk	4 wk	8 wk	2 wk	4 wk	8 wk	2 wk	4 wk	8 wk
Keld	109	106	102	109	105	99	89	87	99
Wellwood	115	91	85	100	98	88	91	84	66
Morton	93	93	94	95	95	94	81	82	83

into a probability plot (Kreyszig, 1968) to obtain standard deviation was employed. D was calculated from the relation, $\sigma = \sqrt{2Dt}$, where σ is the standard deviation, t is the time, and D is the diffusion coefficient of ammonium in porous media. In every instance the probability plot showed a linear relation.

The results (Table 4) indicate that the diffusion coefficient is a function of fertilizer material and soil characteristics.

TABLE 4. CALCULATED DIFFUSION COEFFICIENT OF NH_4^+ FROM THREE NITROGEN CARRIERS IN THREE SOILS AT 20°C

Soil	Diffusion coefficient ($\text{cm}^2/\text{sec} \times 10^6$)		
	$(\text{NH}_2)_2\text{CO}$	$(\text{NH}_4)_2\text{SO}_4$	NH_4OH
Keld	0.64	0.82	0.70
Wellwood II	1.52	0.73	1.93
Morton	1.89	1.24	3.73

With the Keld soil, the highest value of D was obtained with $(\text{NH}_4)_2\text{SO}_4$, whereas with the other two soils the lowest value was obtained with $(\text{NH}_4)_2\text{SO}_4$ and the highest value with NH_4OH . That the lowest value was obtained with $(\text{NH}_4)_2\text{SO}_4$ on neutral and alkaline soils was understandable because the fraction of nitrogen in the NH_4^+ form increases with decreasing pH and the diffusion coefficient of NH_4^+ is smaller than, say, NH_4OH due to ion-exchange reaction. The increased value of D of ammonium from $(\text{NH}_4)_2\text{SO}_4$ -banded Keld is a little more difficult to explain. However, if one assumes that the majority of the negative charge in the acid Keld soil is contributed by organic matter.

whose isoelectric point is near pH 5, then the addition of $(\text{NH}_4)_2\text{SO}_4$ might increase the value of D. If this assumption is correct, the increased value of D of ammonium from $(\text{NH}_4)_2\text{SO}_4$ is due to a reduction in the cation exchange capacity of the acid soil caused by H^+ produced from $(\text{NH}_4)_2\text{SO}_4$. The values of D of ammonium obtained using urea and NH_4OH indicate that the value increased with increasing soil pH. It is not known whether pH was the only factor that affected the increase in the value of D by modifying the ratio of NH_4^+ to NH_4OH . Unfortunately, the soils chosen for this study had decreasing cation exchange capacity with increasing soil pH.

The higher value of D obtained for NH_4OH as compared with that for urea is difficult to explain. One probable cause may lie in the fact that urea was solid and NH_4OH was liquid, thereby modifying the moisture transport near the banded zone during the diffusion process.

The importance of the value of D lies in the fact that it determines the degree of spread of banded ammonium. The standard deviation, whose relationship with D was presented earlier, not only determines the spread but also the maximum concentration at the zone of application. Because the nitrification is inhibited by a high osmotic concentration and unfavorable pH (Bartholomew and Clark, 1965; Morrilland Dawson, 1967), the diffusion coefficients of ammonium, hydrogen, and hydroxide all play an important role in controlling nitrification near the point of application of fertilizer, and hence, the persistence of the ammonium form of nitrogen in the soil following fertilizer application.

Summary

Banding of fertilizer materials greatly modified the local pH, the magnitude of which was dependent upon the soil characteristics and the kind of fertilizer. Ammonium sulfate lowered the local pH whereas urea and NH_4OH raised the local soil pH initially, although as nitrification proceeded there was a decrease in pH throughout the soil column.

The maximum rate of ammonium oxidation took place not at the middle of the fertilizer band but near the edges of the diffuse zone. The progress of the oxidation was characterized by the decrease in pH at the zone where this process takes place. The oxidation of ammonium was slow in the acidic Keld soil but relatively fast in neutral Wellwood II and alkaline Morton soils. Soil pH was not the only factor that slowed down the transformation since $(\text{NH}_4)_2\text{SO}_4$ -banded Wellwood II soil produced more of the oxidized form of nitrogen than either urea or NH_4OH -banded Keld soil, even though they had similar pH values near the edges of the zone of ammonium accumulation. Nitrite was observed during the incubation period in a relative high concentration with urea and NH_4OH -banded Wellwood II soil. A comparison of this zone with the pH distribution curve indicated that the pH was near 7 where nitrite was produced.

Recovery of inorganic forms of nitrogen varied a great deal. Relatively constant recoveries, even though they are not necessarily 100%, were obtained with acidic Keld and alkaline Morton soils. With the neutral Wellwood II soil, however, the percentage recovery decreased with time irrespective of the fertilizer materials.

Diffusion coefficients of ammonium in the soils were calculated.

They varied depending upon the types of soil or the fertilizer materials. Generally, restricted movement of ammonium away from the zone of application eventually retarded the transformation.

Experiment II. Effect of rate of nitrogen application on transformation of band-applied fertilizers in neutral Wellwood II soil

In the previous experiment, it was observed that Wellwood II (pH 6.6) soil accumulated the largest quantity of NO_2^- -N during incubation when band-applied with 92 mg N of $(\text{NH}_2)_2\text{CO}$ and NH_4OH . It was postulated that this could have been due to the effect of a high concentration of ammonium nitrogen and possibly a pH effect near the fertilizer zone. This investigation was undertaken to ascertain the rate of N under which NO_2^- -N accumulation would occur in Wellwood II soil.

Band application of 11.5 mg N (100 kg N/ha on an area basis)

This concentration of fertilizer seldom occurs in the field when fertilizer is broadcast, although concentrations equal to or greater than this may occur if fertilizer is drilled with the seed at 20 lbs N/acre or more in a 6" row spacing. When fertilizer is banded at wider row spacings much greater concentrations can readily occur.

When 11.5 mg N of $(\text{NH}_2)_2\text{CO}$, NH_4OH and $(\text{NH}_4)_2\text{SO}_4$ was banded in the neutral Wellwood II soil, nitrate was the only end product (Fig. 16, 17, 18) after 3 days to 2 weeks incubation, and it amounted to 36%, 27% and 24% of the band-applied $(\text{NH}_2)_2\text{CO}$, NH_4OH and $(\text{NH}_4)_2\text{SO}_4$, respectively.

This concentration did not result in a large change in the soil pH at the fertilizer zone and the area affected by the fertilizer zone was small. Figure 19 shows that the area within which the pH was affected by the banded fertilizers was approximately 1-1.5 cm below and above the fertilizer zone. During the incubation period of 3 days

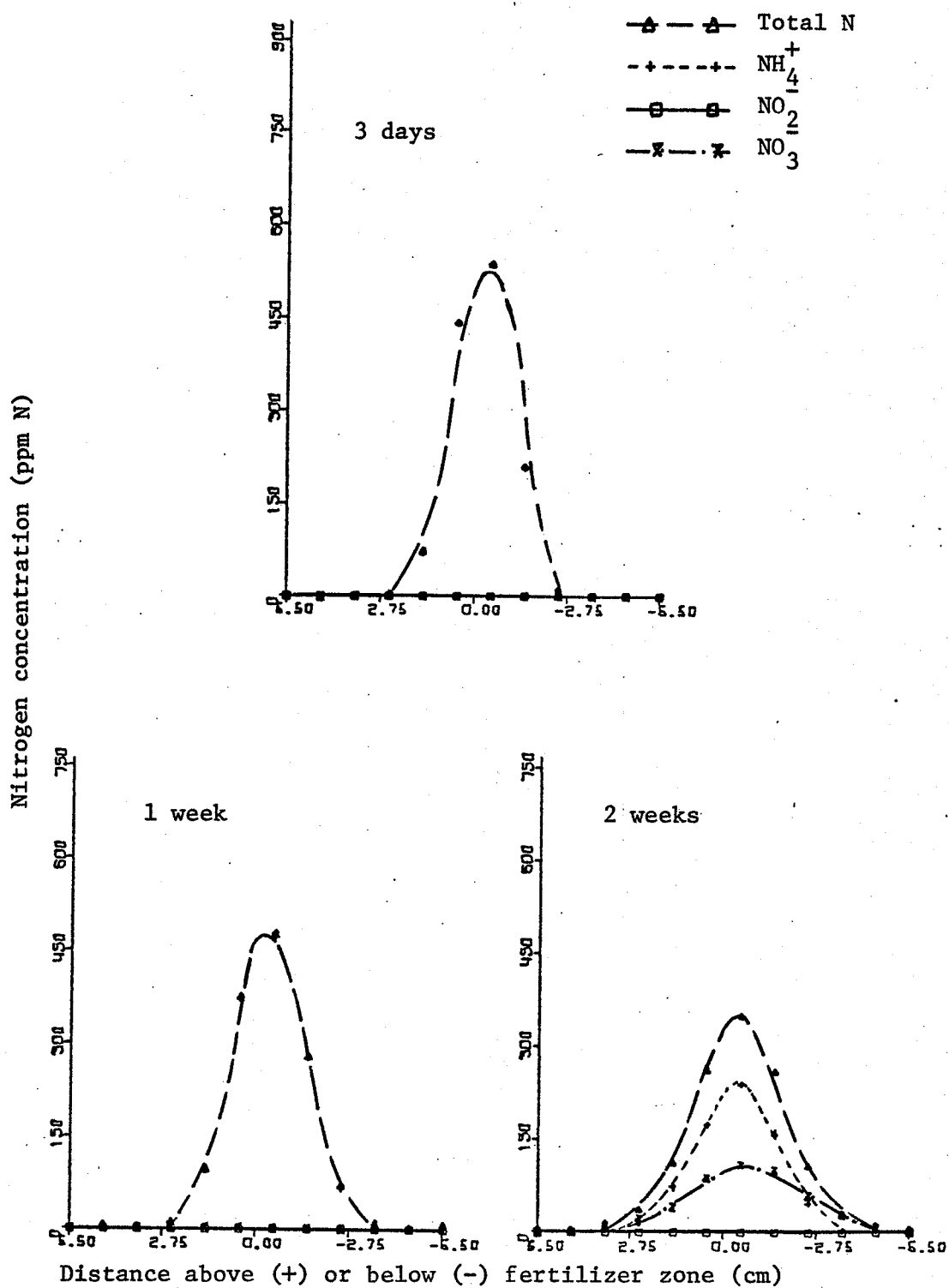


FIG. 16. DISTRIBUTION OF TOTAL INORGANIC N, NH₄⁺-N, NO₂⁻ AND NO₃⁻-N IN THE WELLWOOD II SOIL FROM 11.5 mg N BAND-APPLIED UREA AT 20°C.

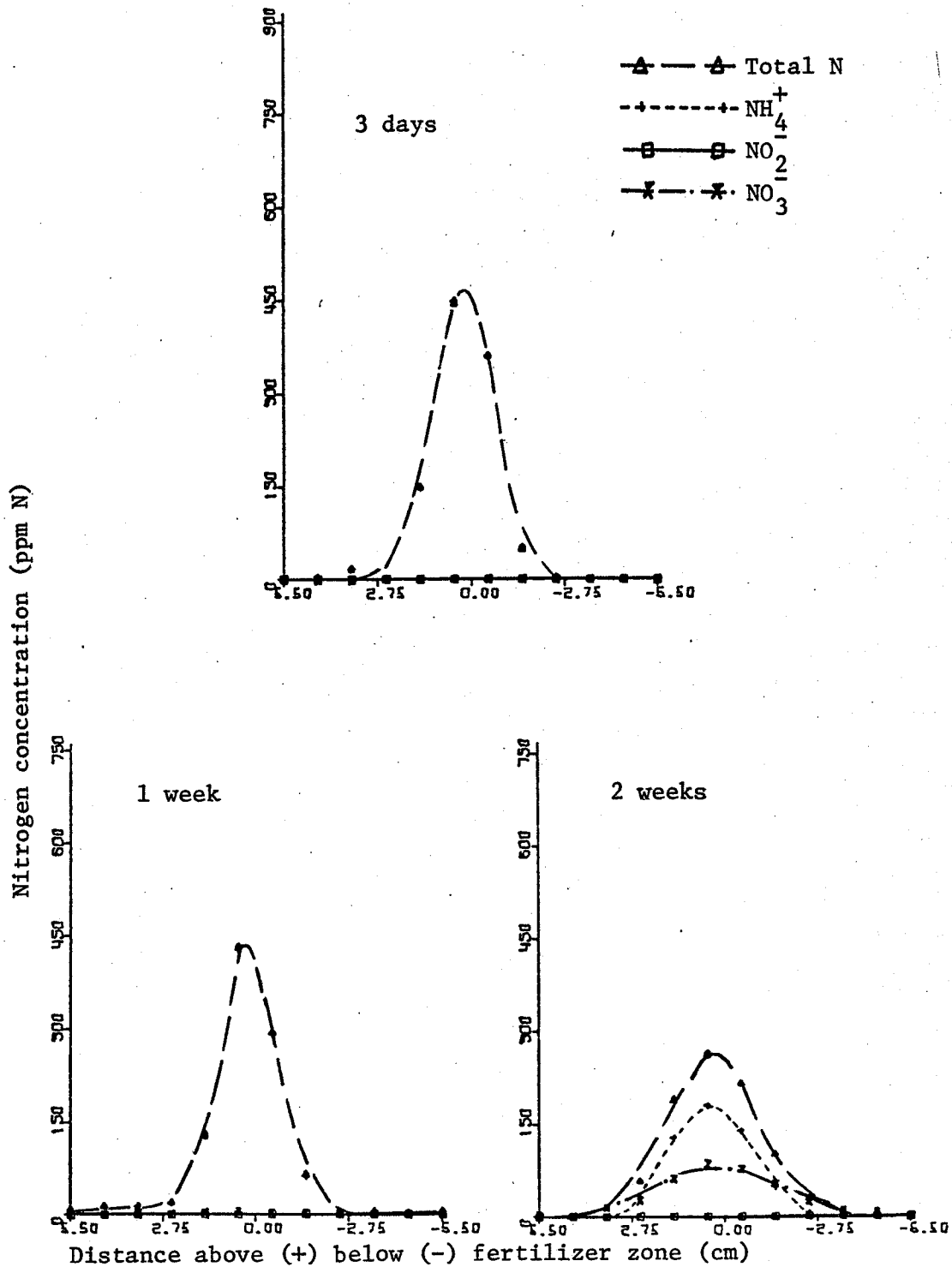


FIG. 17. DISTRIBUTION OF TOTAL INORGANIC N, NH_4^+ -N, NO_2^- -N AND NO_3^- -N IN THE WELLWOOD II SOIL FROM 11.5 mg N BAND-APPLIED NH_4OH AT 20°C.

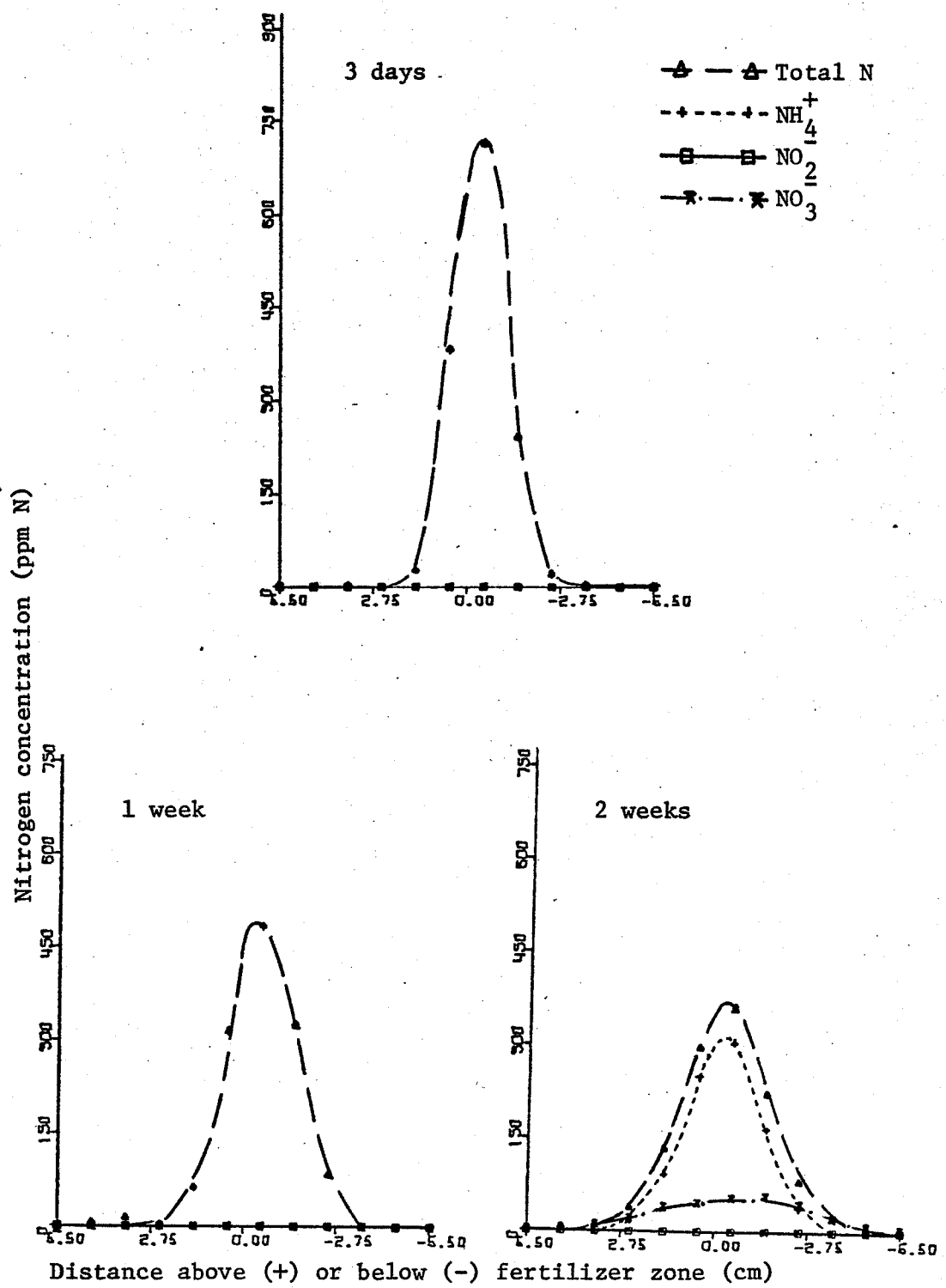


FIG. 18. DISTRIBUTION OF TOTAL INORGANIC N, NH₄⁺-N, NO₂⁻-N AND NO₃⁻-N IN THE WELLWOOD II SOIL FROM 11.5 mg N BAND-APPLIED (NH₄)₂SO₄ AT 20C.

