A STUDY OF THE QUANTITY OF NITROGEN MINERALIZED DURING THE GROWING SEASON, ITS EFFECT ON CROP GROWTH, AND FACTORS AFFECTING THE NITROGEN MINERALIZATION - IMMOBILIZATION RELATIONSHIP.

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

In Partial Fulfillment
of the Requirements for the Degree
of Master of Science in Agriculture

by
William Bruce McGill
May 1969

ACKNOWLEDGEMENTS

The writer wishes to express his indebtedness to Dr. R. J. Soper, Associate Professor, Department of Soil Science, University of Manitoba, under whose supervision this investigiation was conducted, for helpful suggestions and criticism of the manuscript.

The writer also wishes to express his appreciation to the National Research Council for financial assistance.

Table of Contents

List of Tables

List of Figures

ABSTRACT

Net nitrogen mineralization was measured in seeded and fallow portions of four plots on two Manitoba soils during the summer of 1967. The main flush in net nitrogen mineralization occured during July and August. Nitrogen uptake by the wheat crop was greatest during June and July. Mineralized nitrogen was important in completing the growth and development of the crop but did not appear to control yield.

Net nitrogen mineralization in the fallow portions of the plots exceeded that in the seeded portions. Twenty-five pounds of nitrogen per acre were mineralized in the seeded portion of three plots. Mineralized nitrogen in the seeded portion of the fourth plot totalled sixteen pounds per acre. In the fallow portions, the net quantity of nitrogen mineralized was equivalent to 1.7 to 2.0 percent of the organic nitrogen in the top six inches of soil.

Results of a greenhouse experiment indicated that yield reductions due to straw amendments to soil were greater at 60°F. than at 75°F. This was attributed to greater efficiency of nitrogen utilization at the lower temperature. Nitrification, relative to immobilization, appeared to be greater at 75°F. than at 60°F.

Chapter I

INTRODUCTION

A growing cereal crop obtains almost all of its nitrogen from soluble mineral nitrogen stored in the soil at seeding, from nitrogen mineralized during the growing season, or from fertilizer nitrogen. Nitrogen added in rainfall or irrigation water, absorbed from the atmosphere, or supplied through fixation processes accounts for only a minor portion of the nitrogen required by a growing crop(10,83).

The quantity of nitrogen mineralized during the growing season is generally considered to be the major factor in controlling the quantity of fertilizer nitrogen required (30, 42, 43, 44). However, research in Manitoba, in connection with the development of a comprehensive soil testing program, has indicated that at least 50 per cent of the variation in cereal yield can be accounted for on the basis of nitrate-nitrogen in the soil at seeding time. Soper(73)in 1960 observed that the available nitrogen at seeding time was a major factor in controlling the response of barley to nitrogen fertilizer. He also reported that the initial mineral nitrogen content of the soils he studied was a better criterion for predicting nitrogen requirements than were incubation methods. Ferguson[1], in summarizing studies conducted at Brandon, Manitoba from 1954 to 1963, observed a strong correlation between the nitrate-nitrogen content of the soil at seeding time and nitrogen uptake by cereals. Young et al. (89) observed a better correlation between the quantity of nitrate-nitrogen to two feet at seeding time and response to fertilizer nitrogen than between response to nitrogen fertilizer and the quantity of notrogen mineralized during laboratory incubation. These reports indicate that the quantity of nitrogen mineralized during the crop year is probably of only minor importance in determining yield response to nitrogen fertilizer. However, it was not possible, on the basis of reports in the literature, to assess the relative importance of the quantity of available mineral nitrogen at seeding time and the quantity of nitrogen mineralized during the growing season in determining response to nitrogen fertilizer. It was suggested by Ferguson[1] that the quantity of nitrogen mineralized during the growing season may possibly be related to the quantity of stored mineral nitrogen and therefore determination of the quantity of mineral nitrogen available at seeding time would result in a measure of the relative quantity of nitrogen mineralized during the growing season. He also suggested that the quantity of nitrogen mineralized may be constant among soils and years.

---

[1] Data presented at the seventh annual Manitoba Soil Science Meeting , 1963.

Without a direct measurement of the quantity of nitrogen mineralized during the growing season it was not possible to determine which of the above two explanations, if either, was correct.

The present study was initiated in an attempt to determine the relative importance of the quantity of nitrogen mineralized during the growing season and the quantity of nitrogen available at seeding time in controlling yield and nitrogen uptake. This investigation consisted of a field study during the summer and fall of 1967 in which the quantities of nitrogen mineralized at various dates during the growing season and the quantities of mineral nitrogen available at seeding time were measured. The quantities of nitrogen mineralized between seeding and the various sampling dates were compared with nitrogen uptake during the same period. During the winter of 1967-1968 a greenhouse investigation was conducted in an attempt to determine the factors affecting the quantity of nitrogen mineralized and made available to the growing crop.

Chapter II

LITERATURE REVIEW

Nitrogen in soil is largely in organic combination. However, in some soils considerable quantities of nitrogen may be in the form of unexchangeably fixed ammonium-nitrogen in the clay mineral fraction of the soil (19,59). Plants, however, require nitrogen in the mineral form as either nitrate or ammonium ions. The biological conversion of nitrogen from the organic form to the mineral form is termed nitrogen mineralization and renders the nitrogen mobile, available to plants, and vulnerable to large losses (15). Organic material in the soil undergoes decomposition by soil micro-organisms. The carbon in the substrate serves as an energy source and the nitrogen contained therein is utilized in the synthesis of proteins. Some materials contain nitrogen in excess of that required by the organisms, resulting in the excretion of nitrogen as waste in the form of ammonia. The nitrogen contained in other materials, relative to the quantity of carbon, is insufficient to meet the requirements of the microorganisms. This often results in the assimilation of mineral nitrogen from the soil by heterotrophic bacteria capable of utilizing this form of nitrogen in protein synthesis. This microbiological conversion of nitrogen from the mineral form to the organic form will herein be termed nitrogen immobilization. The quantity of nitrogen mineralized or immobilized depends on the relative proportion of carbon and nitrogen in the substrate material, the availability of these elements to microbial attacks, environmental factors, and the length of time that the organisms are operating on the material.

Plants growing in a soil have a pronounced effect on the numbers and activities of bacteria in the soil in the immediate vicinity of the root. Plant roots exude amino compounds and cell wall materials during growth (51, 67, 74). These materials serve as a readily available substrate material for the bacteria on and immediately adjacent to the root, resulting in an increased potential for both nitrogen mineralization and immobilization. Plants, therefore, through their removal of mineral nitrogen from the soil and their effect on microbial numbers and activities, influence the balance between the mineralization and immobilization of nitrogen.

The complete mineralization process consists of ammonification -- the production of NH_3 from organic nitrogen; and nitrification -- the oxidation of NH_3 to NO_3. The mineralization of nitrogen depends on factors such as total nitrogen content of the soil, C/N ratio, previous history of the soil, soil aggregate size, effect of partial sterilization of the soil and the accelerated rate of decomposition of stable humus due to the addition of easily decomposed residues supplying a readily available source of carbon and nitrogen to the organisms. Factors such as pH, temperature and moisture have varying effects on ammonification and nitrification. These factors will be considered separately. The rhizosphere effect on mineralization and immobilization will also be considered separately.

2.1 Internal Nitrogen Cycle

Jansson (see ref. 42) has proposed an internal nitrogen cycle (fig. 1) consisting of active (NH_3 and fresh organic residues) and passive fractions (NO_3 and humic N) in which conversions between organic and inorganic nitrogen proceed. Each revolution of the cycle reduces the carbon available to the microbial population since a considerable quantity (50 per cent) of the carbon is lost as CO_2 (9). Eventually an equilibrium is reached at which time no more mineral nitrogen is converted to organic nitrogen and inorganic nitrogen will tend to accumulate if there is still a sufficient quantity of carbon left to support bacterial development. The substrate material must contain carbon and nitrogen in a readily decomposable form, and losses of mineral nitrogen must not be great in order for the accumulation of mineral nitrogen to occur.

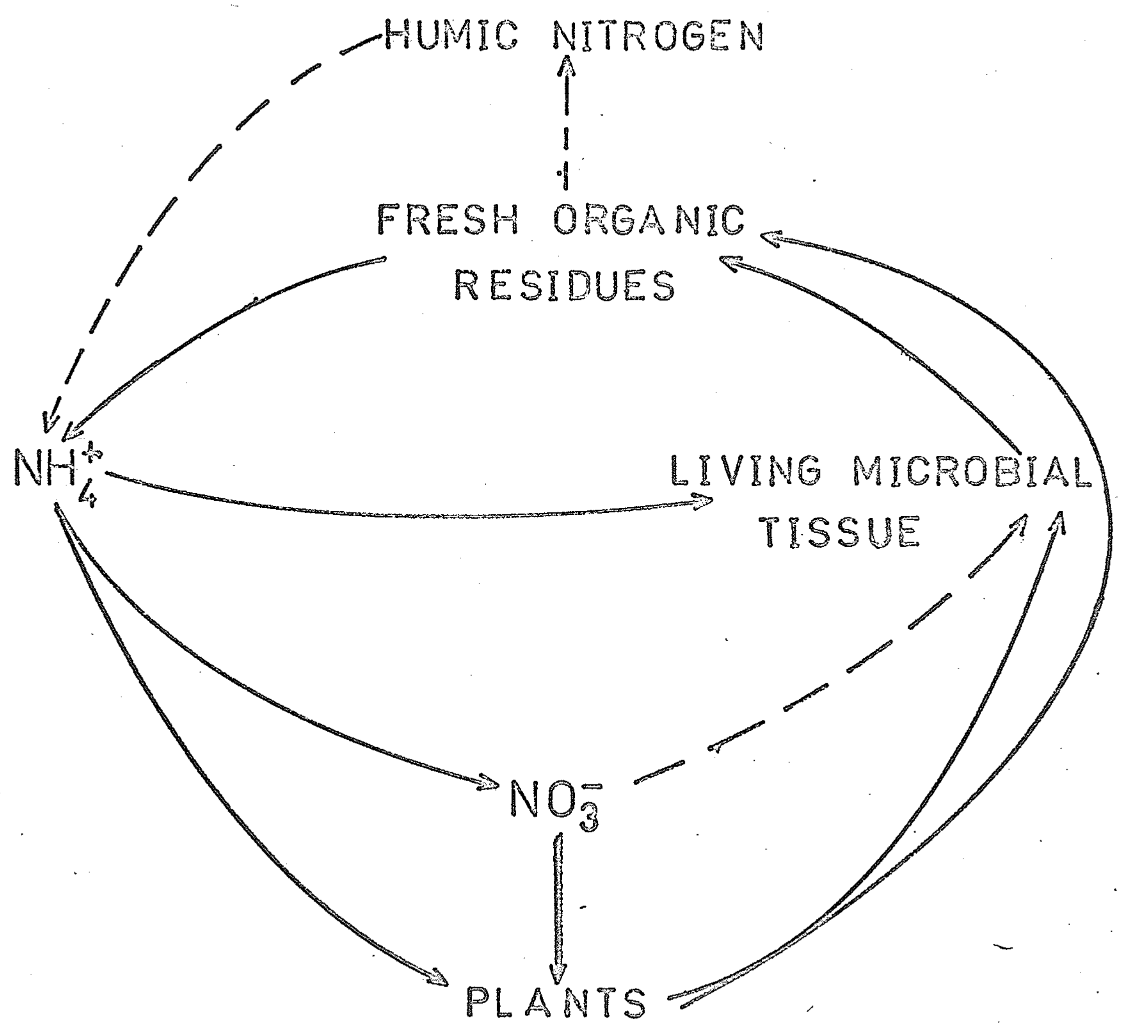


FIG. 1 INTERNAL NITROGEN CYCLE

2.2 Factors Affecting Mineralization

a) Total Nitrogen Content of Soil

The total quantity of nitrogen in a soil is determined by climate, vegetation, topography, parent material, and age. Stevenson (78) reports that climate is the most important single factor in controlling the total nitrogen content of virgin grassland soils. The total nitrogen content follows the Van't Hoff temperature rule and increases two or three times for every ten degree drop in temperature (78). Vegetation controls to some extent the quantity of nitrogen in a soil in that soils developed under legumes contain a greater quantity of nitrogen than soils developed under non leguminous plants (78). The total nitrogen content of soils developed under plants with an extensive root system is generally greater than in soils developed under plants with a more restricted root system. Topography controls the total nitrogen content of soil through its effect on climate, runoff, evaporation, and transpiration. Increasing age of a soil is generally associated with an increased total nitrogen content of the soil until an equilibrium is reached, at which point, no further increases in total nitrogen can be expected with increasing age (78).

Generally the quantity of nitrogen mineralized in a given period increases with an increase in the total quantity of organic nitrogen in the soil. It is estimated that in most arable soils between one per cent and ten per cent of the total nitrogen of a soil is mineralized per year, however, the mineralization of more than three per cent of the total organic nitrogen could be expected in only a very few soils (2, 15, 19). The quantity of nitrogen mineralized in a given time correlates very well with total nitrogen content (0.988) when soils are grouped according to total nitrogen content of the soil, and groups of soil compared. The correlation is very poor (0.368) when individual soils are considered (72). Therefore, factors other than total nitrogen also influence the quantity of nitrogen mineralized in a given period.

Many workers consider the quantity of nitrogen mineralized to be more intimately assoiciated with the characteristics of a portion of the total nitrogen than with all of it (8, 44, 52). An active fraction of the organic nitrogen of the soil has been postulated and it is felt that the quantity of nitrogen in this fraction determines the quantity of nitrogen mineralized. Attempts to characterize the mineralizeable nitrogen fraction in soils have met with only limited success. Keeney and Bremner (52) found that an index of soil nitrogen availability based solely on determination of hydrolysable and non hydrolysable nitrogen was not satisfactory. They also reported that the fraction of the organic nitrogen contributing most to the accumulation of mineral nitrogen during incubation varied with different soils.

Organic nitrogen in soils exhibits a remarkable stability toward microbial attack, resulting in the mineralization of only a limited quantity of nitrogen each year. Reasons for this stability are not immediately evident since the substances from which the soil organic matter is derived are relatively easily and quickly attacked (19). Considerable effort has been directed toward explaining this stability and several theories proposed. In a review by Bremner (19), it is reported that as early as 1892 Hebert and Déherain postulated a protien-lignin complex to account for this stability. This theory has been revived by several workers since then but does not appear to completely explain the phenomenon. Ensminger and Gieseking (33) postulated the adsorption of organic compounds by clay minerals. They found that the enzymatic hydrolysis of protein was inhibited by the presence of clay and that the degree of inhibition varied directly with the base exchange capacity of the clay. Ensminger and Gieseking (33) suggested that the inhibitory effect of clay was due to either absorption and inactivation of enzymes by clay, or the absorption of protein by clays orients it in such a way as to make it inaccessible to enzymatic attack.

A third theory is that the quantity of carbon available to the soil microflora is insufficient to supply the needs of an active microbial population (19, 44). Accelerated decomposition of consolidated humus has been reported due to the additions of fresh green manure (16, 44). Cropped land with its dense microbial population around the roots is reported to exhibit a more rapid breakdown of stable humus than uncropped soil (16, 44). This theory postulates that the stability of organic nitrogen in soil is more apparent than real and that additions of a readily available energy source results in accelerated decomposition of stable humus.

Probably no one theory adequately accounts for the stability (or apparent stability) of organic nitrogen in soil. Since there is ample evidence to support all the theories advanced it is likely that the stability of organic matter in soil is the result of a combination of several factors acting concurrently.

b) Carbon: Nitrogen Ratios

The microbial material in the soil has a characteristic ratio of carbon to nitrogen. A portion of the carbon in soil organic material is oxidized to CO_2 to supply energy for the metabolism of the microorganisms and a portion is utilized in the synthesis of cell wall material and other cellular components. The nitrogen contained in the organic substrate is utilized in the synthesis of proteins, nucleic acid, and other nitrogenous constituents of microbial cells. Since the enzymatic hydrolysis of the organic substrate releases carbon and nitrogen in definite proportions, and since carbon and nitrogen must be utilized in fixed proportions with a portion of the carbon escaping as CO_2, excess nitrogen in the substrate relative to the supply of carbon results in the exudation of nitrogen from the microbial bodies as a waste material (8). However, if the quantity of nitrogen in the substrate material, relative to the quantity of carbon were inadequate for the synthesis of microbial tissue of the proper C:N ratio, then either microbial activity would be curtailed or nitrogen in the mineral form would have to be utilized. It would be expected, therefore, that some critical ratio of carbon to nitrogen should exist, below which nitrogen would be exuded as a waste product and above which nitrogen would be removed from the pool of mineral nitrogen in the soil, i.e. net nitrogen mineralization should occur at C:N ratios below a critical level and nitrogen immobilization would be expected at C:N ratios above this level.

Critical C:N ratios have been established as a result of both experimental observations and theoretical calculations. Net nitrogen mineralization is generally considered to result from the microbial degradation of residues with a C:N ratio of less than 20. Between a C:N ratio of 20 and 30 nitrogen mineralization may or may not occur depending on environmental conditions and type of substrate. Residues with C:N ratios greater than 30 are generally considered to induce immobilization of nitrogen (8, 15, 29, 44).

Only if an equal percentage of the total carbon and nitrogen of a residue is available will the C:N ratio be a valid indication of the potential for either the mineralization or immobilization of nitrogen. If such is not the case then the C:N ratio as determined by total carbon and total nitrogen methods will not be equivalent to the effective ratio of C:N available to the microbial population. If the ratio of available carbon to available nitrogen is not equivalent to the ratio of total carbon to total nitrogen then the ratio of these elements calculated on the basis of their total quantities in an organic material will be meaningless. The critical C:N ratio also varies with qualitative variations in the soil microflora since the C:N ratios of different groups of organisms may be quite dissimilar. Alexander (4) states that "As a rule for mixed populations, 5 - 10% of substrate carbon is assimilated by bacteria, 30 - 40% by fungi, and 15 - 30% by actinomycetes. C:N ratios of 5:1, 10:1, and 5:1 may be proposed for the cellular components of bacteria, fungi, and actinomycetes respectively." It would be very dangerous, therefore, to over emphasise the importance of a single C:N ratio. Much more instructive is a range in the C:N ratio since several variables other than ratio of total carbon to total nitrogen tend to control or modify the process of nitrogen mineralization.

Carbon to nitrogen ratios are often translated into per cent nitrogen values due to the greater variations in per cent nitrogen than per cent carbon and the fact that total nitrogen determinations are more commonly performed than total carbon. As the total quantity of nitrogen in a residue added to soil increases the quantity of nitrogen mineralized is expected to increase. It is generally accepted that the critical range of total nitrogen is from 1.2 to 1.8 per cent of dry weight (27, 36, 44). Less than 1.2 per cent nitrogen in a residue is considered to result in either reduced decomposition, or immobilization of nitrogen and residues containing greater than 1.8 per cent nitrogen when added to soil are expected to cause an increase in the quantity of mineral nitrogen. These values are based on the assumption that most organic residues added to soil have a total carbon content of from 35 to 40 per cent of dry weight. The critical values cited for total nitrogen in a residue are subject to the same restrictions, such as relative availability of the carbon and nitrogen in the residue and type of microflora, as in the C:N ratio. It is therefore impossible to cite one C:N ratio or one total nitrogen percentage above or below which net mineralization of nitrogen will or will not occur. The above arguments indicate that several factors other than the C:N ratio or per cent nitrogen in a residue control the point at which net mineralization of nitrogen is observed. The accessibilty of the residue to microbial attack is very important. The organic matter of most agriculturally important soils in the temperature regions is considered to have a C:N ratio of from 10 to 12 (72). Mineralization of nitrogen from this source, however, is very slow due to its stability toward microbial attack. This further emphasizes the necessity of considering C:N ratios in conjuction with other factors such as type of material, environmental conditions and type of microbial population.

c) Previous Histroy of the Soil

The quantity of nitrogen mineralized during controlled incubation studies is reported to be markedly affected by conditions prevailing in the field at the time of sampling; cultivation, cropping, fertilization, and meteorological seasonal factors (44). Wide variations have also been reported in the mineralization capacity of soils between consecutive years due to differences in the climatic conditions. Virgin soils, when incubated, tend to mineralize a greater per cent of their total nitrogen than do cultivated soils. The same relationship is observed in the field between recently broken soils and those under cultivation for a considerable period of time. The relative per cent of the total nitrogen in the active fraction of newly broken soil is greater than in soils under cultivation for several years. The soil microflora act upon this readily decomposed source of nitrogen and mineralize large quantities of nitrogen each year thus depleting the total quantity of nitrogen in the soil. This eventually results in the predominance of the stable soil organic nitrogen and a slower release of mineral nitrogen by soil microorganisms. Gradually, the rate of nitrogen mineralization decreases until an equilibrium is reached, at which point, the nitrogen content of the soil no longer declines and the quantity of mineral nitrogen made available annually is equal to the quantity of the nitrogen added. It therefore becomes evident that the length of time a given soil has been cultivated influences the quantity of nitrogen mineralized each year (78).

Rapid flushes in nitrogen mineralization have been observed as a result of thawing of frozen soil and rewetting a dry soil. Steaming a soil also results in a flush of nitrogen mineralization. These phenomena will be discussed further during the discussion of partial sterilization.

The major effects of time of year on accumulation of nitrogen are the supply of carbonaceous residues, their C:N ratio, availability to microbial attack, and environmental conditions such as aeration and temperature. During the spring, substrate material is available and microbial activity increases with increasing temperature. As the summer progresses, however, the quantity of root material with an increasing C:N ratio increases, resulting in reduced net nitrogen mineralization. During the fall, immediately after harvest, carbonaceous root material is at a maximum (44) and net immobilization often results. Cultivation of a soil often improves aeration and redistributes microorganisms and organic materials resulting in increased mineralization or immobilization of nitrogen depending on the C:N ratio of the material in the soil. A leguminous crop contains a greater percentage nitrogen in the roots than does a cereal crop. This results in increased mineralization of nitrogen after growth of legumes (44, 72). Perennial grasses possess a tremendous ability to utilize all the mineral nitrogen produced; and due to the large quantity of sloughed off root material associated with the massive root growth, nitrogen immobilization is greater than under annuals. Theron (80) also suggests that perennial grasses actually inhibit nitrogen mineralization. Mineralization after a fallow year may be different from that after cropping but the magnitude and direction of the difference is as yet uncertain.

d) Partial Sterilization

A flush in mineraliztion of nitrogen is often reported following freezing, drying, steaming, or fumigation of soil (17, 18, 42, 44). This effect is termed partial sterilization and is attributed to the predominance of very young cells still in their logarithmic phase of growth. Chemical and physical alteration of the organic constituents in the soil is also considered to be partially responsible. The large number of dead microorganisms, and a readily attacked organic substrate, also helps account for the peak in microbial activity and mineral nitrogen production (17, 42, 44).