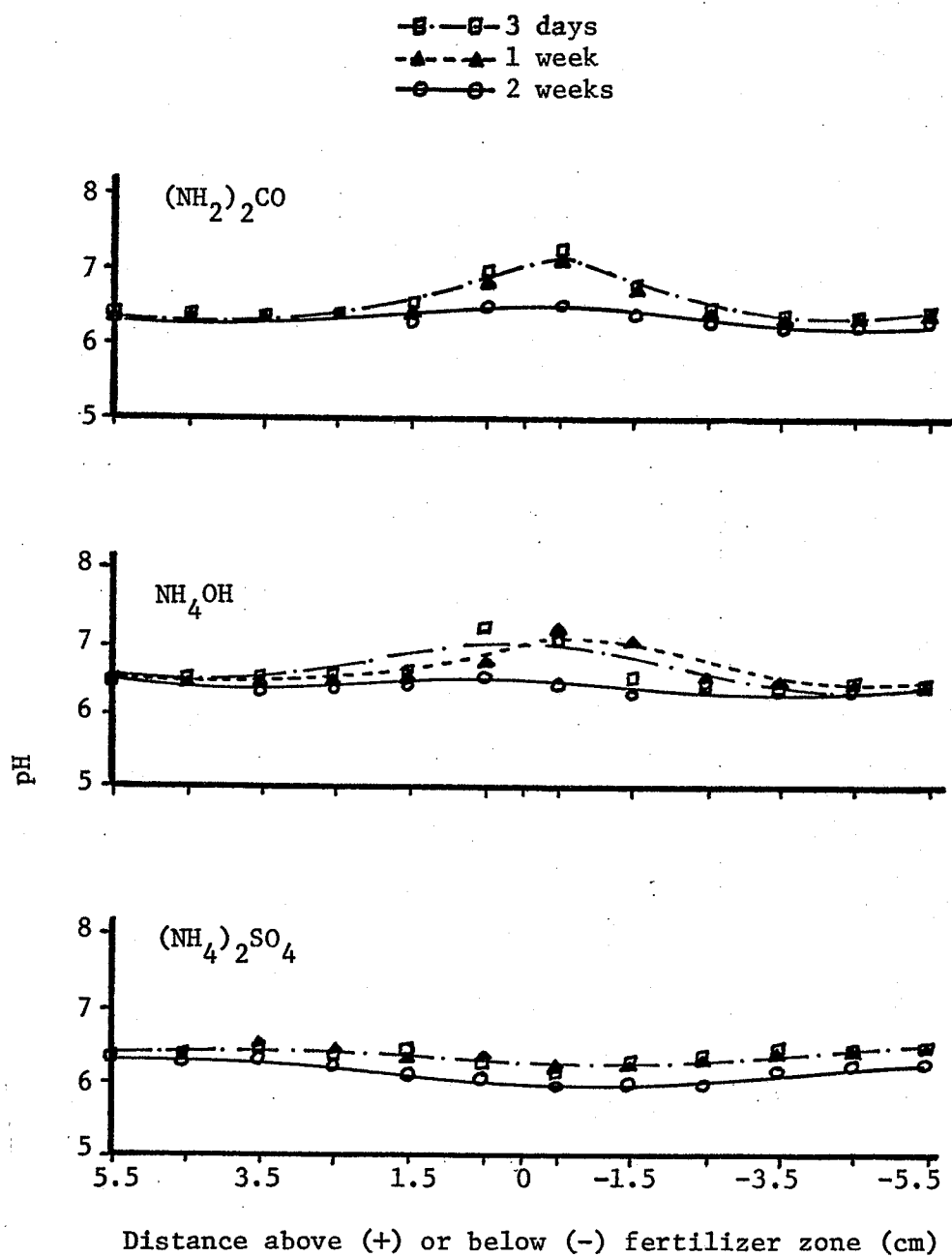


FIG. 19. CHANGE IN SOIL pH DUE TO 11.5 mg N BAND-APPLIED NITROGENOUS SUBSTANCES IN NEUTRAL WELLWOOD II SOIL AT 20C.

to 2 weeks, the pH within the entire column was near or above pH 6.0 irrespective of the nitrogen carriers.

Band application of 23 mg N (200 kg N/ha on an area basis)

The course of oxidation of the ammonia form at this rate was quite different from that which occurred at the lower rate (11.5 mg N). Five to ten percent of the banded nitrogen, irrespective of the carriers, was oxidized to nitrite and nitrate after 1 week incubation (Figs. 20, 21, 22). Nitrite amounting to 1-2% of the banded $(\text{NH}_2)_2\text{CO}$ and NH_4OH was detected at this period. The zone of maximum of nitrite accumulation coincides with that of maximum ammonium concentration. This observation indicated that NO_2^- -N was formed at the fertilizer zone, and diffused outward from the zone of fertilizer application in response to a concentration gradient. In Experiment I the zone of maximum nitrite accumulation during 2 weeks incubation was not located at the fertilizer zone but at a distance from the fertilizer zone where the ammonium concentration was lower and the soil pH was more favorable for ammonium oxidation. This suggests that the combination of NH_4^+ -N and the soil pH was toxic to the Nitrobacter spp. but the degree of toxicity was less than when 92 mg N of $(\text{NH}_2)_2\text{CO}$ and NH_4OH was banded in the Wellwood II soil. After 2 weeks incubation, nitrite accumulation was at its maximum, approximately 10% and 6% of the banded $(\text{NH}_2)_2\text{CO}$ and NH_4OH , respectively. With increasing time of incubation up to 4 weeks only a detectable amount of nitrite, 3% for $(\text{NH}_2)_2\text{CO}$ and only a trace (0.1%) for NH_4OH was observed.

There were two zones of maximum nitrate concentration at approximately 2.5 cm from the zone of fertilizer application after

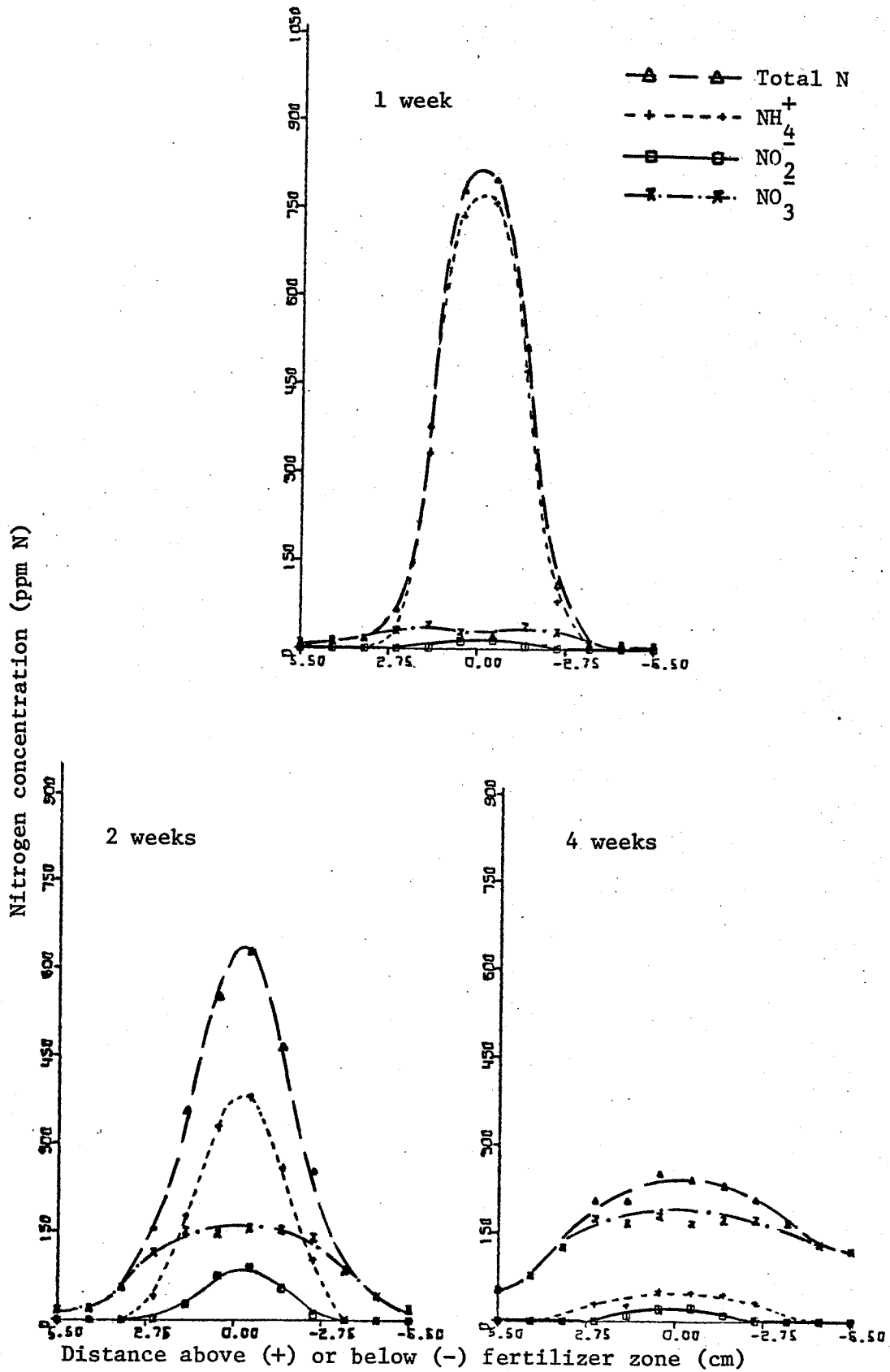


FIG. 20. DISTRIBUTION OF TOTAL INORGANIC N, NH₄⁺-N, NO₂⁻-N AND NO₃⁻-N IN THE NEUTRAL WELLWOOD II SOIL FROM 23 mg N BAND-APPLIED (NH₃)₂CO AT 20C.

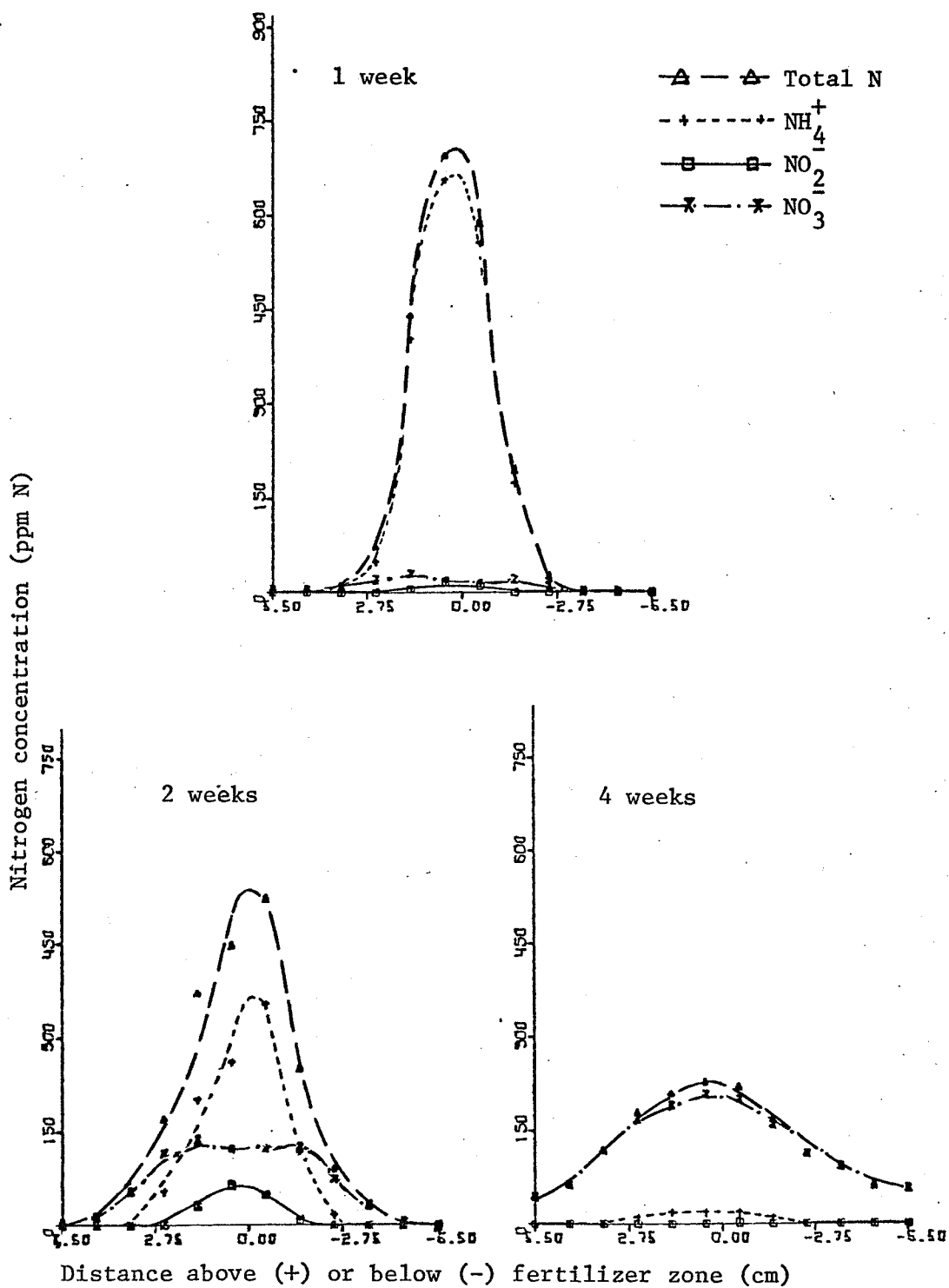


FIG. 21. DISTRIBUTION OF TOTAL INORGANIC N, NH_4^+ -N, NO_2^- -N AND NO_3^- -N IN THE NEUTRAL WELLWOOD II SOIL FROM 23 mg N BAND-APPLIED NH_4OH AT 20C.

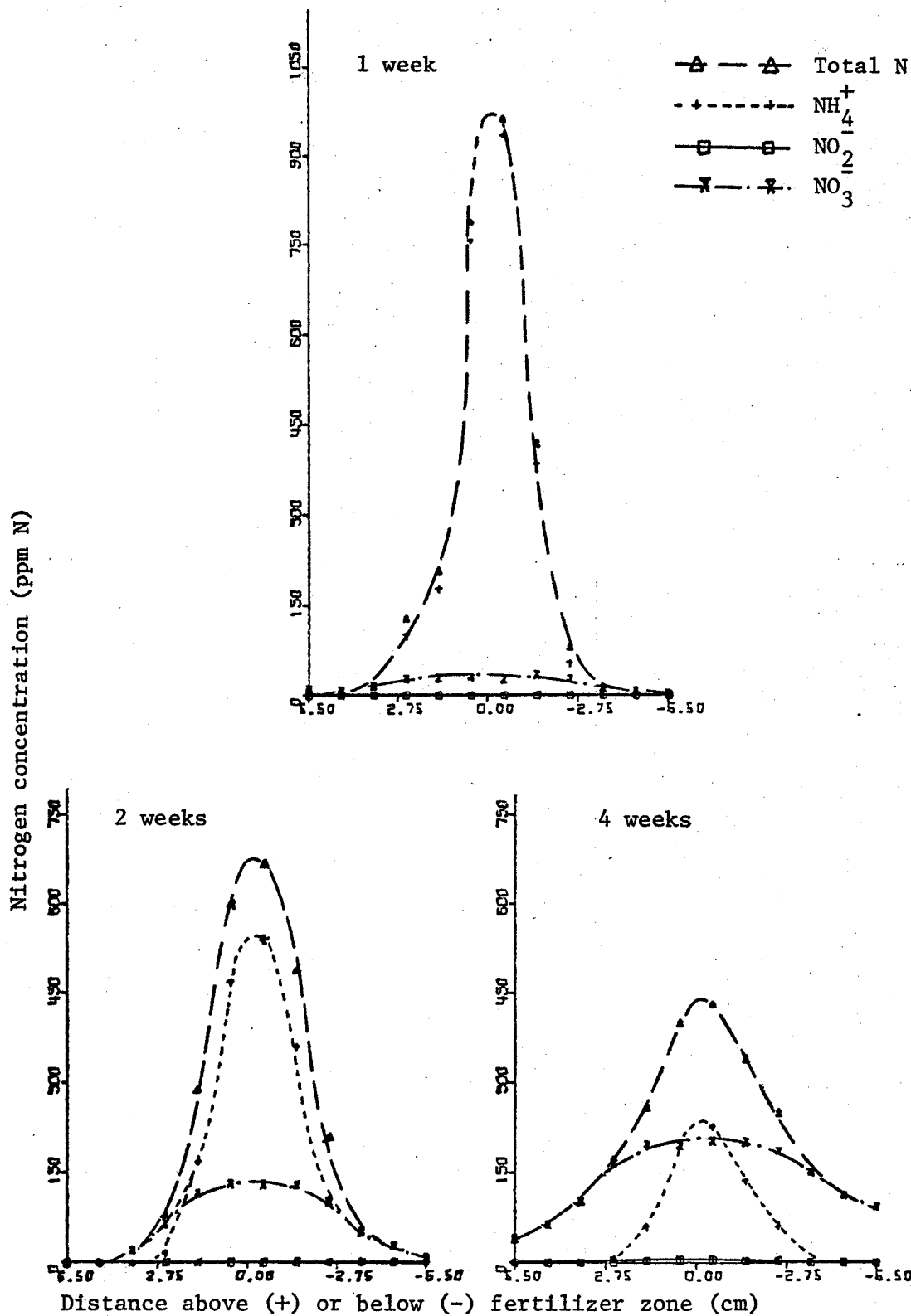


FIG. 22. DISTRIBUTION OF TOTAL INORGANIC N, NH₄⁺-N, NO₂⁻-N AND NO₃⁻-N IN THE NEUTRAL WELLWOOD II SOIL FROM 23 mg N BAND-APPLIED (NH₄)₂SO₄ AT 20C.

one week of incubation. The nitrate peaks merged into one after 2 weeks incubation with $(\text{NH}_2)_2\text{CO}$ and after 4 weeks with NH_4OH -banded soil. By the end of the incubation period of 4 weeks the majority of the total inorganic nitrogen was present as nitrate, approximately 67% and 57% of banded $(\text{NH}_2)_2\text{CO}$ and NH_4OH , respectively.