Birch (17, 18) has studied the drying and rewetting of soil intensively. His conclusions apply mainly to the effect of wetting and drying on nitrogen mineralization but are also applicable to some of the physical treatments which soils undergo such as freezing and thawing, as well as grinding. Birch (17, 18) reports that several theories have been advanced to account for the partial sterilization effect; among them are:

a) the cyclic development of toxic substances resulting from microbial activity.

b) successive dryings effect, on each occasion, the release of small amounts of decomposable material from within the clay lattice.

c) cyclic microbial growth.

d) increased soluble humus as a result of drying.

Birch reported that the magnitude of the flush in nitrogen mineralization upon rewetting a dry soil increased with increasing length of the dry period, with increasing temperature at which the soil was dried, and was directly proportional to the organic matter content of the soil. From this he concluded that the drying resulted in increased soluble material available to attack. Freezing, through its dehydrating effect, may produce a similar effect on the organic components of soils.

In discussing the effect of moistening a dry soil on humus decomposition and nitrogen mineralization Birch (18) states that "it appears that the state of the organic colloids after drying is the main factor governing subsequent decomposition and nitrogen mineralization, with progressive changes taking place as the dry colloids age; these changes being accelerated by heat." The drying effect on nitrogen mineralization is manifest only in soils that have been dried to the air dry state (11). Birch claims that at this point a transition from the sol to the gel form occurs and thereafter with prolongation of the dry state physical changes conforming to a definite pattern occur in the gel. According to Birch, the drying and heating result in dehydration, shrinkage, and cracking of the gel thus exposing greater surface area. Upon rewetting a proportional increase occurs in the quantity of organic matter available to microbial attack. The same explanation may be proposed for the effect of freezing on mineralization of nitrogen. Steaming and fumigation, however, produce an effect similar to that observed after drying and wetting and freezing and thawing (44). These can not be explained solely on the basis of increased solubility of soil organic matter. The complete explanation of the partial sterilization effect probably involves both biological and physical considerations. The number of bacterial cells in the logarithmic phase of growth is probably increased as is the supply of readily assimilated substrate resulting from an increased number of dead cells (42, 44) and increased surface area due to physical and chemical changes during the sterilization process (18).

e) Soil Aggregate Size

Large aggregates and fine textured soils are often associated with slow oxygen diffusion (19, 66). The aerobic nature of the nitrogen mineralization process has led researchers to conclude that increased aggregate size and fine texture would inhibit nitrogen mineralization. Robinson (66) found that increased soil aggregate grinding below 2mm. had no appreciable effect on the quantity of nitrogen mineralized during incubation studies. Other workers, however, have reported increases due to grinding of soil (19, 42, 43, 44, 66). The effect of grinding is probably influenced by the conditions under which incubation occurs. In soils with an active microbial population the oxygen supply would be quickly reduced in fine textured soils and soils with large aggregates. However, with a less active population, increasing the rate of oxygen diffusion would not affect the quantity of nitrogen mineralized or the rate of nitrogen mineralization.

f) Priming Effect

The stable humus of soil is slowly attacked by microorganisms but the addition of green manure or other readily decomposable residue accelerates its rate of decomposition (19, 44). This priming effect is ascribed to the increased supply of energy material due to the easily attacked amendments. This explanation is premised upon the theory that the stability of soil organic matter is a result of an insufficient energy supply for active microbial development. Another explanation is that addition of fresh material overcomes the biostasis resulting from the developement of antibiotic or inhibitory substances during normal bacterial activity (22,48).

The validity of the claim that a priming effect occurs has recently been questioned (22,34). The main objection to the priming effect hypothesis is that of Jansson, (see ref. 42) that the continuous internal turnover of nitrogen in the soil (internal nitrogen cycle) makes a calculation of isotope ratios inapplicable.

2.2.1 Ammonification

Ammonification is the biological conversion of nitrogen from the organic form to the mineral form as ammonia.

a) Organisms Responsible

A host of heterotrophic organisms are responsible for ammonification. They may be aerobic or anaerobic, acid sensitive, acid tolerant, spore-forming, or non-spore-forming (3). The nitrogen in the substrate is utilized to satisfy their own needs and any excess is exuded in the form of ammonia (8). This is the sole method whereby nitrogen is converted from the organic to the mineral form in soils.

b) Factors Affecting Ammonification

Anaerobic, aerobic, spore-forming, non-spore-forming, acid sensitive, and acid tolerant bacteria are capable of degrading nitrogenous material. Therefore, at least some segment of the population is active regardless of the peculiarities of the habitat, so long as microbial proliferation is possible (8). Consequently ammonification is never entirely eliminated in most arable soils but the rate is markedly affected by environment.

Measureable ammonification has been reported at the wilting point and even slightly below (63, 65). The optimum moisture content is considered to be approximately 60 per cent of water holding capacity; however, ammonification has been reported in waterlogged soils (44, 66). Due to the wide variety of ammonifying organisms, optimum moisture contents probably fall over a range of values depending on the predominant organisms in the soil.

The optimum pH for ammonification appears to be slightly above 7.0 (42). There is a wide range in pH, however, at which ammonification can take place. Very little ammonification can be expected in soils with pH values $\leq$ 3.5 - 4.0 or $\geq$ 9.0 - 9.5. The pH effect may operate indirectly through nutrient availability, especially phosphorus, rather than through a hydrogen ion toxicity or deficiency.

Ammonification can proceed over a wide temperature range and proceeds vigorously into the thermophilic range up to a temperature of 50°C to 70°C (42, 44). It has been reported at temperatures as low as 1° or 2°C but 5°C is generally considered the minimum for significant ammonification (42, 84).

c) Fate of End Product and Intermediates

The NH_3 produced by ammonification may be oxidized to NO_3, lost to the atmosphere, unexchangeably fixed, or enter the exchangeable phase. Losses during the intermediate stages may also occur.

The first step in the breakdown of protiens is the hydrolysis and deamination of amino acids and amines (42, 44, 59). These may be:

i) broken down to NH_3 by transamination systems.
ii) fixed to clay minerals and lignin, thereby becoming resistant to further microbial attack.
iii) absorbed by higher plants.

The NH_3 formed by transamination may be:

i) utilized by higher plants.
ii) used for humus synthesis during lignin decomposition and oxidation.
iii) absorbed by clay minerals or lignin.
iv) oxidized to NO_3 by nitrifying bacteria.
v) utilized by heterotrophic organisms and returned to the organic fraction.

vi) volatilized in alkaline soils.

Some of these processes result in a permanent loss of nitrogen from the soil, whereas others result in a rechannelling of the mineral nitrogen into the organic pool or cause nitrogen to be fixed in the soil in a form unavailable to microbes or plants. Only a portion of the nitrogenous material undergoing degradation, therefore, becomes available for plant growth.

2.2.2. Nitrification

a) Organisms Responsible

The process of nitrification, involving the oxidation of NH_3 to NO_2 and NO_2 to NO_3, is associated with the metabolic activity of two groups of chemoautotrophs. The oxidation of NH_3 to NO_2 supplies the energy requirements of one group while the second group derives its energy from the oxidation of NO_2 to NO_3 (8).

The autotrophic nitrifiers are classified in the family Nitrobacteraceae of the order Pseudomonadales. Seven genera have been recognized: (8)

Nitrosomonas)	Oxidize NH_3
Nitrosococcus)	
Nitrosospira)	
Nitrosocystis)	
Nitrosoglea)	
Nitrobacter)	Oxidize NO_2
Nitrocystis)	

Of these Nitrosomonas and Nitrobacter which oxidize NH_3 to NO_2 and NO_2 to NO_3 respectively are the most important agriculturally.

The carbon for cell wall synthesis and synthesis of all organic constituents of the cell is derived from the reduction of CO_2 while the oxidation of inorganic nitrogen compounds supplies the sole source of energy required for the reduction of the CO_2 and for other energy-consuming metabolic processes. The oxidation of NH_3 to NO_2 liberates 66 kcal of energy per gram atom of nitrogen. The conversion of NO_2 to NO_3 liberates 18 kcal. per gram atom of nitrogen. Nitrosomonas utilizes 5 to 14 per cent of the energy supplied by the first reaction, while Nitrobacter, being slightly less efficient conserves 5 to 10 per cent of the energy of the second reaction (8).

b) Hetertrophic Nitrifiers

Many heterotrophs, with a wide taxonomic range, are capable of increasing the oxidation state of nitrogen. Most of these organisms convert NH_4, or an organic nitrogen compound, to NO_2 but a few are capable of producing NO_3 from NH_4, NO_2, or amino compounds. The heterotrophs capable of this oxidation include gram-negative and gram-positive bacteria, spore-formers and non-spore-formers, and an obligate anaerobe (8). Fungi and actinomycetes as well as bacteria have been reported capable of nitrifying but none of these organisms appear capable of utilizing the energy of the oxidation as the sole source of energy for cell synthesis (8). There is no evidence that the energy released by heterotrophic oxidation of NH_4 or NO_2 is coupled with biosynthetic processes i.e. phosphorylation is not directly linked with the oxidation processes (8).

The significance of these organisms in NO_2 and NO_3 production is not as yet determined but is considered to be minor (8). Since the oxidation of nitrogenous compounds is not obligately associated with the development of these organisms their numbers in the soil indicate a potential for nitrogen oxidation and not an actual transformation.

c) Numbers of Autotrophic Nitrifiers

The numbers of these organisms in the soil may vary from zero to a million or more per gram. Generally *Nitrosomonas* and *Nitrobacter* are found together, but under unusual conditions, such as extremes in pH, *Nitrobacter* may be absent while the conversion of NH_3 to NO_2 by *Nitrosomonas* continues (8). Their numbers increase in the spring and decrease in the summer and winter; dessication and freezing decreases their abundance but never entirely eliminates them.

d) Environmental Influences

Organic Matter: Organic constituents in culture media were observed to inhibit the growth of nitrifiers, thus giving rise to the common belief that organic substances were toxic to nitrifiers. It has since been shown however, (6, 8) that the inhibitory effects of organic constituents in culture media was due to the effect of autoclaving on the composition of organic materials contained in the media and not toxicity of the organic compounds per se. Thus there is very little indication that organic compounds inhibit the growth of nitrifiers, especially since they function in soils containing large quantities of organic material.

Nutrient Supply: The supply of oxidizable substrates (NH_3 and NO_2) will control the rate of nitrification since these nutrients are required in greater quantities than any others. Nitrosomonas oxidizes 35 units of nitrogen and Nitrobacter 100 units for every unit of carbon consumed (8). The limited quantity of NH_3 present in most arable soils indicates that NH_3 is probably oxidized at a rate exceeding the rate of its production; the rarity of ever finding 1.0 p.p.m. NO_2 or greater indicates the limitation of Nitrobacter by NO_2 availability. This would lead to the conclusion that under favorable conditions the potential rate of NO_2 oxidation exceeds that of NH_3 oxidation which exceeds the rate of ammonification.