No nitrite was detected in $(\text{NH}_4)_2\text{SO}_4$ -banded soil after 1 and 2 weeks incubation (Fig. 22), but a trace (0.7%) of the banded $(\text{NH}_4)_2\text{SO}_4$ was detected as nitrite after 4 weeks incubation. The rate of oxidation of the banded $(\text{NH}_4)_2\text{SO}_4$ was comparable to that of $(\text{NH}_2)_2\text{CO}$ and NH_4OH although nitrite did not accumulate. The percentage of $(\text{NH}_4)_2\text{SO}_4$ recovered as nitrate after 1, 2 and 4 weeks incubation was 9%, 30% and 67% respectively.

Band application of 92 mg N (800 kg N/ha on an area basis)

With a still higher nitrogen concentration, 92 mg N, at the fertilizer zone the distribution of the various ionic species of nitrogen was different than with concentrations of 11.5 and 23 mg N. Since the distribution of nitrogen ionic species of the three nitrogen carriers were discussed in the Experiment I, mention will be made here only of the times required for the same percentage oxidation to occur in different concentrations of nitrogen. With $(\text{NH}_2)_2\text{CO}$ 65% oxidation to nitrate took place in 8 weeks where 92 mg N was added and approximately the same amount in 4 weeks where 23 mg N was added. With nitrogen in the form of NH_4OH 50% oxidation was achieved in 8 and 4 weeks with 92 and 23 mg N concentrations, respectively. Thirty percent oxidation of the nitrogen in $(\text{NH}_4)_2\text{SO}_4$ occurred in 6 weeks at the 92 mg N level and in 2 weeks at the 23 mg N level. This shows that the concentration of nitrogen in ammonium form at the localized

area of soil had an effect on the rate of nitrification. It has been shown by Parr (1959) that a high ammonia concentration has a sterilizing effect on the nitrifier population and thus retards the rate of nitrification.

Recovery of inorganic form of nitrogen from three rates of N

Figure 23 shows that the recovery of added nitrogen fertilizer as inorganic nitrogen decreased with time irrespective of the nitrogen concentration and fertilizer source. The rate of loss was the greatest with $(\text{NH}_2)_2\text{CO}$ and NH_4OH , and least with $(\text{NH}_4)_2\text{SO}_4$, except where the recovery of added nitrogen is greater than 100%. In this case most of N loss may have been apparent rather than real. After the first sampling period the recovery of $(\text{NH}_2)_2\text{CO}$ and $(\text{NH}_4)_2\text{SO}_4$ with nitrogen concentrations of 92 and 11.5 mg N, respectively were greater than 100%, this might have been the result of a higher rate of mineralization in these samples as compared to the untreated soil. The recovery of NH_4OH from the soil at the first sampling period, irrespective of the concentration, was always lower than that of $(\text{NH}_2)_2\text{CO}$ and $(\text{NH}_4)_2\text{SO}_4$. This might have been the result of ammonia volatilization during the transfer of NH_4OH solution into the soil. This will be discussed in Experiment VII.

Diffusion coefficient of nitrogen fertilizers as ammonium

The diffusion coefficient of the fertilizers as ammonium banded at rates of 92 and 11.5 mg N per soil column was calculated and is presented in Table 5.

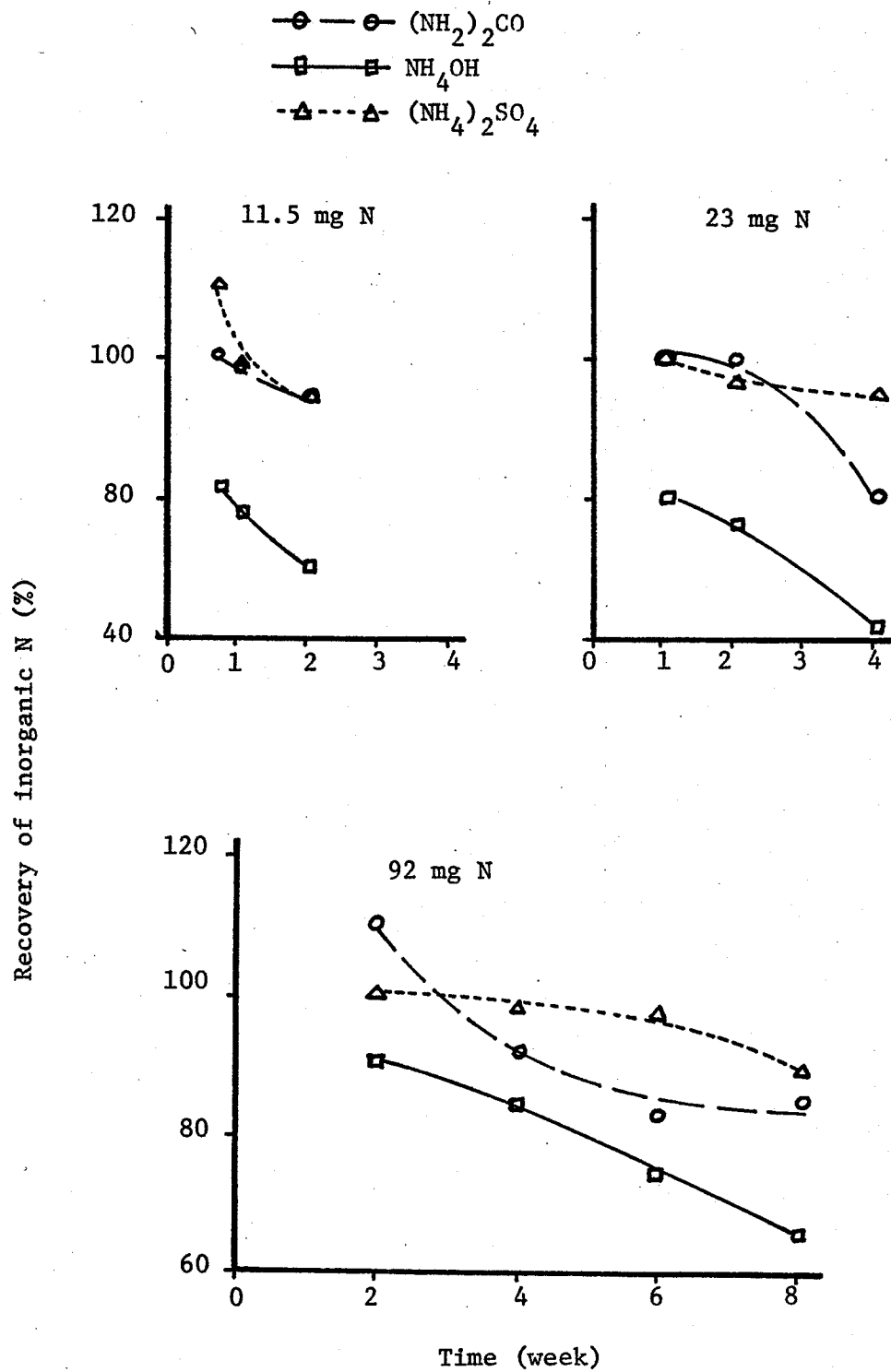


FIG. 23. RECOVERY OF TOTAL MINERAL NITROGEN FROM BAND-APPLIED $(\text{NH}_2)_2\text{CO}$, NH_4OH AND $(\text{NH}_4)_2\text{SO}_4$ AT THREE RATES OF N.

TABLE 5. CALCULATED DIFFUSION COEFFICIENT OF NH_4^+ FROM THREE CARRIERS BANDED AT TWO RATES OF N IN THE NEUTRAL WELLWOOD II SOIL AT 20C

N-Carriers	Diffusion coefficient ($D \times 10^6 \text{ cm}^2/\text{sec}$)	
	92 mg N	11.5 mg
$(\text{NH}_2)_2\text{CO}$	1.54	1.25
NH_4OH	1.59	1.36
$(\text{NH}_4)_2\text{SO}_4$	1.02	1.00

The diffusion coefficient (D) for 92 mg N is slightly different from that shown in Table 4 (Experiment I). The values listed in Table 4 were based on the NH_4^+ remaining after 2 weeks incubation during which time oxidation of some of the ammonium nitrogen took place. The values of D for 92 mg N shown here in Table 5 are for samples treated with 100 ppm of N-serve (on soil weight basis) so that nitrification was inhibited (see Fig. 24). N-serve was not used for 11.5 mg N rate since nitrification had not taken place in the soil banded with N carriers after 3 days incubation (Figs. 16, 17, 18). Table 5 shows that the order of the diffusion coefficients of three carriers is the same with the 2 rates of N, 92 and 11.5 mg N. They are in the order $\text{NH}_4\text{OH} > (\text{NH}_2)_2\text{CO} > (\text{NH}_4)_2\text{SO}_4$. The approximated D based on the NH_4^+ -N at the first sampling period of 3 days and 2 weeks for 11.5 and 92 mg N, respectively, was smaller with 11.5 mg N rate than that of 92 mg N rate. This was probably due to a smaller NH_4^+ -N concentration gradient with a smaller quantity of fertilizer.

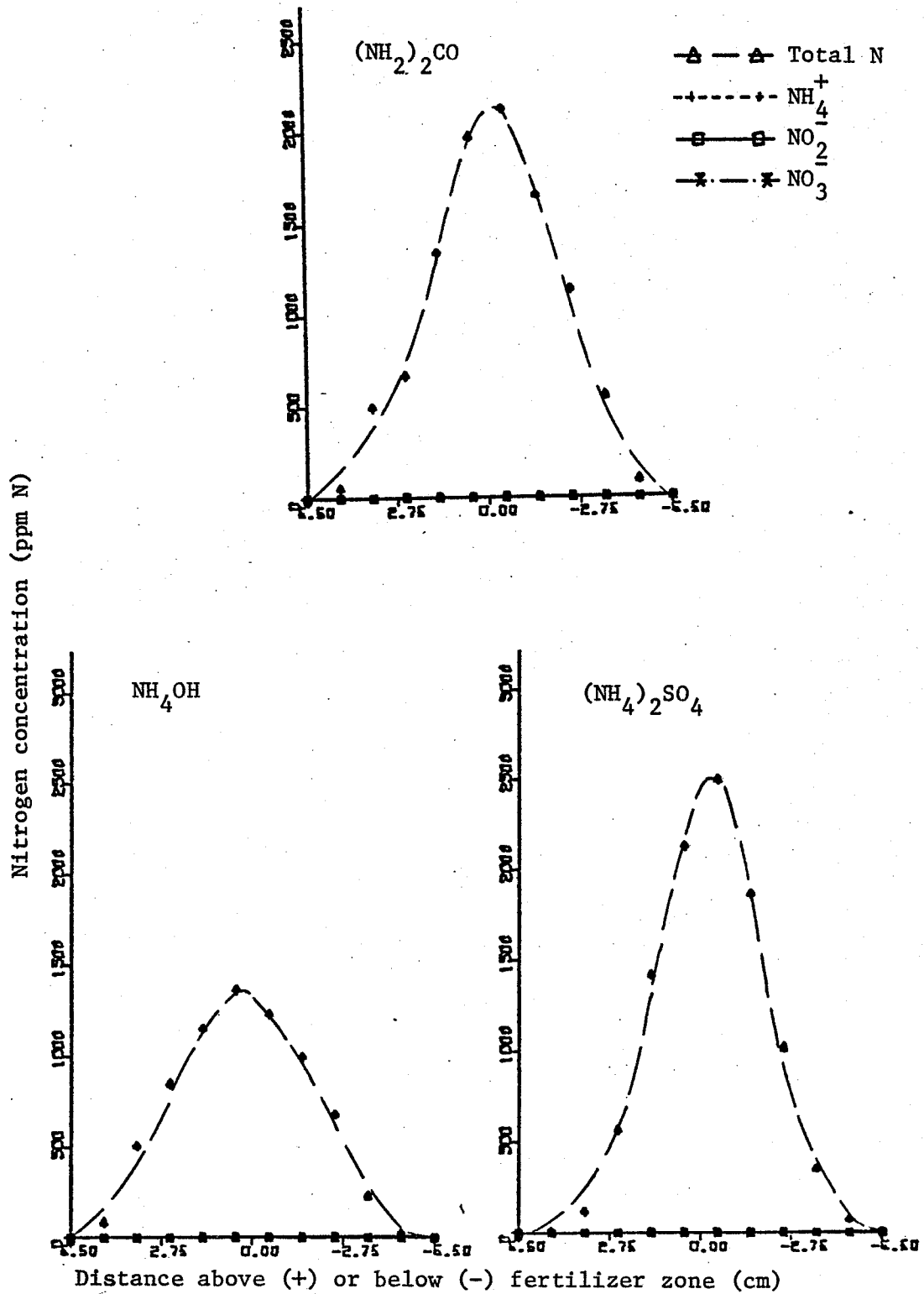


FIG. 24. DISTRIBUTION OF TOTAL INORGANIC N, NH₄⁺-N, NO₂⁻-N AND NO₃⁻-N IN NEUTRAL WELLWOOD II SOIL FROM BAND-APPLIED N₂ IN PRESENCE OF 100 ppm N-SERVE AT 20°C AFTER 2 WEEKS INCUBATION (RATE = 800 kg N/ha).

Summary

The oxidation of banded nitrogen fertilizer was fastest when neutral Wellwood II (pH 6.6) was banded with 23 mg N per column, slowest with 92 mg N and intermediate with 11.5 mg N, irrespective of the N carriers, during 2 weeks of incubation. Theoretically the rate of 11.5 mg N has the least effect on the activity of the nitrifiers and hence should be nitrified most rapidly. For some unknown reason this was not the case. It may have been because the quantity of nitrogen at the diffusion zone was not large enough to stimulate microbial growth. On the other hand, band application of 92 mg N was toxic to the nitrifiers and resulted in very slow oxidation of the band-applied nitrogen.

Nitrite accumulation increased with increases in rate of either $(\text{NH}_2)_2\text{CO}$ or NH_4OH nitrogen application. There was only a trace of nitrite detected with $(\text{NH}_4)_2\text{SO}_4$, irrespective of the rate of N. However, the modified soil pH upon fertilizer addition should not be overlooked. It may also play a role in nitrite accumulation since the final soil pH of a fertilized soil is related to the quantity of N banded in the soil. The effect of soil pH on transformation of band-applied fertilizers will be studied in a later experiment.

The highest recovery of added fertilizers as total inorganic nitrogen (NH_4^+ , NO_2^- and NO_3^-) occurred in neutral Wellwood II soil banded with 11.5 mg N, irrespective of the N carriers. At this rate nitrite did not accumulate. The recovery of fertilizer N as total inorganic N with the other 2 rates, 92 and 23 mg N, was almost equal with $(\text{NH}_2)_2\text{CO}$ and NH_4OH banded soil. This lower recovery of added nitrogen was associated with nitrite accumulation.

Experiment III. Effect of temperature on transformation and movement of band-applied nitrogenous fertilizers in neutral Wellwood II soil

In order to study the effect of temperature on band-applied N fertilizers, Wellwood II (pH 6.6) soil was banded with 800 kg N/ha of urea and incubated at temperatures 10, 15 and 20C. In the area where the Wellwood II soil was collected during the growing season the soil temperature at 0-15 cm depth ranges from 25-10C, except at the immediate soil surface. Wellwood II soil was used in this experiment because among the three soils used in Experiment I it was the only one that produced significant amounts of nitrite. One of the purposes of this experiment was to determine the effect of temperature on the tendency to produce nitrite.

After 2 weeks incubation the greatest amount of nitrification of banded urea took place at 20C (Fig. 10), and little or no nitrification occurred at 10 and 15C (Figs. 25, 26). With increased time of incubation much more nitrite was accumulated at 10 and 15C than at 20C. After 6 weeks incubation at 10C, more than 1,900 ppm NH_4^+ -N was present at the zone of fertilizer application while at 15 and 20C only about half of this amount remained at the fertilizer zone. After 12 weeks incubation at 10C the distribution of the NH_4^+ -, NO_2^- - and NO_3^- -N was almost the same as those at 15 and 20C after 6 weeks incubation although the quantities of the different ionic species of nitrogen differ at each temperature. This shows that the rate of nitrification is very slow at 10C as compared to that of 15 and 20C. A large quantity of NO_2^- , equivalent to approximately the same quantity of NO_2^- -N that accumulated at 20C after 4 weeks incubation, was present at 10C after 12 weeks (3 months) incubation.

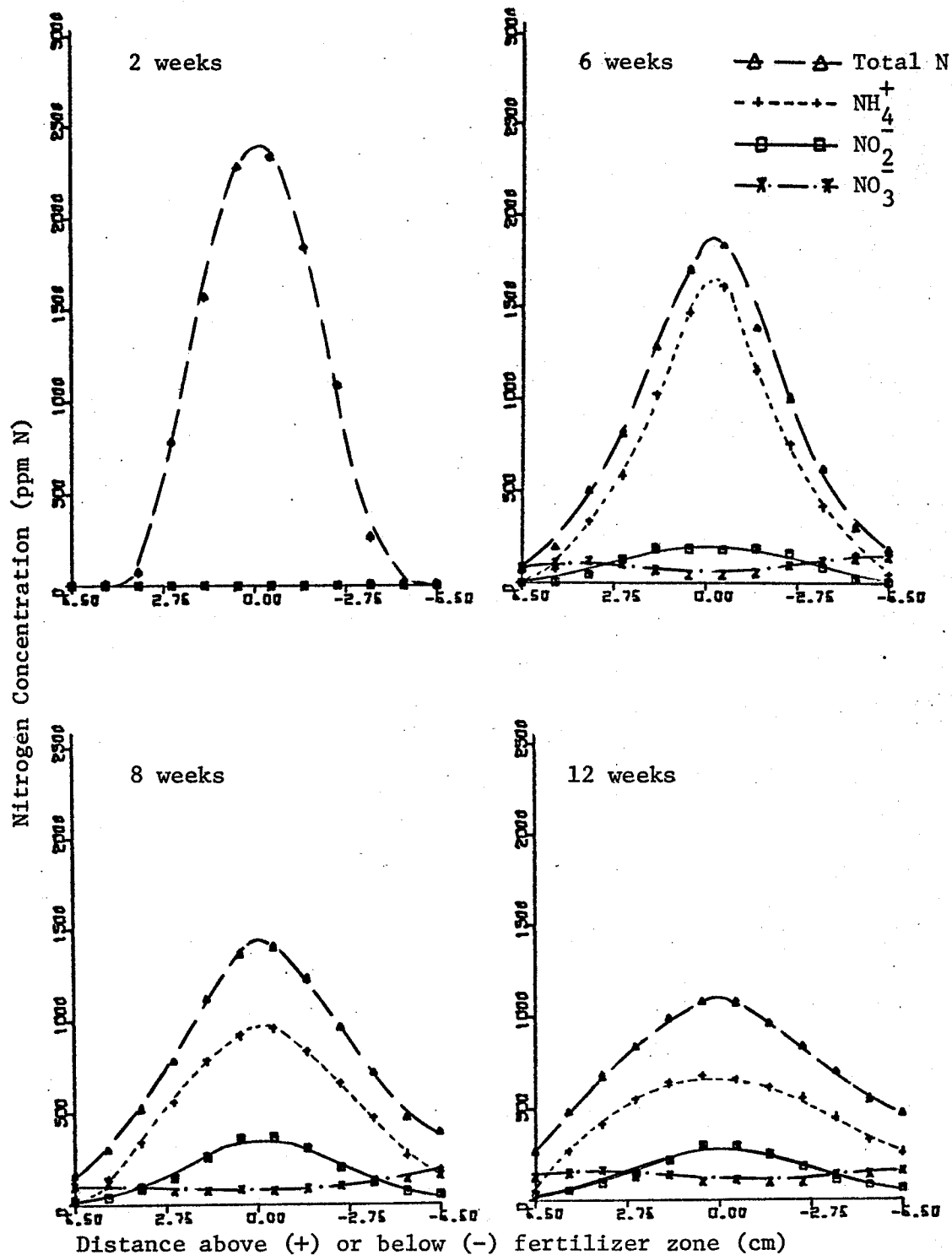


FIG. 25. DISTRIBUTION OF TOTAL INORGANIC N, NH₄⁺-N, NO₂⁻-N AND NO₃⁻-N IN THE NEUTRAL WELLWOOD II SOIL FROM 92 mg N BAND-APPLIED (NH₂)₂CO AT 10C.

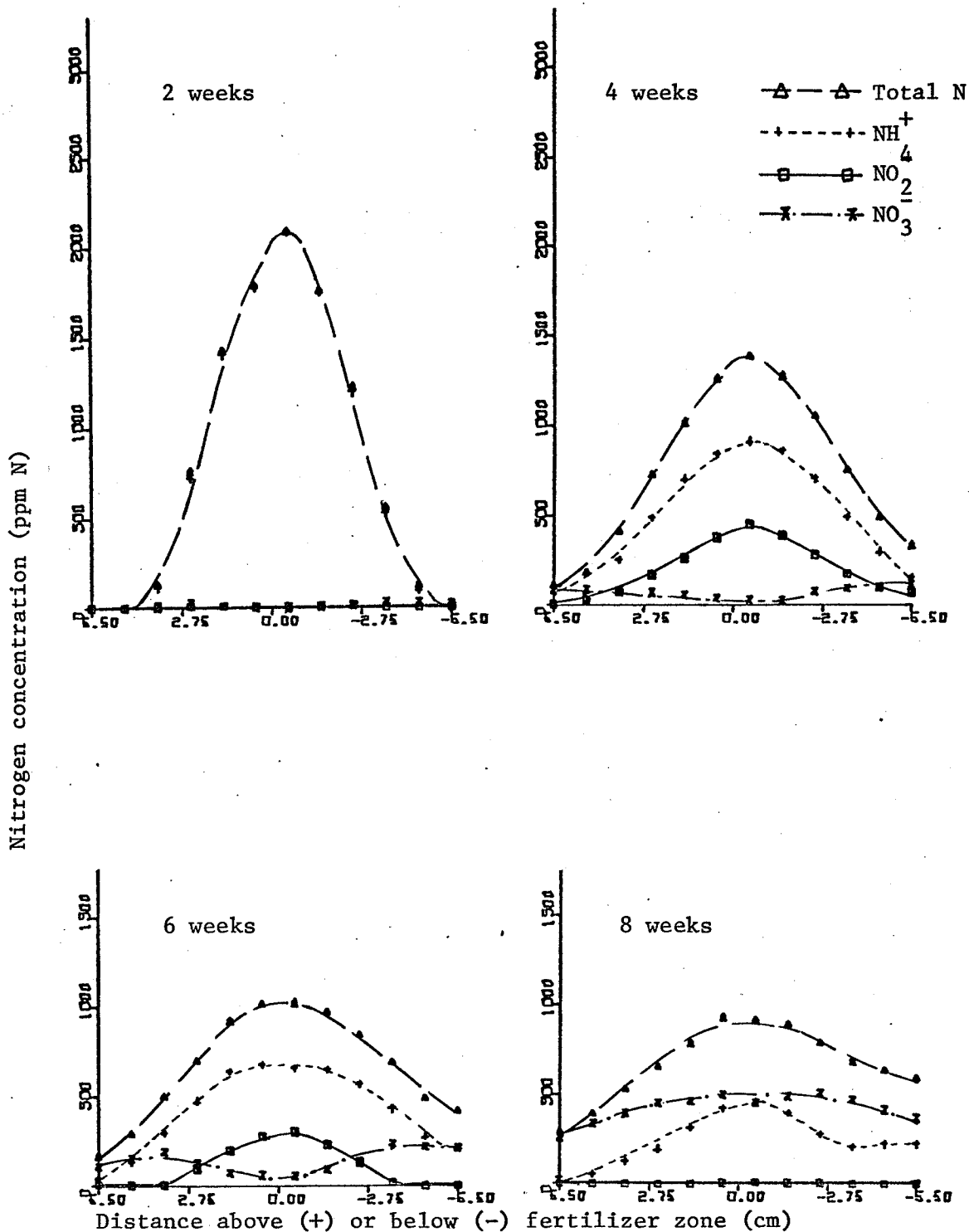


FIG. 26. DISTRIBUTION OF TOTAL INORGANIC N, NH_4^+ -N, NO_2^- -N AND NO_3^- -N IN THE NEUTRAL WELLWOOD II SOIL FROM 92 mg N BAND-APPLIED $(\text{NH}_2)_2\text{CO}$ AT 15C.

This suggest that nitrite persists in soil for a long period when the soil temperature is low. Low temperature causes a slower diffusion of fertilizer N from the zone of application resulting in an accumulation of ammonium near the fertilizer zone. Low temperature also restricted the activities of nitrifiers. Tyler and Broadbent (1960) and Justice and Smith (1963) have shown that nitrite oxidizers are very sensitive to low temperature conditions. Restricted Nitro-bacter activity at 10C together with a high ammonium concentration at the zone of application favors a gradual accumulation of nitrite during ammonium oxidation. This can also account for the higher accumulation of nitrite at 15C as compared to 20C after the same incubation period. The rate of change in soil pH within the soil column as the fertilizer diffused from the zone of application was related to the rate of oxidation and the rate of diffusion of the fertilizer material. The soil pH decreased 0.5 unit from 2-8 weeks incubation at 20 and 15C (Fig. 27) where the oxidation of $(\text{NH}_2)_2\text{CO}$ was the greatest. However, the soil pH remained quite constant throughout incubation period of 2-12 weeks at 10C where the nitrification process was relatively slow. Regardless of the incubation temperature, soil pH was highest at the fertilizer zone early in the incubation period as a result of hydrolysis of urea to ammonium carbonate. With increase in incubation time, the soil pH decreased gradually as a result of the lowering of $\text{NH}_4^+\text{-N}$ from the fertilizer zone by diffusion and nitrification of ammonium.

The diffusion coefficient of $\text{NH}_4^+\text{-N}$ from $(\text{NH}_2)_2\text{CO}$ calculated from 2 weeks data shows that the rate of fertilizer diffusion as $\text{NH}_4^+\text{-N}$ decreased with decreasing soil temperature from 20 to 10C.

Table 6.

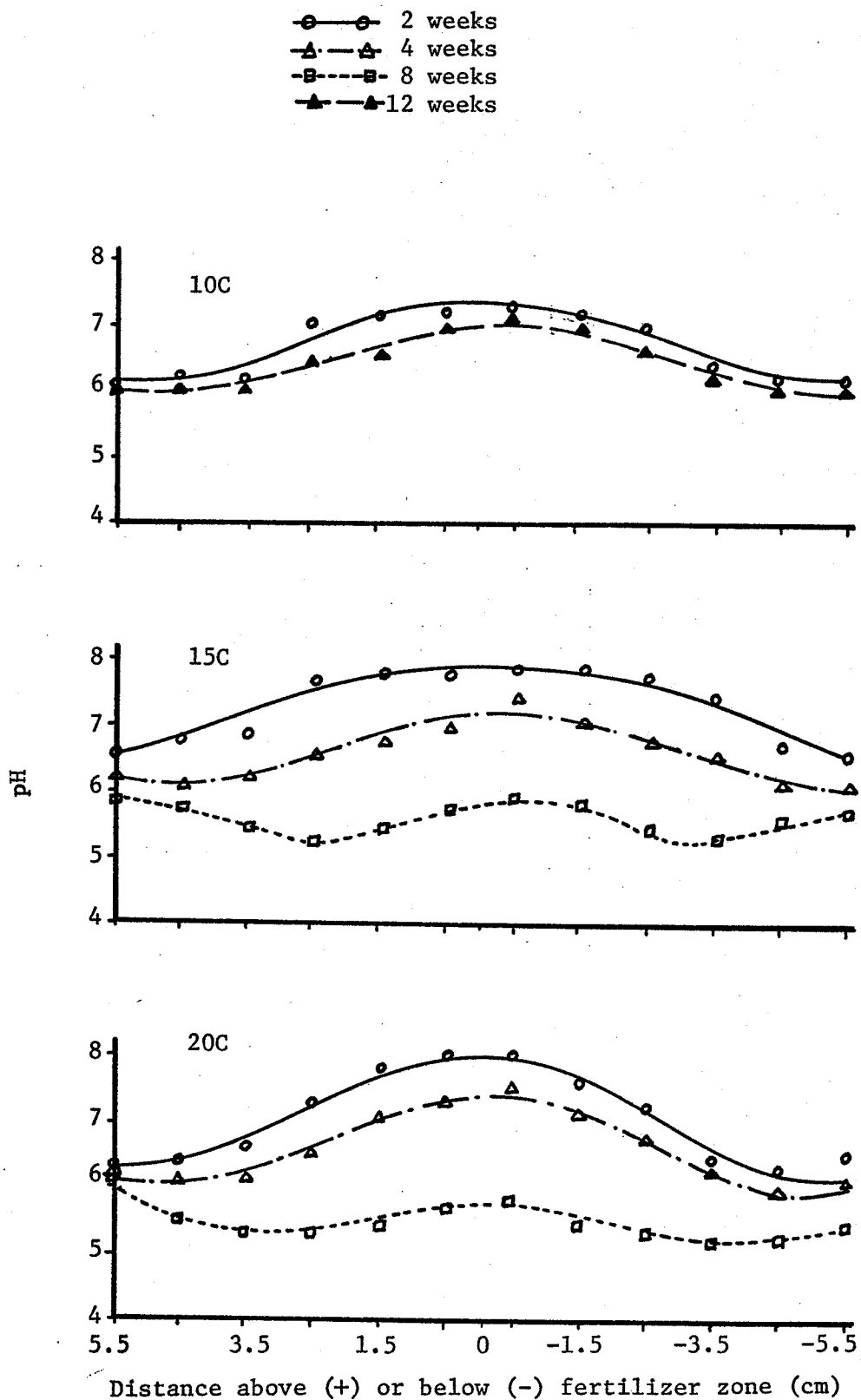


FIG. 27. CHANGE IN pH DUE TO 92 mg N BAND-APPLIED $(\text{NH}_2)_2\text{CO}$ IN NEUTRAL WELWOOD II SOIL AT THREE TEMPERATURES.

TABLE 6. DIFFUSION COEFFICIENT OF NH_4^+ -N FROM THREE NITROGEN CARRIERS BANDED IN WELLWOOD II SOIL AT 10 AND 20C

	Diffusion coefficient ($D \times 10^6 \text{ cm}^2/\text{sec}$)	
	10°C	20°C
$(\text{NH}_2)_2\text{CO}$	1.06	1.54
NH_4OH	1.38	1.59
$(\text{NH}_2)_2\text{SO}_4$	0.80	1.02

A slow diffusion of banded ammonium or ammonium-yielding fertilizer will cause a slower oxidation of the N fertilizer at the proximity of the fertilizer zone.

Irrespective of the incubation temperature, the recovery of band-applied urea nitrogen decreased with time (Table 7). The extent of N loss was greatest at 15C and least at 10C at which temperature the nitrification of banded urea was the slowest.

When NH_4OH was the nitrogen source, the rate of oxidation and distribution of the reaction products were quite similar to those of $(\text{NH}_2)_2\text{CO}$ -banded soil (Figs. 11, 28, 29). In the case of band-applied $(\text{NH}_4)_2\text{SO}_4$, the oxidation of the fertilizer was slow at three temperatures (Figs. 12, 30, 31). This was the result of reduced local soil pH (Fig. 32) which inhibits the nitrification process.

Summary

It was concluded that, irrespective of the nitrogen fertilizers, nitrification was fastest at 20C, intermediate at 15C and slowest at 10C. However, the difference between 15 and 20C diminished after 2 weeks incubation. The extent of nitrite accumulation was related to temperature and the fertilizer used. Maximum accumulation of

TABLE 7. PERCENT RECOVERY OF ADDED N AS TOTAL INORGANIC NITROGEN FROM WELLWOOD II SOIL INCUBATED AT VARIOUS TIMES AND TEMPERATURES.

N Carrier	20C			15C			10C		
	2 wk	4 wk	8 wk	2 wk	4 wk	8 wk	2 wk	4 wk	8 wk
$(\text{NH}_2)_2\text{CO}$	115	91	85	97	88	79	101	93	88
NH_4OH	91	84	66	93	83	81	81	69	62
$(\text{NH}_4)_2\text{SO}_4$	100	98	88	103	97	91	109	94	90

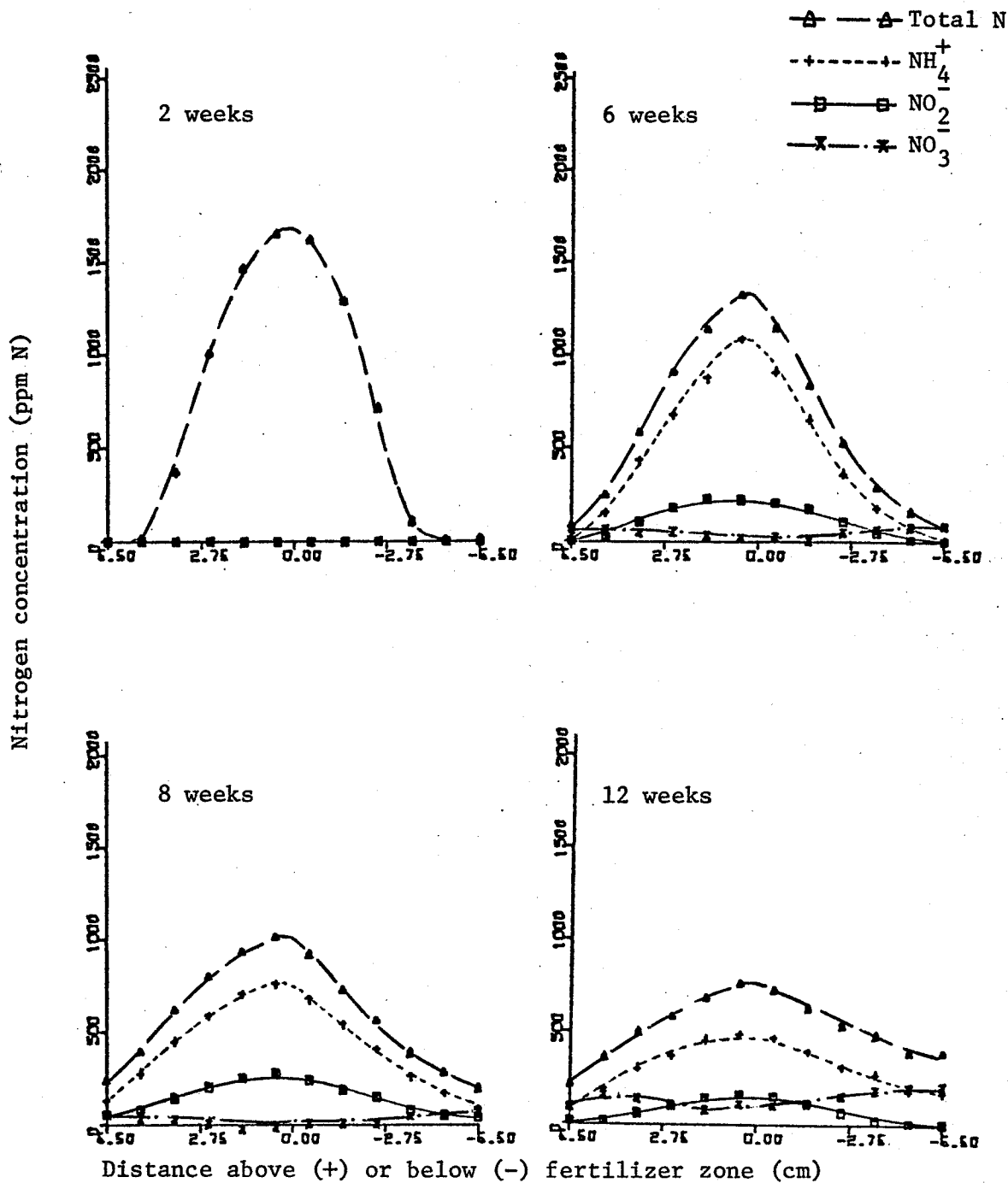


FIG. 28. DISTRIBUTION OF TOTAL INORGANIC N, NH₄⁺-N, NO₂⁻-N, AND NO₃⁻-N IN THE NEUTRAL WELLWOOD II SOIL FROM 92 mg N BAND-APPLIED NH₄OH AT 10C.