The rate of nitrification has been shown to increase with increased base exchange capacity of the soil (44).

This has lead to the conclusion that adsorbed NH_4 ions are preferentially utilized and that base exchange capacity influences the availability of oxidizable substrate.

Temperature: The remarkable physiological similarity in the nitrifiers results in pronounced environmental control of the nitrification process (5). Nitrifiers are mesophilic, resulting in an optimum temperature range of 30° to 35°C and cessation of nitrification at 40° to 45°C. Below 30°C nitrification rate declines rapidly until it practically ceases at 4° to 5°C. The process has, however, been reported to progress at temperatures as low as 1° to 2°c (36, 44, 71).

pH: Nitrifying organisms are very sensitive to the H ion concentration. The optimum pH for nitrification appears to vary with different isolates (5, 8). Organisms isolated from acid soils tend to be more tolerant to low pH than do those from alkaline soil. Generally nitrification is favored by a neutral to slightly slkaline pH, however, activity has been detected in some strains from pH5 - 10 (8).

Another pH-related factor is the accumulation of NO_2 in soils. This occurs in soils with a high pH or a low buffering capacity resulting in a pH rise subsequent to the addition of large quantities of urea (8). Nitrite fails to accumulate below a pH of 7.2. The extent of NO_2

accumulation is dependent upon the quantity of NH_3 formed in or added to soil and the pH. The toxicity to Nitrobacter appears to be a result of inhibition of the bacterium by free NH_3 rather than NH_4 since the NO_2 begins to disappear after the NH_3 concentration starts to decline (8).

Aeration: The nitrogen autotrophs are obligately aerobic, thus aeration is a critical factor controlling their development. Low or unusually high oxygen levels inhibit nitrification. Optimum oxygen content is approximately that of air. Nitrosomonas and Nitrobacter utilize 1.5 and 0.5 moles of oxygen respectively in the oxidation of one mole of energy substrate (8).

Moisture: Nitrification rate increases almost linearly with increasing moisture content between wilting point and field capacity (63, 65). The optimum moisture level varies with different soils but appears to be between one-half to two-thirds of the soil's water holding capacity. Increased moisture content results in restricted oxygen diffusion and hence limited nitrification as well as the possibility of denitrification.

Depth: The effect of depth on nitrification operates through its effect on mineral mutrient supply, aeration, pH, and moisture content. This will vary from soil to soil. Nitrifiers have been reported in soil down to eight feet but seldom is NO_3 accumulation experienced at these depths (8).

e) Fate of Intermediates and End Products

Denitrification - the microbial reduction of NO_3 and NO_2 to N_2, and in some cases N_2O - accounts for considerable losses of nitrogen from the soil system. These losses are of considerable magnitude under anaerobic conditions coupled with a large supply of organic substrate and a high concentration of NO_3 - N in the soil.

A small group of faculative aerobes are responsible for denitrification. The active species are limited to the genera _Pseudomonas_, _Achromobacter_, _Bacillus_, and _Micrococcus_; with _Pseudomanas_ and _Achromobacter_ dominating in most agriculturally important soils (8, 24). These organisms are all aerobic but in the absence of a sufficient oxygen supply will use NO_3 or NO_2 as election acceptors. The denitrification process becomes quantitatively significant only when aeration is considerably reduced or when the soil is rich in readily decomposable organic material and NO_3 is present.

The physiological similarity between the denitrifying bacterial results in strong environmental control of the denitrification process. Denitrification becomes significant above pH 5.5. At pH 6.0 - 6.5 N_2O may be evolved. At pH values above 6.5, N_2O is reduced microbiologically and N_2 is the dominant gaseous product (8, 24). The optimum pH for denitrification appears to be between 8.0 and 8.6 (23).

Very little denitrification occurs below 2°C but due to the thermophilic nature of these bacteria it will proceed to about 60° to 65°C, with an optimum temperature of approximately 25°C and above (8, 23, 24, 44). Denitrification does not appear to occur at less than 60 per cent of water holding capacity. This, however, is due as much to the effect of water on aeration as to the moisture requirements of the bacteria. Elemental nitrogen may be lost from aerobic acid soils through the following reactions:

$$RNH_2 + HNO_2 \dashrightarrow ROH + H_2O + N_2$$

$$RNH_4 + HNO_2 \dashrightarrow RH + 2H_2O + N_2$$

but the quantity of nitrogen thus lost from the soil system is not appreciable (44).

Leaching losses account for a large portion of the nitrogen lost from arable temperature soils during the winter and fall. The NO_3 ion, completely soluble in water and only weakly adsorbed to soil constituents, is subject to rapid and complete removal from the soil profile whenever precipitation exceeds evaporation and transpiration (42, 44).

The NO_3 ion, as well as the NH_4 ion, may be assimilated by heterotrophic organisms and thus returned to the organic fraction of the soil. This removal of NO_3, however, does not constitute a loss from the soil system but does result in a reduction in the pool of mineral nitrogen available to the growth of higher plants.

Figure (2) illustrates the fate of nitrogen as it is

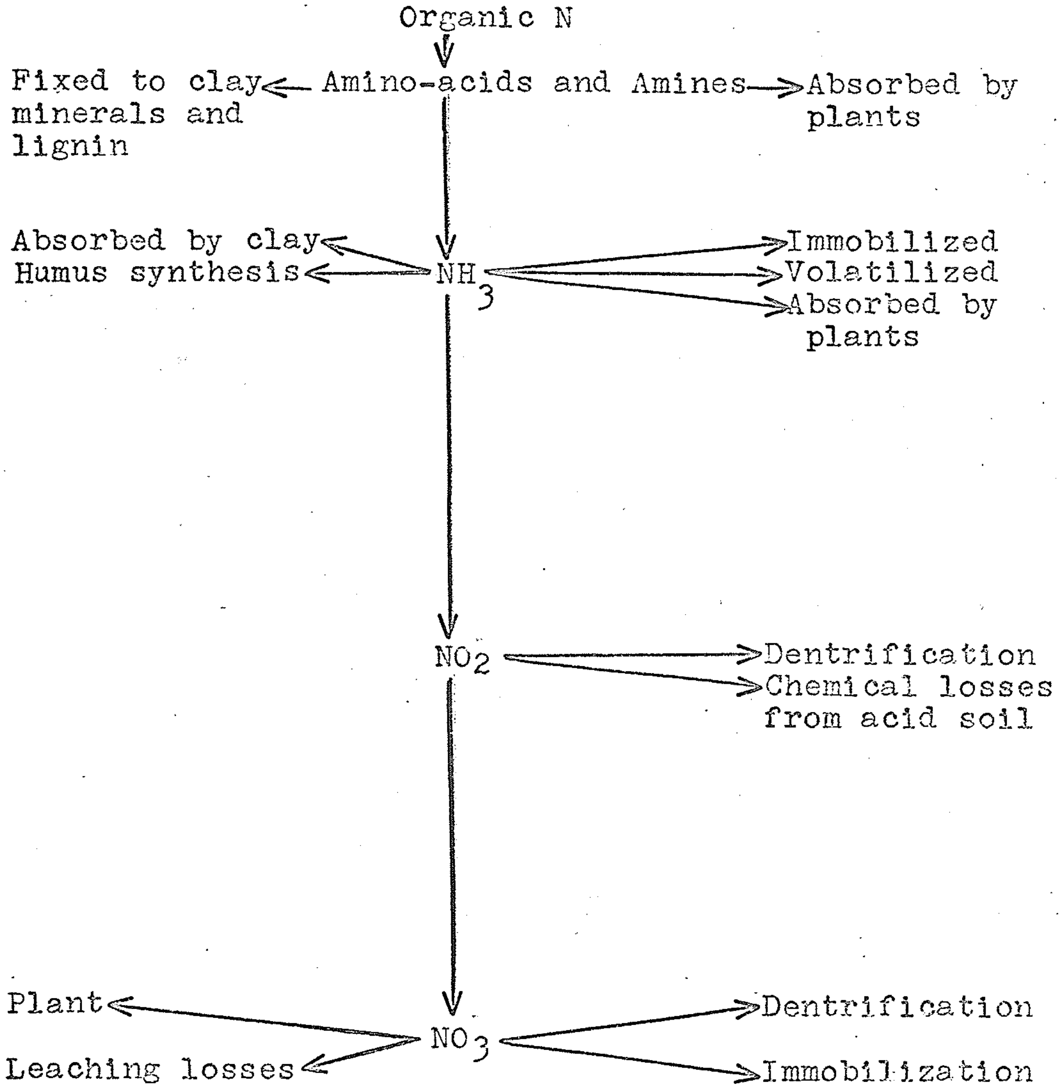


fig. (2) fate of intermediates and end products in the mineralization process transformed from the organic form to NH_3 and thence oxidized to NO_3.

2.2.3 Measurement of Mineralization

The preferential utilization of NO_3 by plants led early researchers to conclude that a measure of nitrogen fertilizer requirements could be obtained through measurement of NO_3 - N in the soil at various times of the year. The NO_3 - N content of the soil was found to be extremely variable and of little value in predicting the quantity of nitrogen fertilizer required. Many workers (39, 43, 44) mainly in the more humid temperate regions, have concluded that the potential for nitrogen mineralization is the only reliable method of determining nitrogen fertilizer requirements. However, in more arid regions the NO_3 - N content at seeding has been found to be a reliable measure of nitrogen fertilizer requirements (45, 56, 73, 89). Cook et al. (30) in Saskatchewan however, found a strong negative correlation between nitrogen mineralized during incubation and response to nitrogen fertilizers. Even where the quantity of NO_3 - N in a soil is a good measure of fertilizer requirements the quantity of nitrogen mineralized through the year is considered important in either partially determining yield or in supplying a portion of the nitrogen required to complete the growth and development of the crop.

Harmsen and Lindenburgh (43) adequately express the present concept of predicting nitrogen fertilizer needs when they state that "determination of the amount of any form of nitrogen in the soil cannot give a correct estimate of fertilization requirements. Only a measurement of the activity and the rate of mineralization of the nitrogen-containing organic matter in the soil, can serve as a criterion for this purpose." They also state that:

> "development of most annual crops in temperate climates starts at a time when the content of mineral nitrogen in the soil is relatively high, since late spring is the period of the highest mineral nitrogen level in soil as a result of the comparatively active mineralization in the warm days of spring without any appreciable uptake of nitrogen by the very young plants. But this stock of mineral nitrogen can never become very large. During the preceeding winter and early spring the temperature was too low for active disintegration of humus, and rainfall surpassed evaporation, while the period between improvement in climatic conditions and the start of plant growth is too short for an accumulation of appreciable amounts of mineralized nitrogen. Thus only the first part of the development of the crop can be supported by accumulated nitrogen, while as soon as the crop comes into the stage of most rapid growth, it starts to absorb so much nitrogen that the absorption surpasses, in most soils, the production of mineral nitrogen by the mineralizing action of microbes. Consequently the stock of mineral nitrogen, available at the beginning of the vegetation period, is soon exhausted and the crop for the rest of its growth depends upon the nitrogen liberated by microbes from the humus."

Methods of measuring the quantity of nitrogen mineralized have been developed. The best method is through measurement of nitrogen uptake and accumualtion in the field. This method is very expensive and time consuming and is therefore used mainly as a standard for routine measurements (72). The measurement of nitrogen made available to plants in the greenhouse or growth chamber is an approximation to the field method but the root zone is restricted and the environment altered considerably.

More popular among researchers is the incubation method whereby soil samples undergo a standardized proceedure of drying, grinding, remoistening, and incubation under controlled environmental conditions. It has the advantage of being inexpensive and is readily adapted to routine procedures. However, incubation provides a measure of only the potential of a soil to mineralize nitrogen and values obtained with incubation techniques must be adjusted to suit the prevailing field conditions. The main disadvantage of the incubation technique is that the incubation conditions are entirely artificial and the results are in no way comparable to the mineralization process under field conditions (44). Considerable energy has, therefore, been expended in correlating incubation results with response to nitrogen fertilizer in the field. The result has been that very little information concerning the quantity of nitrogen mineralized during the year or the time at which it is made available relative to a growing crop's needs has been obtained. The best that can be hoped for with these methods is an empirical relationship between response to nitrogen fertilizer and the quantity of nitrogen mineralized in a given time under controlled laboratory conditions.

2.3 Immobilization

The additions of carbonaceous residues to soil is generally associated with a reduction in the level or in the rate of mineral nitrogen accumulation in a soil. If the quantity of nitrogen supplied by the residue is insufficient to meet the requirements of the heterotrophic microflora then mineral nitrogen from the soil is assimilated to meet these requirements. Immobilization of nitrogen is therefore the converse of nitrogen mineralization. Even when a pure protein is added to soil, not all of its nitrogen is liberated; some always goes into the biosynthesis of microbial cells. Whenever nitrogen mineralization occurs, nitrogen immobilization runs counter to it (8).

a) C:N Ratios of Added Residues

C:N ratios have been discussed in some detail under the section on effect of C:N ratios on nitrogen mineralization (page 11). It is sufficient here to state only that the critical C:N ratio above which nitrogen immobilization occurs and below which nitrogen mineralization occurs, is believed to fall between values of 20 to 30 for cereal residues containing approximately 40 per cent carbon. This corresponds to a nitrogen content of from 1.2 per cent to 1.8 per cent.

The values of the critical C:N ratio depends on the availability of the carbon and nitrogen in the added residue. A C:N ratio of approximately 30 in cereal straw amendments in temperate climates generally results in nitrogen immobilization while a C:N ratio of one or two in sucrose plus nitrogen amendments to soil will result in immobilization of mineral nitrogen (86). Allison and Murphy (12) found that the decomposition of softwood, which is slowly attacked by microbes, did not require added nitrogen whereas the decomposition of a more readily attacked hardwood species required nitrogen additions. In the former case the rate at which the soil supplied mineral nitrogen was equivalent to or greater than the rate at which it was removed by the microbial population, but in the latter case, the rate at which mineral nitrogen was removed by the heterotrophs exceeded the rate at which the soil could supply it.

A C:N ratio of 50 is considered critical in tropical climates (70) but Greenland and Nye (41) found no immobilizing effect due to addition of residues with a C:N ratio of 70 to tropical soils. This may have been due to a very active termite population or to the method of incorporating the straw into the soil. This further illustrates the danger of assigning a single value to the critical C:N ratio rather than setting probable limits for the extremes in the C:N ratio. It also is an indication of the effect of climate and environment on critical C:N ratios.

b) Form of NItrogen in the Soil

The preferential utilization of NH_4 - N over NO_3 - N by most heterotrophs has been conclusively established. The preference for NH_4 - N is probably a result of the reduced state of the NH_4 ion thus minimizing the energy expenditure in converting it to amines and thence to proteins in the organism. A given concentration of NH_4 will result in a more rapid and extensive immobilization of nitrogen than will the same NO_3 concentration (50).

Immobilization is accompanied by a pH change. Immobilization of NO_3 - N increases pH while the immobilization of NH_4 - N reduces soil pH. The immobilization of NO_3 - N is favored by dry conditions. However, NH_3 assimilation exceeds that of NO_3 regardless of the moisture regime (50).

The rate, and time of maximum immobilization, is affected by the quantity of nitrogen present. Increased immobilization occurs with increased nitrogen additions. However, nitrogen additions in excess of that required for complete decomposition does not influence the rate of nitrogen immobilization (50).

c) Relationship Between Per Cent Decomposition and

Immobilization

Maximum nitrogen immobilization is reached at different times depending on the mineral nutrient status of the soil, substrate availability, and environment. Generally, however, the maximum quantity of nitrogen is immobilized at approximately 20 per cent decomposition of the residue. At this point, mineralization of nitrogen from the soil organic matter starts to exceed the rate of nitrogen immobilization and mineral nitrogen again accumulates. Sucrose additions produce a maximum in nitrogen immobilization in about 2 days whereas 24 days incubation is required for a maximum with addition of straw (11).

d) Competition of Heterotrophs with Plants and Nitrifying Autotrophs

Yield reductions have been reported to be greater with additions of straw and NH_4 - N to soil than with additions of straw and NO_3 - N (57). This indicates that plants can compete more favorably for NO_3 than for NH_4 - N. Heterotrophs can generally compete more favorably for NH_4 - N than can the nitrifying autotrophs. The activity of the nitrifying population is controlled in part by the natural fertility of the soil (26). Soils with a high organic matter content generally support a more vigorous nitrifying population than a less fertile soil. Ferguson (34) observed no yeild reduction due to the addition of straw to soils during field trails in Western Manitoba whereas additions of straw to the same soil in the laboratory reduced the NO_3 - N content. He postulated that the cooler temperatures of early spring favored plants relative to the heterotrophs in the competition for available mineral nitrogen. Plant uptake of nitrogen appears to be enhanced somewhat by reduced soil temperature (38, 58) within a definite range. Below approximately 60°F increased temperature increases the uptake of nitrogen.

e) Environmental Effects

Temperature and moisture are the two most important environmental variables determining the quantity and rate of nitrogen immobilization. Very little decomposition appears to occur at temperatures below 7°C (84) but slow decomposition at 1° or 2°C has been reported (36, 71). The optimum temperature appears to be in the mesophilic range but immobilization continues at a decreasing rate into the thermophilic range and ceases between 60° and 70°C (15, 42, 84).

Optimum moisture content is in the region of field capacity (84). At the wilting point immobilization practically ceases and excess moisture inhibits immobilization by reducing the oxygen supply to the organisms.

Decreasing soil pH results in decreased immobilization by either favoring fungal and inhibiting bacterial proliferation, or by reducing the phosphorus supply (88). Phosphorus is necessary to microbial metabolism in that it functions as an energy storage and transport agent. Winsor and Pollard (88) found a correlation of 0.89 between acetic acid extractable phosphorous and immobilization at a probability level of 0.999.

The mineral nutrient status of a soil is important in controlling immobilization. Cultivated soils with a low C:N ratio have been reported to immobilize more nitrogen than virgin soils with a high C:N ratio (88). This was attributed to the increased mineral nutrient content in the cultivated soil which enhanced the microbial population.

f) Quantity of Nitrogen Immobilized

Alexander (4) reports that the complete decomposition of 100 units of plant residue consisting of approximately 40 per cent carbon by bacteria, fungi, and actinomycetes would require 0.4 - 1.8, 1.2 - 1.6, and 1.2 - 2.4 units of nitrogen, respectively, which would be equivalent to 8 - 16, 24 - 36, and 24 - 48 pounds of nitrogen per ton of straw. Bartholomew (14) reports that the decomposition of one ton of residue would require 24 - 34 pounds of nitrogen of which 12 to 17 pounds may be supplied by the residue. Therefore, the quantity of nitrogen in the residue and the dominant organisms in the soil influence the quantity of nitrogen immobilized. Ferguson (34) and Ferguson and Gorby (35) reported that additions of straw to Manitoba soils resulted in no significant yield reduction indicating that the quantity of nitrogen immobilized was either small or that it was rapidly remineralized. Pinck *et al.* (61) calculated from greenhouse data that an extra 16 to 18 pounds of nitrogen per ton of straw added was required in order to produce a wheat yield equivalent to that of soil to which no straw had been added.

2.4 Effect of Plants on Mineralization and Immobilization of Nitrogen

2.4.1 Effect of Plants on Microorganisms

A growing crop affects soil structure, aeration, and nutrient status. However, the major effect of a crop is on the microbial population of the rhizosphere soil. The rhizosphere may be divided into an inner and an outer region. The inner region is at the very root surface and supports a larger and more active microbial population than does the outer region which embraces the immediately adjacent soil (7).

a) Effect on Microbial Numbers

Microorganisms are more numerous in the rhizosphere than in the soil body as a whole (16, 28, 51, 69, 74, 77). The increase in fungal and actinomycete numbers is not as great as that of bacteria (44, 70). The increase in microbial numbers is evident throughout all stages of plant development (28, 51, 77) but is accentuated as the plants age, and reaches a maximum when the plants reach an appreciable size, reach the limit of vegetative growth, or have bloomed and started to degenerate (51).

The abundance of microbial cells is influenced by the type of plant, its stage of growth, and its vigor. Cereals produce the smallest increase in microbial cell numbers whereas rapeseed and legumes produce much larger increases (28, 51, 77). The greatest increase in microbial numbers, by any given plant, occurs during rapid vegetative growth and the effect disappears upon death of the plant. It is therefore believed to be associated with normal growth (77).

b) Qualitative Effect of Plants on Microorganisms

The relative proportion of the various microbes is often different in the rhizosphere than in the soil body. This indicates some selective action of the rhizosphere, or more precisely, rhizosphere conditions appear to favor some organisms over others. Herein will be discussed the effect of plants on Azotobacter, ammonifying and proteolytic bacteria, nitrifiers, denitrifiers, and cellulose decomposing bacteria.

Azotobacter: There is little indication that there is a stimulatory effect of plant roots on these nitrogen fixing bacteria (28, 51, 77). Some reports indicate increased numbers of these bacteria in the root zones of some plants. Russian workers claim that increased cereal yields have resulted from inoculation of the seed with Azotobacter but these findings are the exception rather than the rule.

It is often argued that sloughed off root material supplies the large quantity of energy required by these organisms. Experimental observations, however, have not verified this claim (51, 77).

<u>Ammonifying and Proteolytic Bacteria</u>: The numbers of these organisms are increased as much as several hundred times by plant roots (7, 51,84). Most of the organisms associated with plant roots belong to species active in decomposition of fresh organic matter (51).

<u>Nitrifiers</u>: Plants are reported to accelerate nitrification during the early stages (51) of growth and depress it during the latter stages (51, 77). This may be a result of stimulated activity and increased numbers of these organisms due to plant root excretions (51).

<u>Denitrifiers</u>: Denitrifiers have been observed in large numbers in the root zones of plants by many investigators (44), but there is still no evidence that denitrification is increased as a result of plant growth.