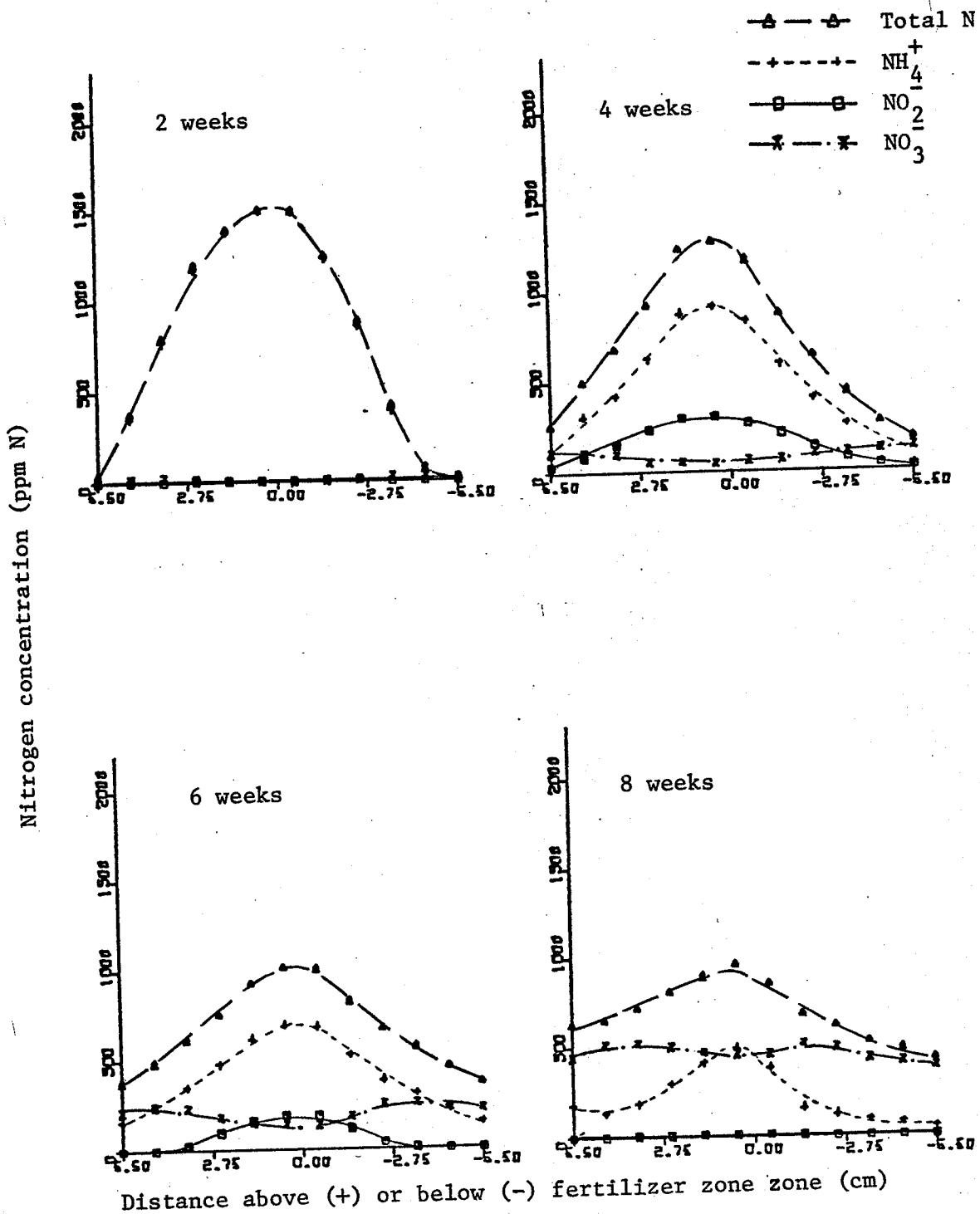


FIG. 29. DISTRIBUTION OF TOTAL INORGANIC N, NH₄⁺-N, NO₂⁻-N AND NO₃⁻-N IN THE NEUTRAL WELLWOOD II SOIL FROM 92 mg N BAND-APPLIED NH₄OH AT 15C.

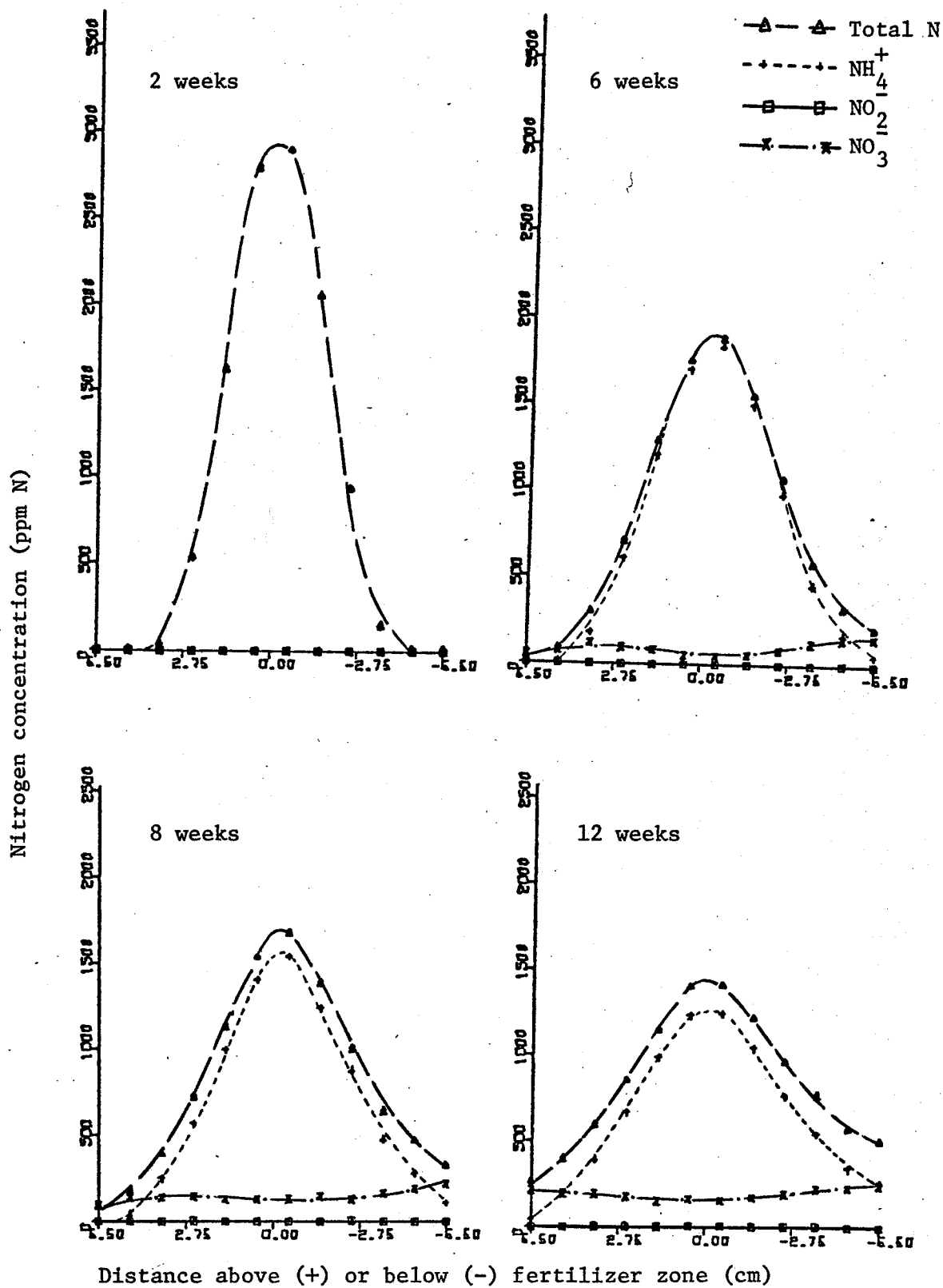


FIG. 30. DISTRIBUTION OF TOTAL INORGANIC N, NH₄⁺-N, NO₂⁻-N AND NO₃⁻-N IN THE NEUTRAL WELLWOOD II SOIL FROM 92 mg N BAND-APPLIED (NH₄)₂SO₄ AT 10C.

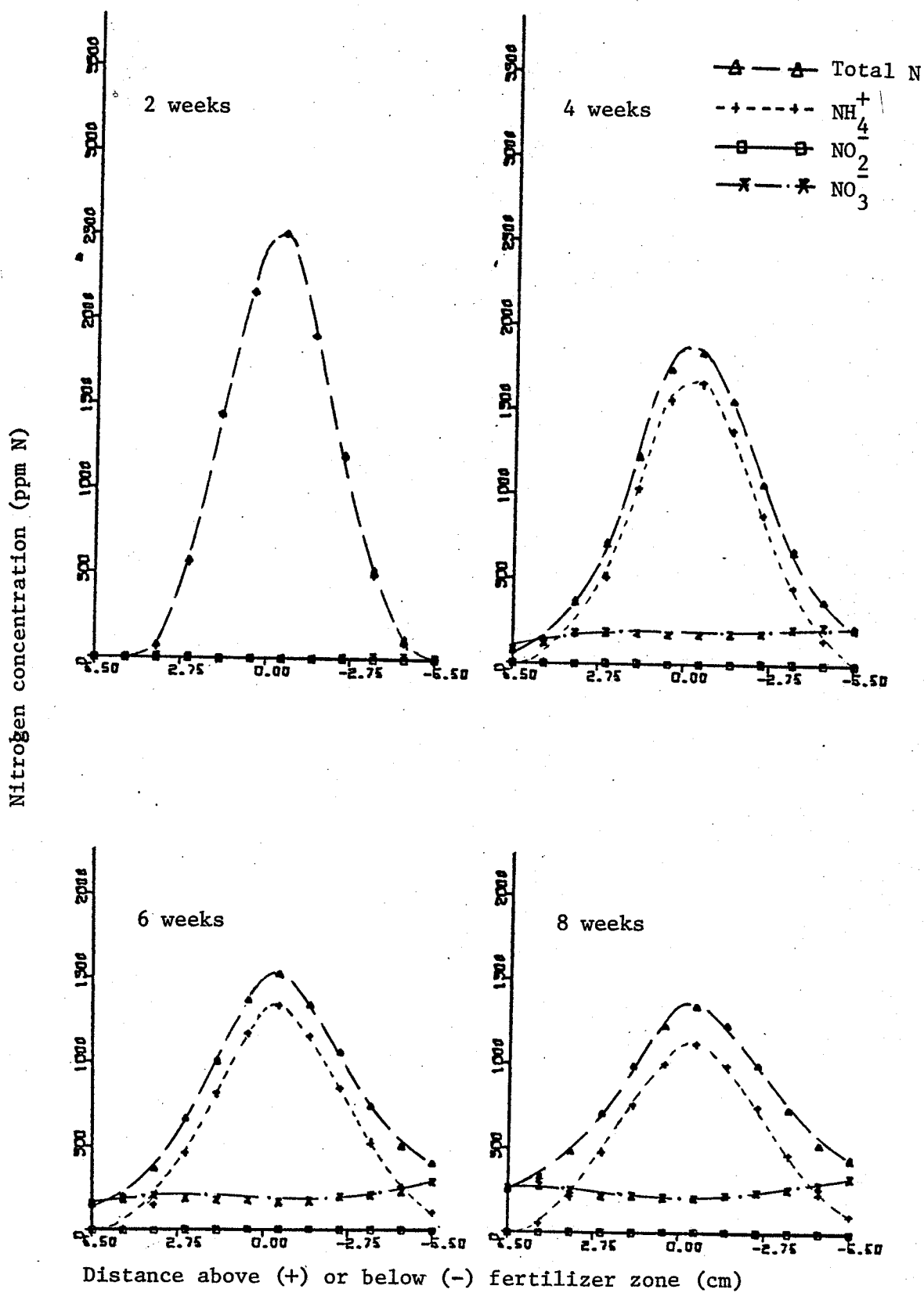


FIG. 31. DISTRIBUTION OF TOTAL INORGANIC N, NH_4^+ -N, NO_2^- -N AND NO_3^- -N IN THE NEUTRAL WELLWOOD II SOIL FROM 92 mg N BAND-APPLIED $(\text{NH}_4)_2\text{SO}_4$ AT 15 C.

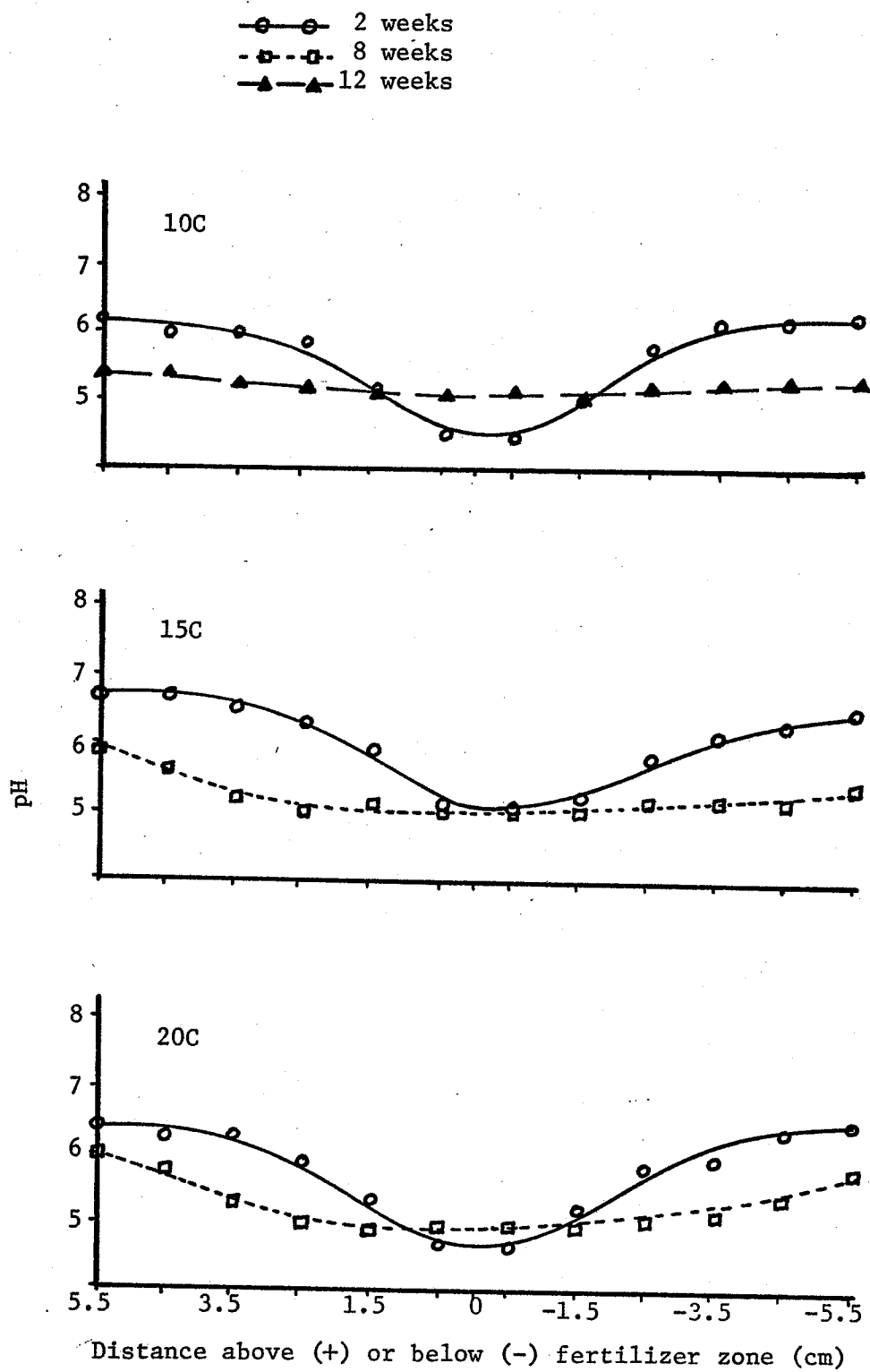


FIG. 32. CHANGE IN pH DUE TO 92 mg N BAND-APPLIED $(\text{NH}_4)_2\text{SO}_4$ IN NEUTRAL WELLWOOD II SOIL AT THREE TEMPERATURES

nitrite near the fertilizer zone occurred after 8 weeks incubation at 10C when urea and NH_4OH were band applied. However, when the incubation temperatures were 15 and 20C the maximum appearance of nitrite occurred at 4 weeks. The formation of nitrite from $(\text{NH}_4)_2\text{SO}_4$ was small and it was independent of temperature.

Experiment IV. Effect of pH on the transformation of band-applied urea in Keld and Wellwood II soils

Results of Experiment I showed that the nitrifying activities of Keld (pH 5.4) and Wellwood II (pH 6.6) soils differed greatly. Wellwood II soil was more active in nitrifying the band-applied urea and produced a considerable amount of nitrite while Keld was the least active. In order to ascertain whether the difference in behaviour of these two soils was due solely to a difference in pH, the Keld soil was treated with $\text{Ca}(\text{OH})_2$ to increase its pH and the Wellwood II soil was acidified. These modified soils were then incubated with urea and the transformation products were analyzed.

Effect of pH in Wellwood II soil

Nitrification in Wellwood II soil was inhibited during the first two weeks of incubation by the lowering of pH to 5.5. The amounts of oxidation products of NH_4^+ in the natural Wellwood II were 987 μg NO_2^- -N and 700 μg NO_3^- -N per 12 g of soil (Fig. 10). However, the amounts were 44 μg NO_2^- -N and 76 μg NO_3^- -N per 12 g of acidified Wellwood II soil (pH 5.5) at the end of 2 weeks incubation (Fig. 33).

After 4 weeks incubation, there were similar amounts of nitrite in the two treatments but at pH 5.5 there was 621 μg NO_3^- -N/12 g soil as compared to 2153 μg NO_3^- -N/12 g of natural Wellwood II soil (pH 6.6). Maximum nitrite accumulation occurred after 4 weeks incubation at both soil pH values. After 8 weeks incubation, only detectable amounts of nitrite were present, and nitrate accounted for the majority of the mineral nitrogen although appreciable amounts of NH_4^+ -N was present in both soils. The results suggest that the lowering of soil pH affected both nitrosification and nitrite oxidation.