<u>Cellulose Decomposers</u>: Cellulose decomposers are present in the root zones of a large number of plants (7, 28, 51, 77). Large numbers of these organisms have been reported on wheat roots during the early stages of development followed by a decline during the most rapid portion of the vegetative phase. A second increase in their numbers occur during the latter stages of growth (51). It has been concluded that they take part in decomposition of sloughed

off root fragments and that the products of this process are reacted upon by other soil organisms (51, 77).

c) Types of Root Exudate

The most consistent finding in examination of nutritional requirements of rhizosphere microbes is their need for amino acids (7, 28, 51, 67, 74, 77). This has led to the suggestion (77) that leakage of amino-acids, but not of growth substances, from plant roots occurs. Vitamins required by rhizosphere organisms are believed to be obtained from plant residues, from root excretion, or from excretions of associated organisms. Various bacteria recovered from the rhizosphere secrete extracellular vitamins and amino acids (77). Rovira (67) isolated actual secretions of amino acids from sand in which young oat plants were growing. He noted that as the plants aged root secretions become less important and sloughed off cellular material increased in quantity. This suggests that a qualitative change in the microbial population may also be assoiciated with the change in substrate material as the plants age.

d) Environmental Factors

Environmental conditions influence the magnitude of the rhizosphere effect (51). Bacteria are most numerous in the rhizosphere of plants growing in neutral to slightly acid soil. Actinomycetes are unaffected by pH and fungi prevail at extremes in pH. Soil texture has an effect on the ratio of bacteria in the rhizosphere to those in the soil as a whole (R:S). The R:S ratios are in the following order:

loam > sand > clay > humus.

Drier soil conditions produce an enhanced rhizophere effect relative to wet conditions (28, 51). Large numbers of bacteria have been found in the rhizosphere of plants grown on steamed soil even though the bacterial numbers were near zero in the rest of the soil (51). This is probably due to the enhanced growth of bacteria in the rhizosphere relative to the soil as a whole.

2.4.2. Effects of Microorganisms

a) Nutrient Supply

The bacteria of the rhizosphere are physiologically more active than those in other portions of the soil (68, 69, 74, 75). Ammonification is markedly stimulated by the presence of plant roots. The ammonifying population may be several hundred times as great in the rhizosphere as in the soil bady as a whole. Investigations with N^{15} have revealed that although the net quantity of nitrogen mineralized in cropped soils is often half that of fallow, the absolute quantity of nitrogen mineralized is greater in cropped than in fallow soils (16). The reduction in net nitrogen mineralization is a result of markedly increased nitrogen immobilization in the root zone (16).

Goring and Clark (40) reported increased net mineralization of nitrogen in cropped soils over fallow soils during the early stages of plant development, but reduced net nitrogen mineralization as the crop matured. Similar effects have been reported in several reviews (7, 28, 51, 77).

Nitrification is also stimulated by growth of plants (74). The effect may be due to increased activity of the nitrifying bacteria or to increased numbers (51, 69, 76, 77). The increased nitrification, however, results in increased NO_3 being made available to the growing plant for at least part of the growing season. The stimulation of immobilizing bacteria often results in a failure of this mineral nitrogen to be utilized by higher plants. The stage of plant development appears to partially regulate the magnitude of the nitrification-immobilization effect.

Immobilization is definitely increased by the grwoth of a crop (7, 16, 28, 39, 51, 77). This increase in nitrogen immobilization is believed due to sloughed off cellular material. As the plants age the C:N ratio of the sloughed off material increases and the quantity of nitrogen immobilized therefore increases. Increased nitrification during the early stages of growth results in a greater accumulation of mineral nitrogen in cropped soil relative to fallow soil early in the season. Nitrogen immobilization increases with increasing age of the plant and surpasses nitrification resulting in a reduced net production of mineral nitrogen in cropped soils relative to fallow soil.

The large number of bacteria in the rhizosphere capable of reducing NO_3 - N to NO_2 - N and N_2, coupled with the generally reduced oxygen supply resulting from the growth of a crop, has led to the hypothesis that denitrification may be a significant factor in accounting for the reduced net quantity of mineral nitrogen made available during the growing season in cropped relative to fallow soil. The large number of denitifiers in the rhizosphere indicates only a potential for the reduction of large quantities of NO_3 to N_2 should the necessary conditions arise (77). There may be slight denitrification in local areas of oxygen deficiency but it is, at present, not considered quantitatively significant in cropped soils; the potential, however, for rapid and extensive losses of mineral nitrogen in this manner definitely exists.

The major effect of plant development on nutrient supply is the increased ammonification and immobilization of nitrogen in the root zone resulting in an increased nitrogen supply to the plants during the early stages of plant development and a reduced supply during the latter stages of growth. Increased fixation of atmospheric N_2 does not often result from the growth of cereals. It has, however, been reported that significant quantities of N_2 become fixed by non-symbiotic bacteria developing in the rhizosphere of non-leguminous plants on organic material coming from the roots (77). Starkey (77) reports that Parker observed the fixation of a greater quantity of N_2 under grass than by additions of 3,000 pounds of sugar per acre. This indicates that the increased root materials under grass may possibly result in sufficient stimulation of non-symbiotic nitrogen-fixing bacteria to cause significant fixation of atmospheric N_2.

Chapter III

Experiment I Field Experiment

The purpose of this experiment was to determine the quantity of nitrogen mineralized during the growing season in both fallow and seeded soil. The effect of crop growth on nitrogen mineralization was an important aspect of this investigation as was the effect of mineralized nitrogen on yield and plant development. This experiment was also designed to determine the rate at which nitrogen is made available to the growing drop through mineralization.

3.1 Methods and Materials

This investigation was conducted on two plots on each of two soil types. One plot on each soil type was located on a previously fallowed field while the other was located on a previously cropped field. In order to reduce variations between soil characteristics in the plots on the same soil type, adjacent stubble and fallow fields were selected.

a) Soils

1. Morden clay loam

Legal description: N.E. 5-3-4-W

Soil Survey Map Area: South Central

Parent Material: Grey-drab alluvial clay deposited as overwash or outwash plain

Drainage: Good

Topography: Flat and Smooth

Vegetation (native): Tall prairie grasses and herbs

Soils Report No. 4 (32)

2. Almasippi Loamy sand

Legal location : 22-8-7-W

Soil Survey Map Area: Carberry

Parent Material: Sandy deltaic deposits

Drainage: Imperfect

Topography: Level

Vegetation (native): Tall prairie grasses and sedges

Soil Report No. 7 (31)

Some characteristics of the soils are summarized in Table I.

b) Experimental Design:

The field experiment consisted of two treatments:

a) seeded (to Manitou wheat) no fertilizer.

b) fallow

which were replicated four times on each of the four plots. Each replicate of each treatment was 30 feet by 17.5 feet thus allowing for ten samplings, each on a different 3.5 x 15 foot area of plot. Wheat was seeded at the rate of one bushel per acre with a self-propelled six row seeder with seven inch row spacing built at the University of Manitoba. The Almasippi stubble plot had been cropped to oats the previous year, and the Morden stubble plot to barley. The Almasippi Summerfallow and Morden Summerfallow plots had been summerfallowed the previous year.

Sampling:

Soil samples were taken to the following depths:

0 - 6"
6 - 12"
12 - 24"
24 - 36"
36 - 48"

Samples were taken at seeding and every two weeks thereafter until the final harvest. The dates of seeding and harvest are summarized below:

	Seeding	Maturity
Morden summerfallow	May 25/67	Aug. 15/67
Morden stubble	May 25/67	Aug. 12/67
Almasippi summerfallow	May 25/67	Aug. 21/67
Almasippi stubble	May 29/67	Aug. 25/67

Plant material samples were taken every two weeks after seeding until maturity. Plant material was collected from one ten foot row of each replicate at each sampling date except at maturity at which time two ten foot rows were harvested.

c) Treatment of Samples

1) Soil -- The samples were immediately brought into the lab where they were divided into two portions.

One portion was weighed, oven dried, reweighed, ground to $\angle 2$ mm., and replicates bulked and stored for $NO_3 - N$ determinations. Each depth was treated separately. Replicates were bulked by first thoroughly mixing each of the ground samples and then taking a measured volume of soil from each replicate of each treatment and bulking them together and thoroughly mixing them before analysis.

Moisture determinations were made on all soil samples as soon as they were brought from the field. Loss of water upon drying for 24 hours at 100°C was calculated as per cent of oven dry weight and was considered to represent the moisture content of the soil at the time of sampling.

The second portion of the samples from the field consisted of approximately 30 to 40 grams of field moist soil which was stored at approximately 4°C in the field moist condition. Determinations of the $NH_4^+ - N$ content were made on these samples.

2. Plant Material -- Plant material samples were brought to the drying shed and cut into pieces two to three inches long and placed on paper to dry. The samples were dried at $30^\circ \pm 5^\circ C$. The drying period lasted approximately two weeks, after which time the material was weighed and ground. Replicates were bulked on a volume basis after grinding and were stored for total nitrogen analysis. At Maturity the plant material was dried at $30^\circ \pm 5^\circ C$. and then

weighed. The samples were then threshed and the seed and straw collected separately. The seed was weighed and straw weight was obtained by subtracting the seedweight from the total weight of each sample. The seed and straw replicates were then bulked separately.

d) Analytical Procedures --

1. Soil pH -- The pH of a saturated soil water paste was measured using a universal - pH meter (47).

2. Soil carbonate content -- The acid neutralization method as outlined by Allison (13) was used.

3. Soil organic matter content -- The procedure developed by Walkley and Black (85) was used in which organic matter is oxidized by chromic acid.

4. 0.5 M. $NaHCO_3$ - extractable phosphorus __ The soil samples were analysed for extractable phosphorus using 0.5 M. $NaHCO_3$ at pH 8.5 according to the procedure described by Olson, et al. (60).

5. Potassium determination -- The soil samples were analysed for exchangeable potassium using the flame photometric method according to the procedure described by Pratt (62). No correction for water soluble potassium was made.

6. Nitrate nitrogen determination -- Nitrate determinations on the soil samples were made using the colorimetric nitrophenol disulfonic acid method as modified by Harper (49).

7. Ammonium nitrogen determination -- This determination was made according to the procedure described by Jackson (48) using the modified Nessler's reagent as described by Yuen and Pollard (90). The NH_4^+ - N was extracted with 2N KCL, steam distilled in the presence of NaOH into boric acid solution, treated with Nessler's reagent, and determined colorimetrically on a spectronic 20 colorimeter.

8. Nitrite nitrogen determination -- The modified Griess-Illosway method described by Bremner (22) was used in which NO_2^- - N was determined on the 2N KCL extract used for NH_4^+ - N determination.

9. Total nitrogen determination -- The macro-kjeldahl procedure described by Bremner (20) was used. The nitorgen in both the soil samples and the plant material was converted to ammonium form, distilled into boric acid solution, and titrated with dilute hydrochloric acid.

e) Calculations:

1. Units of concentration (p.p.m. and per cent) to pounds per acre -- This conversion was made using the following density factors obtained from the Manitoba Soil Testing Laboratory:

Depth (inches)	Almasippi	Morden
0 - 6	2.2	1.6

6 - 12	2.2	1.6
12 - 24	4.6	3.9
24 - 36	4.6	3.9
36 - 48	4.6	3.9

2. Net mineralized nitrogen -- The net quantity of nitrogen mineralized between seeding and the various sampling dates was calculated according to the following:

N.M.N. = lb. NO_3^- - N to 4 feet at the various dates + lb. N contained in the plant material - lb. NO_3^- - N to 4 feet in the soil at seeding.

TABLE I

CHARACTERISTICS OF SOILS USED

Morden Summerfallow
Morden Clay Loam

Depth (inches)	pH	%CaCO$_3$ Equiv.	P ppm.	K ppm.	O.M. %	N %
0 - 6	7.2	1.6	31.0	433	4.4	0.26
6 - 12	6.9	1.4)	4.32	246	2.2	0.18
12 - 24	7.7	7.9)			1.0	0.11
24 - 36	7.8	16.2			1.0	0.08
36 - 48	7.8	17.2			1.0	0.06

Morden Stubble
Morden Clay Loam

Depth (inches)	pH	%CaCo$_3$ Equiv.	P ppm.	K ppm.	O.M. %	N %
0 - 6	6.7	1.4	11.9	321	5.3	0.29
6 - 12	6.8	1.8)	2.3	192	3.4	0.22
12 - 24	7.7	9.9)			1.6	0.13
24 - 36	7.8	15.3			0.7	0.06
36 - 48	7.8	12.0			0.7	0.06

Table I continued .....

Almasippi Summerfallow
Almasippi Loamy Sand

Depth (inches)	pH	%CaCO$_3$ Equiv.	P ppm.	K ppm.	O.M. %	N %
0 - 6	7.7	0.6	1.2	59	1.6	0.08
6 - 12	7.6	0.5)	0.4	42	0.5	0.05
12 - 24	7.4	1.0)			0.2	0.03
24 - 36	8.0	4.2			0.3	0.03
36 - 48	8.0	7.8			0.1	0.02

Almasippi Stubble
Almasippi Loamy Sand

Depth (inches)	pH	%CaCO$_3$ Equiv.	P ppm.	K ppm.	O.M. %	N %
0 - 6	7.4	0.9	1.9	64	2.3	0.1
6 - 12	7.7	0.5)	1.9	49	1.0	0.05
12 - 24	7.7	1.2)			0.7	0.03
24 - 36	7.9	8.0			0.3	0.03
36 - 48	8.0	9.6			0.1	0.02

3.2 RESULTS AND DISCUSSION

3.2.1. Quantities of Ammonium -, Nitrate -, and Nitrite-Nitrogen in the Four Plots

No nitrite-nitrogen was detected in either the Almasippi or the Morden soil (Table III).

The absence of NO_2^- - N in these soils is to be expected since the pH values were near neutral, (Table I) waterlogging was not evident, and no nitrogen fertilizer was added to these soils.

The ammonium-nitrogen content of both soils was very low (Table III). On occasion the variation was as great as, or greater than, the average quantity of ammonium-nitrogen detected (Table IV). Harmsen (44) argues that the consistently small quantities of ammonium-nitrogen found in grassland may be an artifact of the method used to determine it. This may have been the source of the ammonium-nitrogen detected in this experiment.

The quantity of nitrate-nitrogen in these soils was considerably greater than the ammonium-nitrogen contents (Table III). In pounds per acre these values are:

Table II Pounds Nitrate-Nitrogen Per Acre

plot	0 - 24 ins.	0 - 48 ins
Morden summerfallow	117 ± 4*	144 ± 5*
Morden stubble	33 ± 1*	66 ± 2*
Almasippi summerfallow	27 ± 1*	32 ± 1*
Almasippi stubble	9 ± 1*	13 ± 1*

*for 95 per cent confidence limits

TABLE III

SOIL AMMONIUM-, NITRATE-, AND NITRITE-NITROGEN AT SEEDING TIME

p.p.m.

DEPTH (inches)	MORDEN S.F.			MORDEN St.			ALMASIPPI S.F.			ALMASIPPI St.		
	NH_4^+	NO_3^-	NO_2^-	NH_4^+	NO_3^-	NO_2^-	NH_4^+	NO_3^-	NO_2^-	NH_4^+	NO_3^-	NO_2^-
0 - 6	0.6	37.1	0	0.5	7.5	0	0.7	3.0	0	0.9	1.5	0
6 - 12	0.5	16.9	0	0.6	5.2	0	0.8	3.8	0	1.2	1.0	0
12 - 24	0.9	7.9	0	0.6	3.3	0	0.6	2.6	0	0.7	0.8	0
24 - 36	0.8	4.5	0	0.8	5.3	0	0.7	0.7	0	0.8	0.6	0
36 - 48	0.5	2.6	0	0.6	3.2	0	1.1	0.4	0	0.6	0.3	0

TABLE IV

DELTA VALUES FOR 95 PERCENT CONFIDENCE LIMITS

(March 25, 1967) p.p.m.

delta = (standard diviation/$n^{1/2}$) x T(n-1,.975)

DEPTH (inches)	MORDEN S.F.		MORDEN ST.		ALMASIPPI S.F.		ALMASIPPI ST.	
	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-
0 - 6	0.1	1.1	0.1	0.2	0.1	0.1	0.1	0.1
6 - 12	0.1	0.6	0.1	0.1	0.1	0.2	0.2	0.1
12 - 24	0.1	0.4	0.1	0.1	0.2	0.1	0.1	0.1
24 - 36	0.1	0.2	0.1	0.2	0.1	0.2	0.1	0.1
36 - 48	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1

According to limits used by the Manitoba Soil Testing Laboratory, a nitrate-nitrogen content of less than 13 pounds per acre in the top two feet would be considered very low. A medium value would be between 26 and 38 pounds of nitrate per acre in the top two feet. Amounts of NO_3^- - N in excess of 60 pounds per acre would be considered very high. According to these limits the Almasippi stubble plot contained a very low quantity, the Morden stubble and the Almasippi summerfallow plots contained medium quantities, and the Morden summerfallow plot a very high quantity of nitrate-nitrogen.

Nitrate-nitrogen is the dominant form of mineral nitrogen in most agriculturally important soils (except paddy soils) (44). Results reported herein are therefore in accordance with those in the literature.

3.2.2 YIELD AND NITROGEN UPTAKE

1. Yield: Dry matter yield curves were sigmoidal in form (fig. 3), became logarithmic about two weeks after seeding (June 8), linear by July 6, and reached a maximum at ten weeks and then declined during the period between ten weeks and harvest.

The Morden Summerfallow plot produced the greatest dry matter yield and the Almasippi Stubble plot the least. Similar and intermediate yields were obtained on the Morden Stubble and Almasippi Summerfallow plots.

All yield curves were smooth with no rapid fluctuations. The curves for the various plots started to diverge during the logarithmic phase and retained their relative orders until maturity. Potential maximum yield was probably determined during an early stage of development.

The relative seed yields on various plots were the same as the relative maximum dry matter yields:

Table V Total Dry Matter Yields and Seed Yields

PLOT	MAX. DRY MATTER YIELD (LB./AC.)	SEED YIELD (BU./AC.)
Morden Summerfallow	6,273	40
Morden Stubble	3,378	23
Almasippi Summerfallow	3,944	27
Almasippi Stubble	2,066	16

2. Nitrogen Uptake: The nitrogen uptake curves (fig. 4) were probably sigmoidal but sampling was not sufficiently frequent to allow detection of the logarithmic phase. The curves became linear after two weeks and remained so until July 20 in the Morden Summerfallow plot, until August 3 for the Almasippi Summerfallow and Morden Stubble plots, and until the final harvest for the Almasippi Stubble plot. After the above-mentioned dates the curves leveled off, and in two plots, declined somewhat by harvest. A large portion of the nitrogen utilized by the crop was assimilated early in the summer. This was expected since cereals are capable

FIG. 3 DRY MATTER YIELD

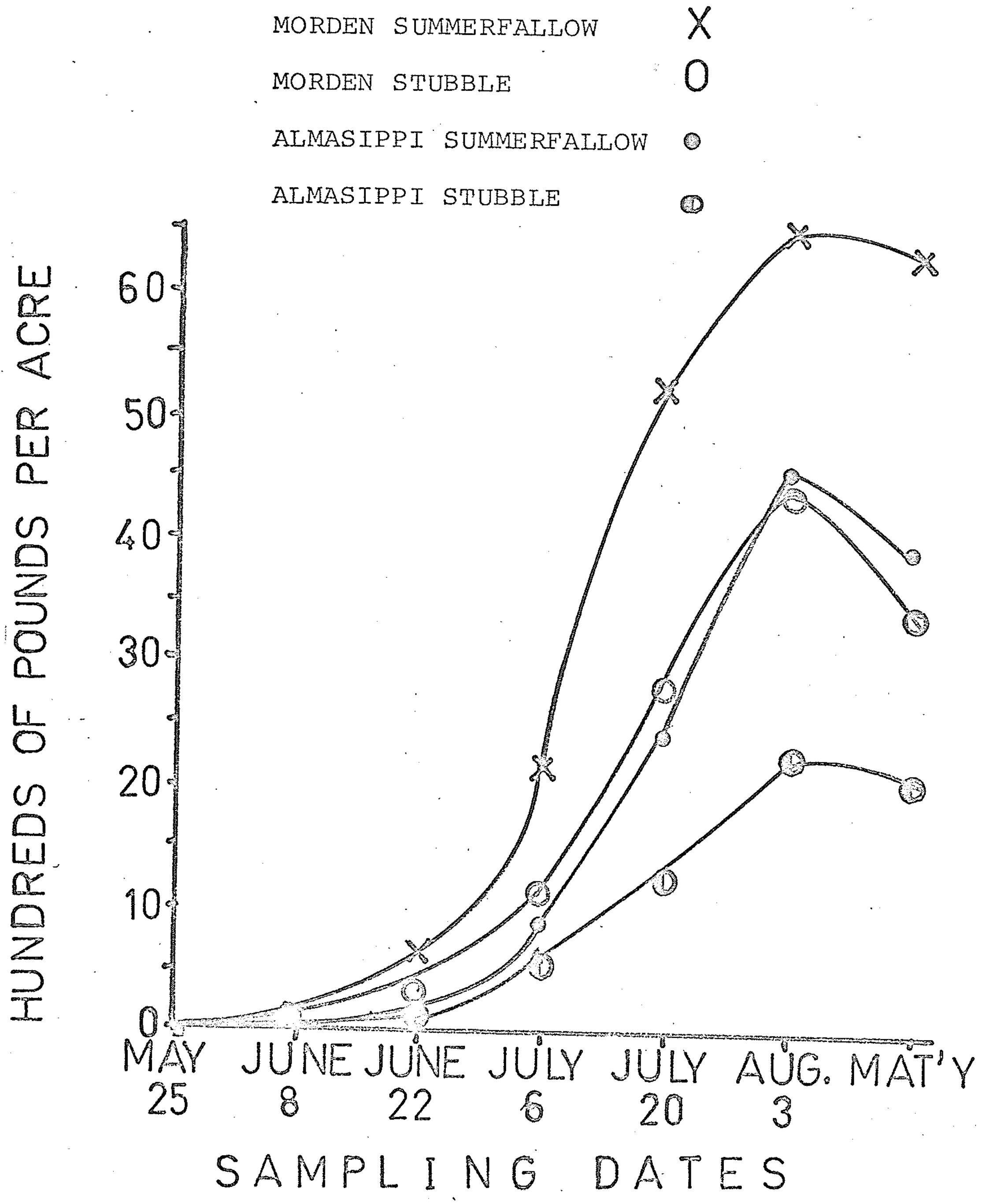
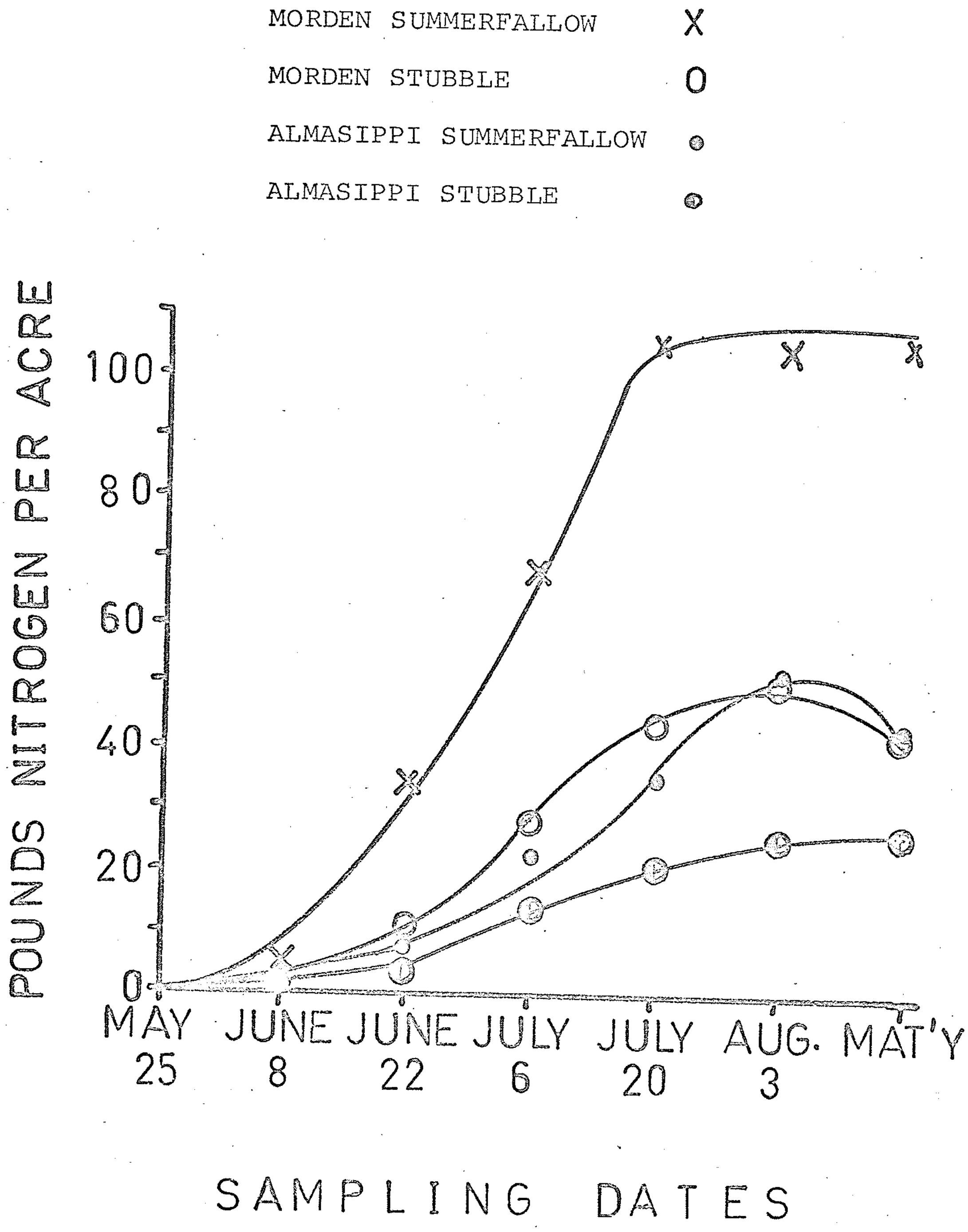