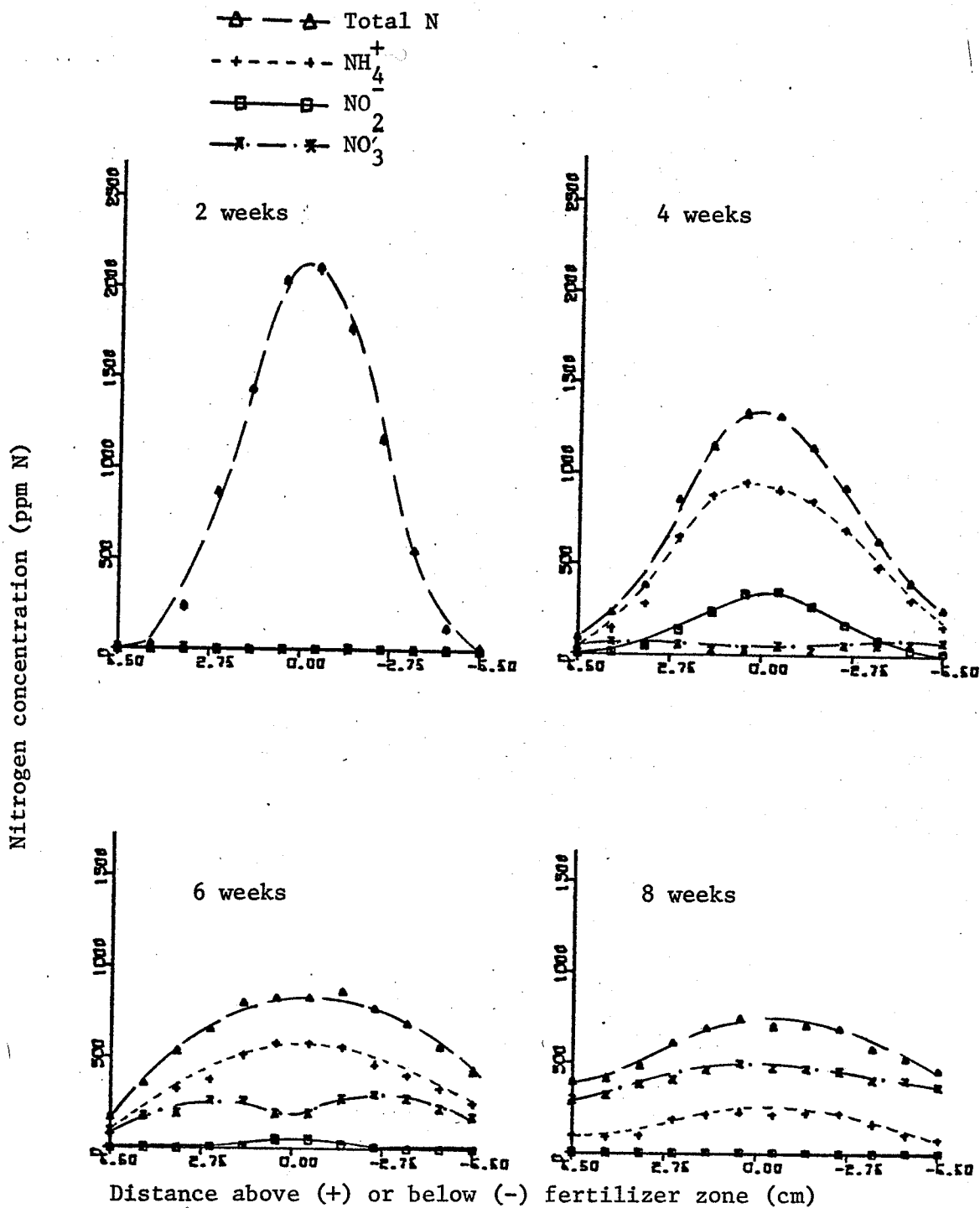


FIG. 33. DISTRIBUTION OF TOTAL INORGANIC N, NH₄⁺-N, NO₂⁻-N AND NO₃⁻-N IN ACIDIFIED WELLWOOD II SOIL (pH 5.5) FROM BAND-APPLIED (NH₂)₂CO AT 20C.

However, the degree of retardation on Nitrobacter spp. activity by lowering the soil pH was greater than on Nitrosomonas spp..

When acidified Wellwood II (pH 5.5) and Keld (pH 5.4) soils of similar soil pH were compared, the acidified Wellwood II soil had a different distribution pattern of the ionic species of nitrogen within the soil column (Figs. 33, 7). There was very little oxidation of banded urea with Keld soil (pH 5.4) in which majority of the mineral nitrogen remained as the ammonium form after 6 weeks incubation. This shows that addition of 2 meq. of conc. H_2SO_4 into Wellwood II soil was not enough to reduce its natural oxidation capacity to the same extent of a natural Keld soil of the same pH.

Effect of pH in Keld soil

When Keld soil (pH 5.4) was limed with $Ca(OH)_2$ to a soil pH of 6.5, there was no increase in oxidation of the banded urea after 6 weeks incubation as compared to that of the natural Keld soil (Figs. 34, 7). This may have been due to low autotrophic nitrifier population of the soil. No nitrite accumulated in either soil. The nitrate produced at both soil pH values after 6 weeks incubation may have been the result of heterotrophic oxidation by fungi at the diffusing edges of the fertilizer band. The nitrate then diffused towards the center of fertilizer zone from the diffusing edges in response to a nitrate concentration gradient.

When the distribution curves of the ionic species of nitrogen in the limed Keld (pH 6.5) soil was compared to natural Wellwood II (pH 6.6) soil, it was observed that the oxidation of band-applied urea in Wellwood II was almost complete, while it was far from complete in limed Keld soil (Figs. 10, 34). This indicates that liming

Nitrogen concentration (ppm N)

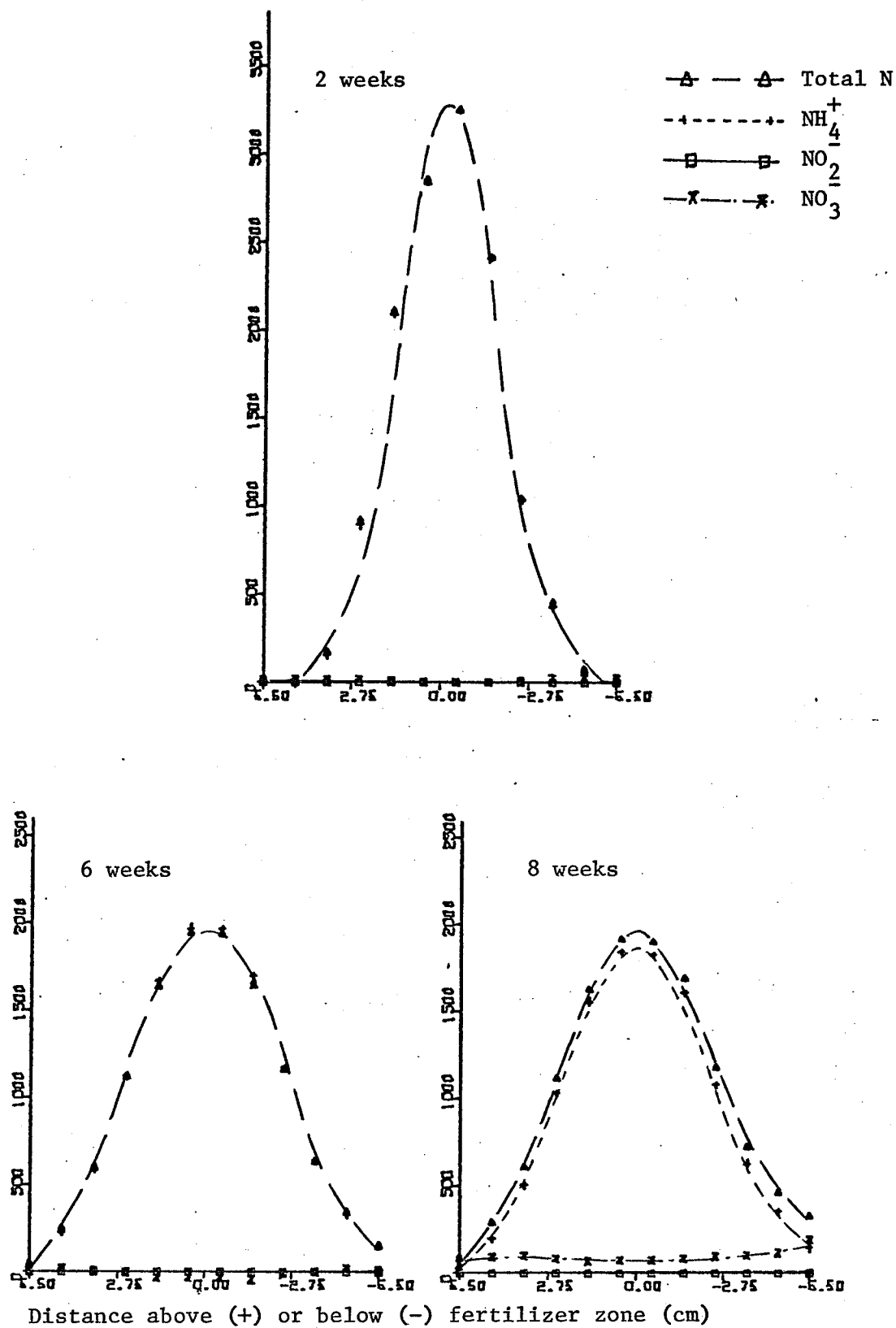


FIG. 34. DISTRIBUTION OF TOTAL INORGANIC N, NH₄⁺-N, NO₂⁻-N AND NO₃⁻-N IN LIMED ACID KELD SOIL (pH 6.5) FROM 92 mg N BAND-APPLIED (NH₂)₂CO AT 20C.

an acid soil did not stimulate a nitrifier population enough to oxidize the urea.

pH 7.3

The Keld soil was further limed to a pH of 7.3. After 6 weeks incubation, the nitrification of banded urea in limed soil (pH 7.3) was still very slow and nearly all the added fertilizer remained in the ammonium form (Fig. 35). This further suggests that the initial autotrophic nitrifier population was very low or non-existent. This might have been due to the toxic effects that exist in the soil.

Inoculation of limed Keld (pH 7.3) with 1% natural Wellwood II soil (pH 6.6)

The limed Keld soil (pH 7.3) was inoculated with 1% natural Wellwood soil (pH 6.6). A small quantity of Wellwood II soil was used in order that the physical and chemical properties of the limed Keld soil were not altered by inoculation. Inoculation of the limed Keld soil did not increase nitrification of banded urea during the first 2 weeks incubation (Fig. 36). However, after 6 weeks incubation $1158 \mu\text{g NO}_3^- \text{-N}/12 \text{ g soil}$ was observed in inoculated limed Keld soil as compared to $109 \mu\text{g NO}_3^- \text{-N per 12 g of uninoculated limed Keld soil}$. No nitrite was detected. The slow response to inoculation may have been because time was required for the nitrifiers of the Wellwood II soil to become established in the limed Keld soil.

Inoculation of limed Keld soil (pH 7.3) with *Nitrosomonas europaea**

Limed Keld soil of pH 7.3 was inoculated with 1 ml of a Nitro-

*The *Nitrosomonas europaea* was kindly supplied by Dr. I. Suzuki, Department of Microbiology, University of Manitoba, Winnipeg, Manitoba.

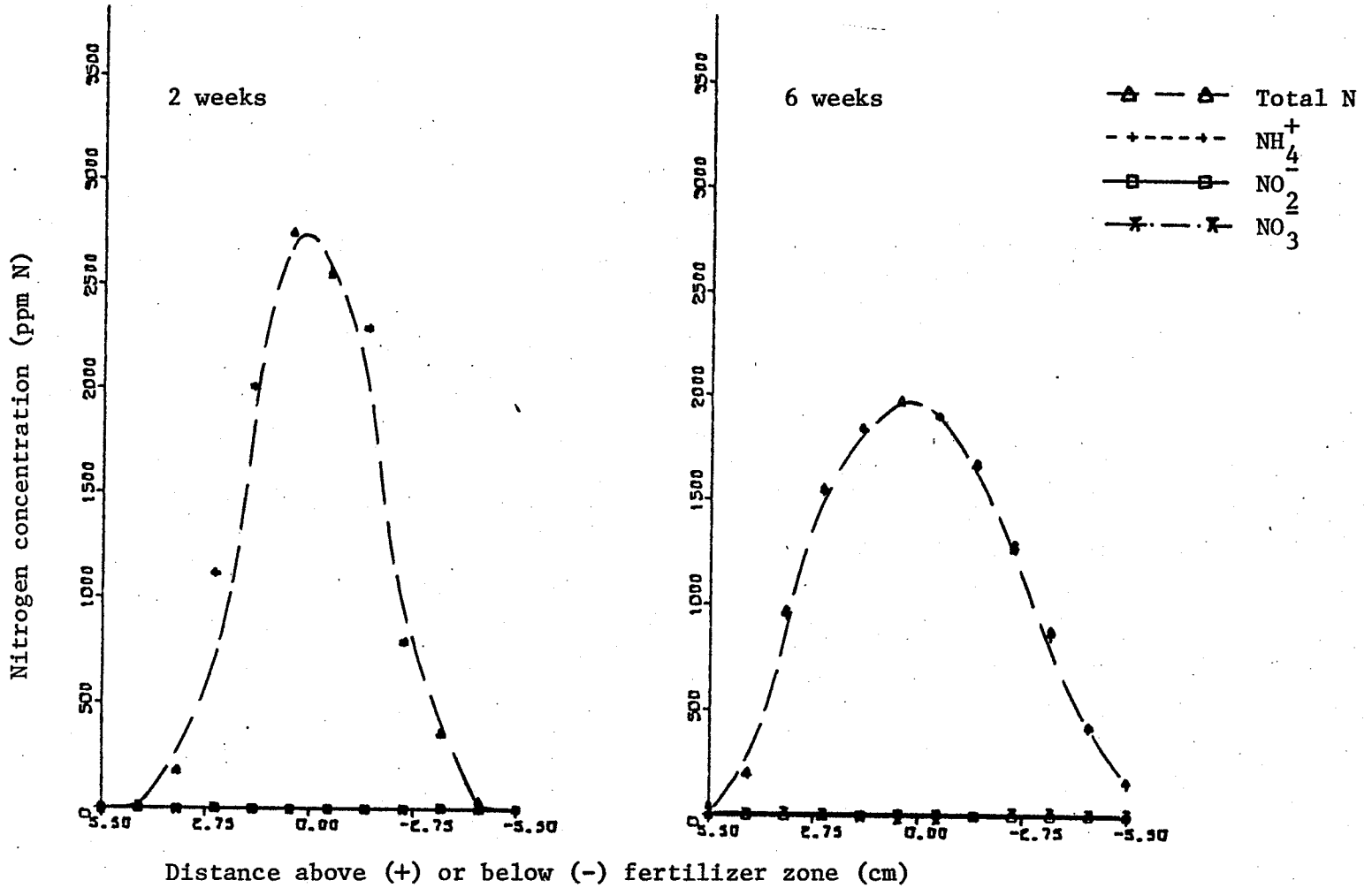


FIG. 35. DISTRIBUTION OF TOTAL INORGANIC N, NH₄⁺-N, NO₂⁻-N AND NO₃⁻-N IN LIMED KELD SOIL (pH 7.3) FROM 92 mg N BAND-APPLIED (NH₂)₂CO AT 20C.

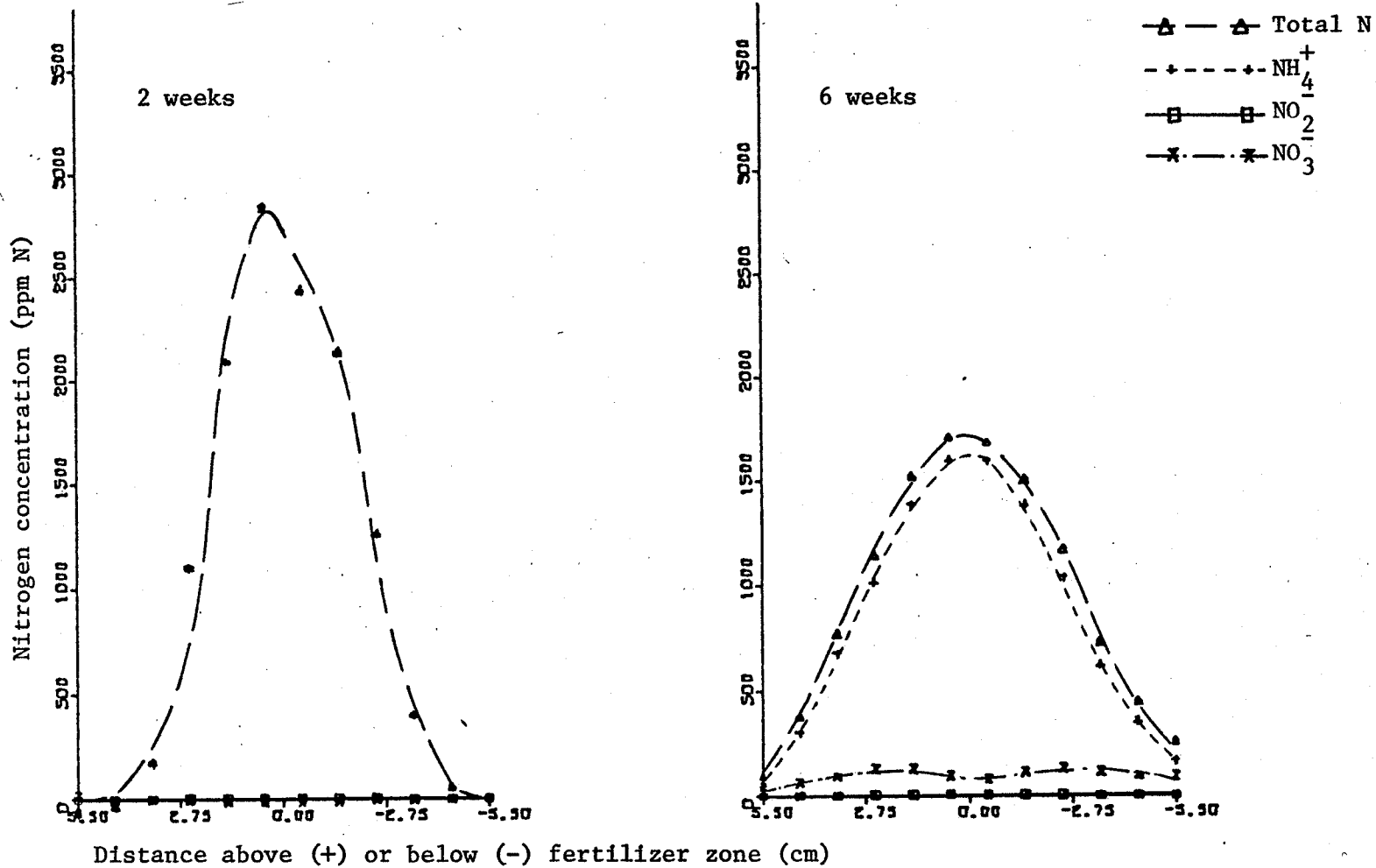


FIG. 36. DISTRIBUTION OF TOTAL INORGANIC N, NH₄⁺-N, NO₂⁻-N AND NO₃⁻-N IN LIMED KELD SOIL (pH 7.3) INOCULATED WITH 1% WELLWOOD II SOIL FROM 92 mg N BAND-APPLIED AREA AT 20C.

somonas europaea culture and incubated for 2 and 6 weeks. The numbers of Nitrosomonas in the culture was estimated to be 16×10^6 per ml. The oxidation of banded urea to nitrite was greatly increased upon inoculation, resulting $552 \mu\text{g NO}_2^- \text{-N}/12 \text{ g soil}$ after 2 weeks incubation, and $932 \mu\text{g NO}_2^- \text{-N}/12 \text{ g soil}$ after 6 weeks incubation (Fig. 37). Although the soil was not inoculated with Nitrobacter spp., there was a large increase in nitrate after 6 weeks incubation as compared to that of the uninoculated soil. The increase in Nitrobacter spp. might have been stimulated by the presence of its substrate, nitrite. The soil was not inoculated with Nitrobacter spp. because no culture was available. After 6 weeks incubation, a nitrite maximum occurred near the fertilizer application zone while two maximum nitrate peaks were present, approximately 3-4 cm above and below the fertilizer zone. This indicates that Nitrobacter spp. is active where the concentration of ammonium and/or ammonia is reasonably low.

Summary

From the above experimental observations, it was concluded:

- 1) Effect of a low soil pH on nitrification of band-applied urea is indirect. Soil acidity results in a small nitrifier population which is inadequate to nitrify the banded fertilizer.
- 2) The natural soil pH has the greatest effect on nitrification. Liming acidic Keld soil to a pH of 6.5 and 7.3 did not increase the oxidation of band-applied urea unless nitrifying organisms were added. Acidifying neutral Wellwood II soil to pH 5.5 lowered the oxidation rate initially but this effect seemed to be temporary.
- 3) Inoculation of a limed acidic soil with nitrifying organisms enhanced the nitrifying capacity of that soil.

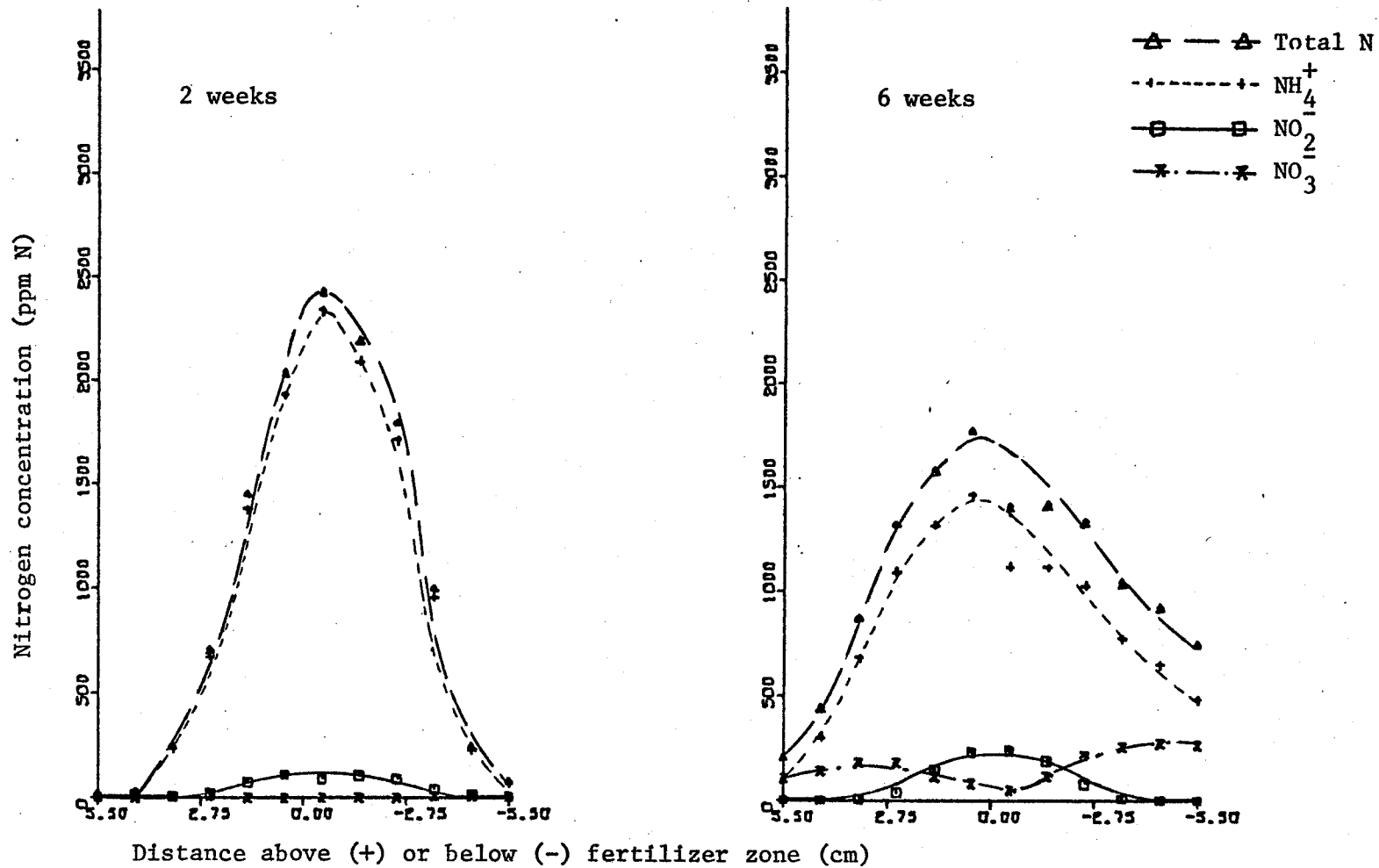


FIG. 37. DISTRIBUTION OF TOTAL INORGANIC N, NH₄⁺-N, NO₂⁻-N AND NO₃⁻-N IN LIMED KELD ACID (pH 7.3) INOCULATED WITH NITROSOMONAS EUROPEAE CULTURE FROM 92 mg N BAND-APPLIED (NH₂)₂CO AT 20C.

Experiment V. The recovery of banded KNO_3 and KNO_2 in presence of added urea with Wellwood I soil

Previous investigations with Wellwood II soil (Experiment I) indicated that whenever NO_2^- -N accumulated there was a low recovery of the added nitrogen as inorganic nitrogen (NH_4^+ , NO_2^- and NO_3^- -N). This investigation was undertaken to study the recovery of band-applied KNO_3 and KNO_2 nitrogen in the presence of band-applied urea. In order to simulate the conditions existing in Wellwood II soil at the time of maximum nitrite accumulation, Wellwood I (pH 6.0) soil was used. The physical and chemical characteristics of Wellwood I and Wellwood II soils are similar except for the soil pH (see Table 1).

Recovery of 11.5 mg (100 kg N/ha) KNO_3 -N in the presence of 11.5 mg $(\text{NH}_2)_2\text{CO}$ -N

The recovery of fertilizer in soil banded with NO_3^- -N alone was almost complete (98%) after 1 week incubation (Table 8), but the recovery decreased slightly (82%) after 2 weeks incubation. A loss of 17% of the added KNO_3 -N might be due to assimilation of NO_3^- -N by microbes for cell synthesis, and/or dissimilatory reduction of NO_3^- -N through biological denitrification. These two processes can occur simultaneously during incubation. However, in the presence of urea, the recovery of added nitrogen, $\text{KNO}_3 + (\text{NH}_2)_2\text{CO}$ was complete (102%) after 2 weeks incubation (Table 8). With both band-applications, nitrite was not detected during incubation.

Recovery of 11.5 mg (100 kg N/ha) KNO_2 -N in the presence of 11.5 mg $(\text{NH}_2)_2\text{CO}$ -N

The recovery of fertilizer in soil banded with KNO_2 -N was very

TABLE 8. CONCENTRATION OF IONIC SPECIES OF NITROGEN AND PERCENT RECOVERY AS TOTAL INORGANIC NITROGEN IN WELLWOOD I SOIL OF BAND-APPLIED KNO_3 , KNO_2 IN THE PRESENCE OF $(\text{NH}_2)_2\text{CO}$

(Mg N/110 g soil) Treatments	Time (wks)	N conc. ($\mu\text{g N}/12 \text{ g soil}$)				% Rec.
		NH_4	NO_2	NO_3	Total Inorg. N	
11.5 mg KNO_3 -N	1	87	0	1156	1243	97.8
	2	0	0	1037	1037	81.6
11.5 mg KNO_3 -N + 11.5 mg $(\text{NH}_2)_2\text{CO}$ -N	1	1257	0	1168	2425	95.4
	2	931	0	1659	2590	102.0
11.5 mg KNO_2 -N	1	555	57.6	-298	314	24.7
	2	756	0	-374	382	30.0
11.5 mg KNO_2 -N + 11.5 mg $(\text{NH}_2)_2\text{CO}$ -N	1	1720	144	-212	1653	65.0
	2	1952	4.6	-286	1670	65.7

low after 1 and 2 weeks incubation (Table 8), amounting to 25 and 30%, respectively. In the presence of urea, the recovery of $(\text{KNO}_2 + (\text{NH}_2)_2\text{CO})\text{-N}$ as total inorganic nitrogen (NH_4^+ , NO_2^- and NO_3^- -N) was also far from complete (65%) after the same incubation periods (Table 8). However if one assumes complete recovery of urea the loss of nitrogen from KNO_2 applied with urea was the same as where it was applied alone. This indicates that the presence of $(\text{NH}_2)_2\text{CO}$ did not affect the recovery of nitrite. Where KNO_2 was applied, there was an increase in NH_4^+ -N as compared to the untreated soil. However the increase in NH_4^+ -N did not correspond quantitatively with the decrease in NO_2^- -N. This together with the fact there was a reduction in total mineral N indicates that there was a loss of nitrite by denitrification. If the decrease in NO_3^- and NO_2^- was caused by microbiological activity, the addition of urea could have prevented this due to its effect on osmotic pressure and NH_4^+ concentration. If, however, the decrease was chemical, the addition of urea should have no effect as long as urea did not participate in the reaction. Therefore it seems likely that NO_3^- -N was decreased by a biological process, such as assimilation, since the addition of urea prevented this decrease. However, the disappearance of NO_2^- -N seems to be caused by a chemical reaction since the presence of urea did not alter the fate of applied nitrite.

Summary

From the above experimental investigation, it was concluded that

- 1) There was loss of inorganic nitrogen when nitrite was present although its mechanism was not known.

- 2) The disappearance of nitrite from the soil was not enhanced by the presence of urea and its probable product, NH_4^+ . This suggests that nitrite did not combine with urea or its product, NH_4^+ , a reaction commonly known as "Van Slyke type", to form elemental nitrogen. (Mechanism of nitrite disappearance will be investigated in Experiment VI).
- 3) Addition of a large quantity of KNO_2 to the soil induced the accumulation of the ammonium form of nitrogen. The source of NH_4^+ is not fully understood.

Experiment VI. A mass-spectrometric investigation on denitrification from nitrite-treated soil

Previous experiments indicated that recovery of mineral nitrogen was incomplete. This disappearance could have been due to immobilization or denitrification. An experiment was conducted using nitrogen-15 as a tracer to determine whether nitrogen unaccounted for could be recovered as N containing gases.

An example of mass-spectrometric scanning charts for air and for gas samples from Na¹⁵NO₂ labelled soil are shown in Figure 38. Peak heights were measured to represent the qualitative values of the different mass to charge ratios (m/e). The m/e of the possible denitrification products and some natural gases are shown in Table 9.

TABLE 9. MASS TO CHARGE RATIO (m/e) OF SOME GASES

Gases	m/e
N ₂	28, 29, 30
N ₂ O	44, 45, 46
NO	30, 31
NO ₂	46, 47
A	40
CO ₂	44
O ₂	32

The gas sample analyzed by the mass spectrometer is a mixture of the original atmosphere and the probably products of chemical or biological processes taking place in the soil. In order to deduce the relative magnitude of the products in the mixture the following cal-

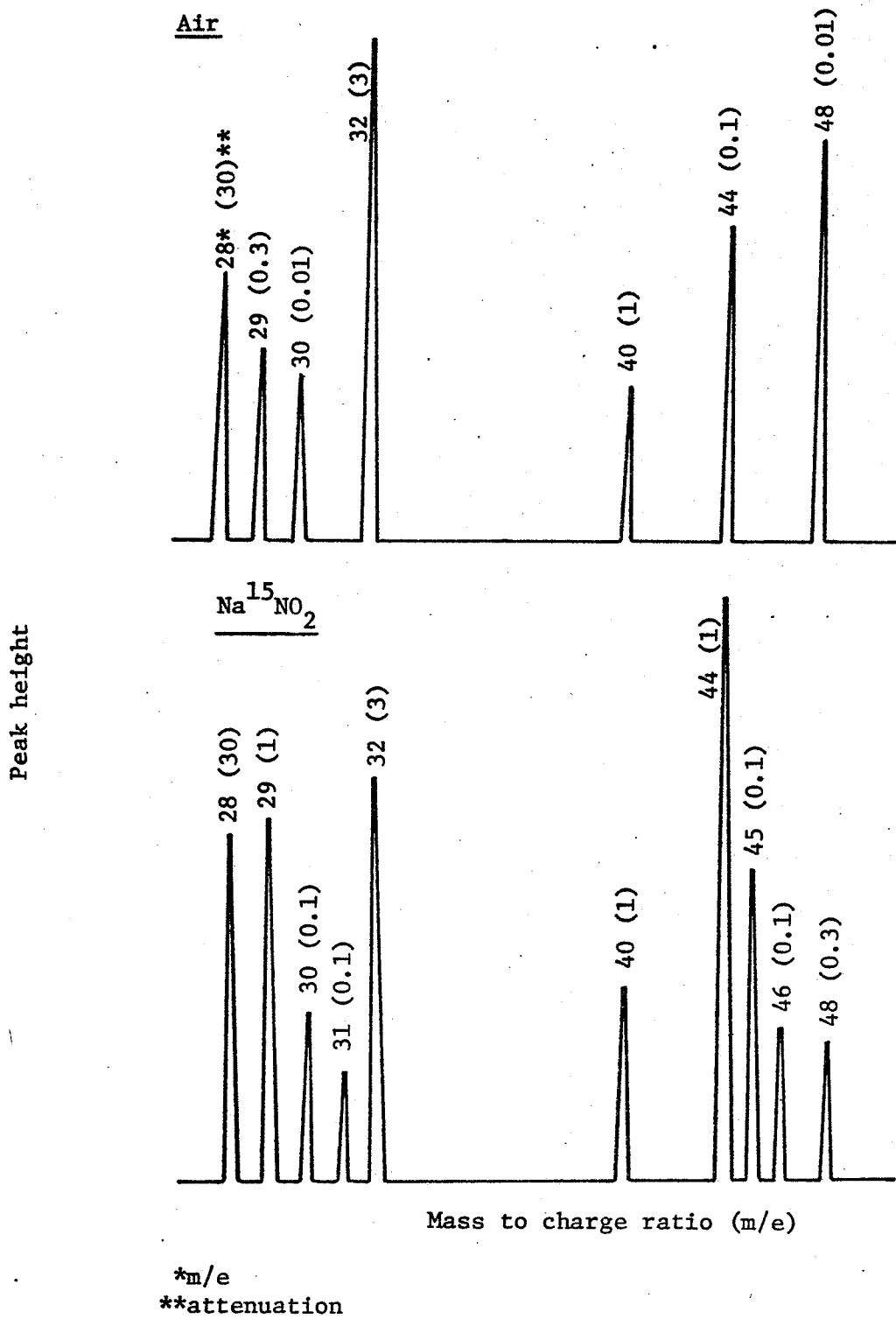


FIG. 38. THE MASS SPECTROMETRIC SCAN OF AIR AND GAS SAMPLES FROM Na¹⁵NO₂ TREATED SOIL.

culations were carried out. The peak height of m/e 28 of an air sample was considered to be 100 and all the other measured peak heights of air were converted to this scale. Since argon (A) in air is known to be neither assimilated nor produced, the measured peak heights from all the samples were recalculated based on an argon peak height identical to that of the air sample in which the peak height of m/e 28 was chosen to be 100. In these calculations the values of argon (40) peak were chosen to 1.963 for nitrogen-15 and its air sample, and 1.944 for non-labelled nitrogen and its air sample. The difference in the value of the argon peak height of the two air samples when m/e 28 was chosen to be 100 may have been due to a slight shift in the operational conditions of the mass spectrometer at different dates of measurement. The results shown in Tables 10 and 11 are the average of four replicates. The method of calculating N_2 and other nitrogenous compounds produced from denitrification utilizing argon (A) as a standard in an aerobic system has not been used by other workers studying the denitrification products using nitrogen-15.

The relative quantities of the various components, either produced or consumed during incubation, are calculated by subtracting the quantities found in the air sample from those found in the gas sample from incubation (4th column of Tables 10, 11). The mass to charge ratio (m/e) 28 in the gas sample obtained after incubation from the soil which received unlabelled N was higher than that of the air sample. The increase in $^{14}N^{14}N$ during incubation was due to the production of $^{14}N^{14}N$ from soil and/or applied N fertilizer (this will be discussed later). The quantity of $^{14}N^{14}N$ produced in the nitrogen-15

TABLE 10. POSSIBLE GASEOUS PRODUCTS HAVING m/e 28 THROUGH 48
OBTAINED DURING INCUBATION OF WELLWOOD I SOIL TO WHICH
NITROGEN-15 LABELLED $\text{Na}^{15}\text{NO}_2$ WAS APPLIED

m/e	Normalized peak heights		Difference*	Probable components	Symbols
	$\text{Na}^{15}\text{NO}_2$	Air			
28	103.68	100	3.68	$^{14}\text{N}^{14}\text{N}$	(28) _T
29	3.707	0.739	2.968	$^{14}\text{N}^{15}\text{N}$	(29) _T
30	0.176	0.0165	0.159	$^{15}\text{N}^{15}\text{N}, ^{14}\text{NO}$	(30) _T
31	0.117	-	0.117	^{15}NO	(31) _T
32	11.839	19.399	-7.560	O_2	
40	1.963	1.963	-	A	
44	6.206	0.347	5.857	$\text{CO}_2, ^{14}\text{N}^{14}\text{NO}$	
45	0.332	-	0.332	$^{14}\text{N}^{15}\text{NO}$	(45) _T
46	0.160	-	0.160	$^{15}\text{N}^{15}\text{NO}, ^{14}\text{NO}_2$	(46) _T
47	-	-	-	-	
48	0.0425	0.0811	-0.0386	$\text{O}_3(?)$	

*Difference = $\text{Na}^{15}\text{NO}_2$ treated sample - air sample

TABLE 11. POSSIBLE GASEOUS PRODUCTS HAVING m/e 28 THROUGH 48 OBTAINED DURING INCUBATION OF WELLWOOD I. SOIL TO WHICH NON-LABELLED NaNO_2 WAS APPLIED

m/e	Normalized peak heights		Difference*	Probably component	Symbols
	NaNO_2	Air			
28	105.05	100	5.05	$^{14}\text{N}^{14}\text{N}$	$(28)_\text{N}$
29	0.783	0.745	0.038	$^{14}\text{N}^{15}\text{N}$	$(29)_\text{N}$
30	0.248	0.0188	0.229	^{14}NO , $^{15}\text{N}^{15}\text{N}$ (trace)	$(30)_\text{N}$
31	-	-	-	-	
32	10.927	19.184	-8.256	O_2	
40	1.944	1.944	-	A	
44	6.531	0.382	6.149	CO_2 , $^{14}\text{N}^{14}\text{NO}$	
45	0.0754	0.0062	0.0692	$^{14}\text{N}^{15}\text{NO}$	$(45)_\text{N}$
46	0.0263	0.0032	0.0231	$^{14}\text{NO}_2$, $^{15}\text{N}^{15}\text{NO}$ (trace)	$(46)_\text{N}$
47	-	-	-	-	
48	0.0334	0.0543	-0.0209		

*Difference = NaNO_2 treated soil sample - air sample.

labelled sample was lower than that of non-labelled. However, only a portion of the N_2 produced from the nitrogen-15 labelled sample was of $^{14}N^{14}N$ (m/e 28), and the remainder was of $^{14}N^{15}N$ (29) and $^{15}N^{15}N$ (30). The m/e 29 produced from the labelled sample was excessively high as compared to that of air sample and the sample from non-labelled soil. Data in Tables 10 and 11 also showed that the microbial activity was quite high in the soil since approximately 40% of the original O_2 (32) was consumed during 1 week incubation. The increase in m/e 44 from the soil samples indicates that CO_2 was produced. Some, but not necessarily all, of the increase was due to CO_2 (44) alone since N_2O (44) may also have been produced. The m/e of 48 was observed in both air and gas samples. In the air sample which consisted of a larger quantity of O_2 (32), the peak height measurement of m/e 48 was larger. Therefore m/e 48 was independent of the sample, either air or soil gas samples, and was dependent on the oxygen level in the sample. Mass to charge ratio of 48 was believed to be due to ozone (O_3 has m/e of 48) which was produced within the mass spectrometer during air and gas analysis.

The following calculations were carried out to ascertain the kinds and relative amounts of the gases actually present (refer to Tables 10 and 11 for symbols):

1) Elemental nitrogen (N_2)

Firstly the atom percent ^{15}N in N_2 gas produced from both non-labelled and labelled $NaNO_2$ treated soil was calculated using the equilibrium assumption. According to this assumption, the equation

$$\text{Atom \% } ^{15}N = \frac{100}{2R + 1}$$

where $R = 28/29$. The calculations show that

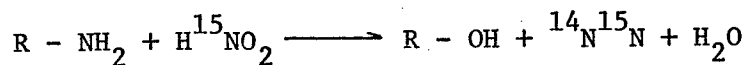
$$\text{Atom } \%^{15}\text{N of non-labelled sample} = 0.374$$

$$\text{Atom } \%^{15}\text{N of labelled sample} = 28.73$$

The value of 0.374 for the non-labelled sample is amazingly close to 0.367, the atom $\%^{15}\text{N}$ in natural air, even though a number of calculations were involved in obtaining the small quantities of N_2 produced from the NaNO_2 treated soil in the presence of a large quantity of atmospheric N_2 . With the ^{15}N labelled sample, the calculated atom $\%^{15}\text{N}$ is 28.730 which is very much smaller than the atom $\%^{15}\text{N}$ of the $\text{Na}^{15}\text{NO}_2$ added to the soil (50 atom $\%^{15}\text{N}$). A part of the N_2 present in the gaseous product resulted from sources other than $^{15}\text{NO}_2\text{-N}$. If the atom $\%^{15}\text{N}$ based on m/e 28 and 29 was 28.73%, then m/e 30 due to $^{15}\text{N}^{15}\text{N}$, $(30)_{\text{TN}_2}$, should be

$$(30)_{\text{TN}_2} = \frac{2(\text{atom } \%^{15}\text{N})(28 + 29) - 100(29)}{2(100 - \text{atom } \%^{15}\text{N})} = 0.5977$$

However, the measured m/e 30 in the labelled sample had a value of 0.159. The measured value of 0.159 should include some nitric oxide (NO). The presence of NO (30) gas in the labelled NaNO_2 treated-sample is evidenced by the appearance of m/e of 31 (Table 10). Thus it is concluded that the mechanism of N_2 gas formation in this experiment was such that little or no $^{15}\text{N}^{15}\text{N}$ was formed. Instead it is probable that one atom from the labelled source and another from the natural sources combined to form an N_2 molecule. A van Slyke reaction, for example,



is a reaction where one atom from each of two different nitrogen sources combine to form a molecule of nitrogen gas. If the above reaction is

the principal mechanism for N_2 evolution, then the resultant atom $\%^{15}N$ in the N_2 gas should be

$$\text{Atom } \%^{15}N = \frac{100 (29)_T}{2 [(28)_T + (29)_T]} = 22.32\%$$

If N_2 gas was only produced by the van Slyke reaction, then one of the N atoms in N_2 was contributed by N from $^{15}NO_2^-$ and the other N atom was due to R - $^{14}NH_2$, resulting in an atom $\%^{15}N$ in $^{14}N^{15}N$ of approximately 25% when the original $Na^{15}NO_2$ has an atom $\%^{15}N$ of 50%. The calculated atom $\%^{15}N$ of 22.32% seems to indicate that the N_2 gas produced was mainly derived by a reaction giving a 50% dilution of the applied ^{15}N . The soil pH was 6.4 throughout the incubation period, a value higher than the accepted value of pH 5.0 above which the van Slyke reaction does not occur (Broadbent and Clark, 1965).

2) Nitric oxide (NO)

The presence of m/e 31 in ^{15}N labelled gas sample indicates that NO was produced. It also means that m/e 30 consists of both ^{14}NO and $^{15}N^{15}N$. Since m/e 31 had a value of 0.117, then m/e 30 due to NO, $(30)_{TNO}$, should also be 0.117 if NO was derived from $Na^{15}NO_2$ of 50 atom $\%^{15}N$. If one assumes that m/e 30 in the labelled sample was mostly due to ^{14}NO , the atom $\%^{15}N$ in NO becomes

$$\text{Atom } \%^{15}N \text{ in NO} = \frac{(31)_T (100)}{(30)_T + (31)_T} = 42.39\%$$

where $(31)_T = 0.117$ and $(30)_T = 0.159$.

This value of atom $\%^{15}N$ in NO is the minimum value and may be higher, depending upon the relative abundance of $^{15}N^{15}N$ in the measured m/e 30 in the labelled sample. The calculated value of 42.39% indicates

that NO was produced mainly from $\text{Na}^{15}\text{NO}_2$ having atom % ^{15}N of 50%.

3) Nitrous oxide (N_2O) and nitrogen dioxide (NO_2)

The mass to charge ratio of 44 contained both CO_2 and $^{14}\text{N}^{14}\text{NO}$, therefore it could not be used for calculation of atom % ^{15}N of N_2O .

Mass to charge ratios of 45 and 46 were used instead. If one assumes that NO_2 was derived solely from NaNO_2 in non-labelled samples, m/e 46 due to NO_2 in the labelled samples, $(46)_{\text{TNO}_2}$, should be $(46)_{\text{N}}/2$. If this assumption is correct, then m/e 46 in the labelled sample due to N_2O , $(46)_{\text{TN}_2\text{O}}$, should be

$$(46)_{\text{T}} - (46)_{\text{N}}/2 = 0.148$$

Since m/e 45 was present in the non-labelled sample, it might contain an unknown which should be deducted from m/e 45 in the non-labelled sample in order to calculate the atom % ^{15}N by the following equation:

$$\text{Atom \% } ^{15}\text{N of } \text{N}_2\text{O} = \frac{200}{2 + [(45)_{\text{TN}_2\text{O}} / (46)_{\text{TN}_2\text{O}}]} = 53.046\%$$

where $(45)_{\text{TN}_2\text{O}} = (45)_{\text{T}} - (45)_{\text{N}} = 0.262$ and $(46)_{\text{TN}_2\text{O}} = 0.148$

The calculated atom % ^{15}N of N_2O almost equals the atom % ^{15}N of $\text{Na}^{15}\text{NO}_2$ uniformly mixed in the soil. This also suggests that N_2O is probably derived solely from the applied $\text{Na}^{15}\text{NO}_2$.

Summary

From the above observation, it was concluded that incomplete recovery of fertilizer N during incubation may be due to denitrification. It was observed that N_2 , NO and N_2O were produced when 1,000 ppm $\text{NaNO}_2\text{-N}$ was mixed uniformly with Wellwood I soil (pH 6.0). Nitrogen

dioxide (NO_2), if produced, was present in very small amounts. Most of the N_2 produced was generated from soil organic matter through the reaction of nitrite with an amino compound, a van Slyke type reaction, although the soil pH was above pH 5. The majority of the NO and N_2O was derived directly from NaNO_2 .

Experiment VII. Recovery of band-applied $(^{15}\text{NH}_2)_2\text{CO}$, $^{15}\text{NH}_4\text{OH}$ and $(^{15}\text{NH}_4)_2\text{SO}_4$ from Wellwood II soil as measured by the nitrogen-15 tracer technique

In earlier experiments it was found that some of the applied nitrogen was not recovered as inorganic forms, NH_4^+ , NO_2^- and NO_3^- . It is not known whether this N was converted to gaseous forms or assimilated by microorganisms. To ascertain the causes of this decrease in inorganic nitrogen, nitrogen-15 labelled substances were used.

Total nitrogen contents and the atom % ^{15}N of the Wellwood II soil to which 23 mg N (200 kg N/ha) as $(\text{NH}_2)_2\text{CO}$, NH_4OH and $(\text{NH}_4)_2\text{SO}_4$ had been added were determined at zero time and after 1 and 4 weeks incubation. The data obtained were used to calculate the recovery of added nitrogen (Table 12).

The calculations were as follows:

$$\%^{15}\text{N} = \frac{100}{2R + 1} \quad \text{where } R = 28/29$$

$$\text{Total } ^{15}\text{N} = [(\%^{15}\text{N}) (\%N) (110)] / 10^4 \quad (\text{in grams})$$

$$\text{Fertilizer N remaining} = (100) (\text{total } ^{15}\text{N}) / (\%^{15}\text{N of the added fertilizer}).$$

$$\% \text{ Recovery} = (\text{Fertilizer N remaining} / \text{Fertilizer N added}) (100)$$

The recovery of added nitrogen from the total nitrogen fraction (total organic N + total inorganic N) decreased with increased incubation time from 0 to 4 weeks. There was a loss of 6.0%, 7.7% and 3.8% of added N from $(\text{NH}_2)_2\text{CO}$, NH_4OH and $(\text{NH}_4)_2\text{SO}_4$, respectively after 4 weeks incubation. This was due to gaseous loss. The recovery of fertilizer N from NH_4OH before incubation was 72.6% indicating there was a loss of N as NH_3 before and during injection of NH_4OH into the soil column.

Total inorganic nitrogen contents were determined. From these

TABLE 12. PERCENT RECOVERY OF TOTAL NITROGEN (INORGANIC + ORGANIC FRACTIONS) FROM NITROGEN-15 LABELLED N-CARRIERS BANDED IN WELLWOOD II SOIL (RATE = 200 kg N/ha)

Time (Weeks)	% ^{15}N	% N	Total ^{15}N (g)	Fertilizer N remaining (g)	Recovery (%)
$(^{15}\text{NH}_2)_2\text{CO}$ (29.7 atom % ^{15}N)					
0	1.624*	0.320	0.00572	0.0230	100
1	1.692	0.345	0.00642	0.0216	94
4	1.696	0.344	0.00642	0.0216	94
$^{15}\text{NH}_4\text{OH}$ (52.20 atom % ^{15}N)					
0	2.541	0.312	0.00872	0.0167	72.6
1	2.386	0.330	0.00866	0.0166	72.1
4	2.072	0.342	0.00779	0.0149	64.9
$(^{15}\text{NH}_4)_2\text{SO}_4$ (52.20 atom % ^{15}N)					
0	3.366	0.327	0.01211	0.0232	100.8
1	3.221	0.324	0.01480	0.0220	95.6
4	3.024	0.350	0.01164	0.0223	97.0

*Atom % ^{15}N of $(\text{NH}_2)_2\text{CO}$ used was 24.79% not 29.70%.

data, the ^{15}N percentage and the amount of applied nitrogen recovered at zero time and after 1 and 4 weeks incubation were calculated (Table 13). The recovery of N before incubation was incomplete with urea and NH_4OH . Explanation for the incomplete recovery of NH_4OH was given earlier. The recovery of $(\text{NH}_2)_2\text{CO-N}$ was only 55% of the added fertilizer at zero time, this was probably the result of the incomplete hydrolysis of urea to NH_4^+ . The recovery of fertilizer N from 3 carriers calculated from the total inorganic fraction was also incomplete after an incubation period of 1 to 4 weeks. Nitrogen immobilized during incubation was obtained by subtracting total inorganic nitrogen from total nitrogen (organic plus inorganic). Table 14 shows the percentage of fertilizer N immobilized during incubation. The recovery of NH_4OH was corrected to 100% based on the value of 72.6% at zero time. The greatest immobilization of N occurred with band-applied $(\text{NH}_2)_2\text{CO}$. It amounted to 20% after 4 weeks incubation. Soil banded with NH_4OH immobilized the smallest percentage of applied nitrogen.

Summary

From the nitrogen-15 study, it is concluded that the incomplete recovery of N from urea was largely due to immobilization rather than gaseous loss although a large quantity of nitrite was produced and accumulated during the 4 weeks incubation. With NH_4OH band-applied soil, gaseous loss was the main cause of low nitrogen recovery after 4 weeks incubation. More than 20% of the $\text{NH}_4\text{OH-N}$ was lost as NH_3 before and during injection. Immobilization and gaseous loss also occurred with band-applied $(\text{NH}_4)_2\text{SO}_4$ during incubation, but the magnitude was smaller than with urea. The order of N loss before and

TABLE 13. PERCENT RECOVERY OF TOTAL INORGANIC NITROGEN FROM NITROGEN-15 LABELLED N CARRIERS BANDED IN WELLWOOD II SOIL (RATE = 200 kg N/ha).

Time (Weeks)	% ^{15}N	% N	Total % ^{15}N	Fertilizer N remaining (g)	Recovery (%)
$(^{15}\text{NH}_2)_2\text{CO}$ (29.7 atom % ^{15}N)					
0	17.112*	0.0168	0.00316	0.0127	55.2
1	18.259	0.0294	0.00590	0.0199	86.4
4	17.469	0.0263	0.00505	0.0170	74.0
$^{15}\text{NH}_4\text{OH}$ (52.20 atom % ^{15}N)					
0	30.851	0.0236	0.00800	0.0153	66.7
1	29.023	0.0267	0.00852	0.0163	71.0
4	27.248	0.0252	0.00755	0.0145	62.9
$(^{15}\text{NH}_4)_2\text{SO}_4$ (52.20 atom % ^{15}N)					
0	36.639	0.0306	0.0123	0.0236	102.6
1	33.288	0.0324	0.0119	0.0227	98.8
4	32.154	0.0302	0.0107	0.0205	89.0

*Atom % ^{15}N of urea used was 24.79% not 29.70%.

TABLE 14. PERCENT N LOSS BY IMMOBILIZATION AND VOLATILIZATION FROM THREE NITROGENOUS MATERIALS BANDED IN WELLWOOD II SOIL (RATE = 200 kg N/ha).

Time (Weeks)	Recovery (%)		Loss %		Total Loss
	Total N	Inorganic N	Immobilization*	Gaseous**	
$(\text{NH}_2)_2\text{CO}$					
0	100	55.2	?		
1	94	86.4	7.6	6.0	13.6
4	94	74.0	20.0	6.0	26.0
NH_4OH					
0	100	100	0		
1	99.3	100	0	0.7	0.7
4	89.4	88.6	0.8	10.6	11.4
$(\text{NH}_4)_2\text{SO}_4$					
0	100.8	102.6	0		
1	95.6	98.8	0	4.2	4.2
4	97.0	89.0	8.0	3.8	11.8

*% Immobilized = (% Rec) Total N - (% Rec) Inorganic N.

**% Gaseous loss = $(\text{Total N})_{t=0} - (\text{Total N})_{t=1, 4}$

during incubation with the 3 nitrogen fertilizers was $\text{NH}_4\text{OH} > (\text{NH}_2)_2\text{CO} > (\text{NH}_4)_2\text{SO}_4$ if NH_3 volatilization at zero time was included as part of N loss with NH_4OH . The loss during incubation was in the order $(\text{NH}_2)_2\text{CO} > \text{NH}_4\text{OH} = (\text{NH}_4)_2\text{SO}_4$.

Experiment VIII. Nitrification of urea in soil suspensions and the nitrifier populations

Experimental results previously obtained indicated that banded fertilizer materials were transformed and transported from the zone of application at different rates depending upon the soil characteristics. There were also differences in the nitrifying capacity of the soils. To obtain more information on the nitrifying capacity of the soils it was decided to study them under a homogeneously mixed system.

The incubation of urea solution with acidic Keld soil resulted in an increased suspension pH with time while the pH decreased with time in the case of neutral Wellwood II soil (Table 15). The pH of Wellwood II soil decreased approximately 1-2.5 unit after 2 weeks incubation and remained constant thereafter. However, the magnitude of the decrease of soil pH in the Morton soil suspension during the incubation was very much smaller than that of the Wellwood II soil (Table 15). This was probably due to the high carbonate content in the Morton soil.

With the Keld soil, urea was hydrolyzed slowly to $(\text{NH}_4)_2\text{CO}_3$ which resulted in a steady increase in solution pH during the incubation (Fig. 39). Nitrite was not detected and very little or no nitrate was produced. From the observation, it was believed that urea hydrolysis was incomplete in four weeks with 75 and 100 ppm $(\text{NH}_2)_2\text{CO-N}$. This belief was substantiated by results on the recovery of total inorganic nitrogen. The hydrolysis of 25 and 50 ppm urea-N was almost complete since the NH_4^+ concentration began to level off at 4 weeks. Coupled with the fact that little oxidation had occurred, it strongly indicated that only urea hydrolysis had taken place in urea solutions containing 0.5 g Keld soil. Urea content was not

TABLE 15. pH OF SOIL SUSPENSION TREATED WITH $(\text{NH}_2)_2\text{CO}$ AFTER ONE TO FOUR WEEKS INCUBATION

$(\text{NH}_2)_2\text{CO}$ (ppm N)	Keld				Wellwood II				Morton			
	1 wk	2 wk	3 wk	4 wk	1 wk	2 wk	3 wk	4 wk	1 wk	2 wk	3 wk	4 wk
0	5.5	5.6	5.6	5.6	6.6	6.5	6.4	6.2	8.2	7.8	7.8	7.8
25	6.0	6.1	6.3	6.4	7.0	6.2	5.4	5.4	8.3	7.8	7.6	8.0
50	6.3	6.6	6.7	6.9	7.3	5.3	4.3	4.5	8.4	7.7	7.3	8.0
75	6.5	6.6	6.8	7.6	7.4	5.0	5.1	5.2	8.5	7.9	7.1	7.5
100	6.5	6.5	7.0	7.6	7.6	5.0	5.2	5.2	8.6	8.2	8.0	7.9