FIG. 4 NITROGEN UPTAKE



of assimilating nitrogen early and storing it for later use (82, 83).

The crop on the Morden Summerfallow plot assimilated the greatest quantity of nitrogen while that on the Almasippi Stubble plot assimilated the least nitrogen. Intermediate quantities of nitrogen were contained in the crops on the Almasippi Summerfallow and the Morden Stubble plots.

The nitrogen uptake curves diverged early and by July 6 a definite rank had developed which persisted until maturity. The slopes of the linear portions of the nitrogen uptake curves (which may be assumed to determine the potential yield) were determined during the first six weeks after seeding.

An important feature of these results is the similarity of both yield and nitrogen uptake in the Morden Stubble and the Almasippi Summerfallow plots. These soils differed greatly in their properties (Table I). One had been cropped for two years previous to this study and the other fallowed for a year. Greater yields are generally expected after fallowing than on previously cropped soil. The only observed similarity in these two soils was the quantity of nitrate-nitrogen in the top two feet at seeding time (approximately 30 pounds per acre).

A mathematical relationship between the quantity of nitrate-nitrogen to two or four feet and yield and nitrogen uptake would be meaningless with only four sites. Intuitively, however, yield and nitrogen uptake appear closely and positively related to the quantity of nitrate-nitrogen in the soil at seeding time. More data are required to adequately determine the correlation between nitrate-nitrogen and yield. Studies conducted in Manitoba on yield response to nitrogen fertilizers have indicated that yield and nitrogen uptake are highly dependent upon the initial nitrate-nitrogen supply to two feet (73).

3.2.3. Quantities of Mineralized Nitrogen

The quantities of nitrogen mineralized at the various dates are reported in Table VI and figures 5, 6, and 7. The values reported represent the net quantity of nitrogen mineralized between seeding and various sampling dates. Minor quantities of nitrogen were mineralized during the first six weeks after seeding (Table VI). Large quantities of nitrogen were mineralized in the Morden Summerfallow plot on the first sampling date, but due to the large error (Table VII), they were probably not significant.

Three factors contribute to the uncertainty in the quantity of nitrogen mineralized:

a) Uncertainty in the original quantity of nitrate-nitrogen in the soil.

b) Uncertainty in the quantity of nitrate-nitrogen in the soil at each sampling date.

c) Uncertainty in the quantity of nitrogen in the crop.

Twenty-four samples from each plot at each depth were taken at seeding time and individual nitrate determinations made on them. Due to the large number of samples, uncertainty in the initial quantity of nitrate-nitrogen measured was not great (see page 60, Table IV).

Error in the quantity of nitrate-nitrogen present at the first sampling date (June 8) was calculated (Table VIII), (samples were bulked for all dates except seeding and June 8). Due to the limited number of replicates, the uncertainty in this value was quite large. In all four plots the variation was greater in the seeded than in the fallow portions. This indicates that the crop had an effect on the nitrate-nitrogen content of the soil in local areas during the first two weeks of growth. This effect may have been on the mineralization-immobilization balance as well as the removal of nitrate from the soil.

Uncertainty in the quantity of nitrogen in the crop was not as great as that in the soil. The quantity of nitrogen in the roots was not determined not was a correction made for it. This resulted in an underestimation of the quantity of nitrogen mineralized in the seeded portions of the plots.

The large quantities of nitrogen mineralized between July 6 and harvest appear to be significant (Table VI and VII).

TABLE VI

QUANTITIES OF NITROGEN MINERALIZED BETWEEN SEEDING AND SAMPLING DATES

(pounds per acre)

DATE	MORDEN a		MORDEN b		ALMASIPPI a		ALMASIPPI b	
	F	S	F	S	F	S	F	S
June 8	62	39	-7	-2	4	0	0	9
June 22	0	23	0	8	2	-2	10	11
July 6	30	-5	-1	0	0	4	2	7
July 20	20	19	35	17	4	3	18	22
Aug. 3	83	28	46	32	33	30	38	22
Maturity	82	24	49	25	23	26	21	16

F fallow portions of the plot

S seeded portions of the plot

a previously fallowed plot

b previously cropped plot

TABLE VII

DELTA VALUES FOR 95 PERCENT CONFIDENCE LIMITS FOR THE QUANTITY OF NITROGEN MINERALIZED

(pounds per acre)

DATE	MORDEN a		MORDEN b		ALMASIPPI a		ALMASIPPI b	
	F	S	F	S	F	S	F	S
June 8	41	64	25	63	13	21	6	17

Lower limit = Mean value - Delta

Upper limit = Mean value + Delta

$$\text{Delta} = \frac{\text{Standard Deviation}}{\sqrt{n}} \times T(n-1, .975)$$

$T = 3.182$

F fallow portions of the plot

S seeded portions of the plot

a previously fallowed plot

b previously cropped plot

TABLE VIII

POUNDS NITRATE-NITROGEN IN THE PLOTS AT THE JUNE 8 SAMPLING DATES

PLOT	AVE. LB. NO_3^-/AC. (0 - 48 inches)	DELTA (LB./AC.)
Morden Summerfallow		
fallow	207 ± 36*	+ or - 36
seeded	181 ± 60*	+ or - 60
Morden Stubble		
fallow	59 ± 23*	+ or - 23
seeded	60 ± 48*	+ or - 48
Almasippi Summerfallow		
fallow	30 ± 12*	+ or - 12
seeded	36 ± 20*	+ or - 20
Almasippi Stubble		
fallow	13 ± 5*	+ or - 5
seeded	36 ± 11*	+ or - 11

* at 95 per cent confidence level

There was a steady increase in the quantity of nitrogen mineralized after July 6 in both the fallow and seeded portions of all four plots. A maximum in net nitrogen mineralization was attained by August 3 followed by a slight decline in some of the plots. (This decline was due to a reduction in the quantity of nitrate-nitrogen in the soil at harvest).

The main flush in nitrogen mineralization started after July 6 and persisted for approximately one month to six weeks. Ferguson[2] reported that during a 30 year study, the main flush in nitrogen mineralization (in fallow soil) occured during the middle of July. Results reported herein are in agreement with those of Ferguson.

The quantities of nitrogen mineralized in the seeded portions of three of the plots were less than in the fallow portions (Table VI). The quantities mineralized in both portions of the Almasippi Summerfallow plot were similar. Considering the quantity of nitrogen in the roots of the crop, net mineralization was probably greater in the seeded than in the fallow portion of the Almasippi summerfallow plot.

3.2.4 Factors Affecting the Quantity of Nitrogen Mineralized

a) Total Organic Nitrogen Content of the Soil

The quantity of nitrogen mineralized in most agriculturally important soils during one crop year is generally equivalent to one to three percent of the total soil organic nitrogen (15, 19). If the quantity of nitrogen mineralized during the growing season could be predicted by the total organic nitrogen content of the soil at a given depth it would facilitate the prediction of nitrogen fertilization requirements. In an attempt to determine the

---

[2] Data presented at Saskatchewan Soil Fertility Conference University of Saskatchewan. 1968.

depth at which most nitrogen mineralization occured, the quantities of nitrogen mineralized in the various plots were compared with the quantities of organic nitrogen to 6, 12, and 24 inches.

The maximum net quantity of nitrogen mineralized in the fallow portions of three plots were equivalent to 1.7 - 2.0 per cent of the total soil organic nitrogen in the top six inches (Table IX). In the Morden Stubble plot a much smaller per cent of the organic nitrogen was mineralized. This was probably due to greater immobilization resulting from the greater quantities of residue left from the previous crop.

A smaller proportion of the organic nitrogen was mineralized in the seeded than in the fallow portions of all four plots (Table IX). The quantity of nitrogen mineralized exceeded one per cent of the total organic nitrogen in the top six inches of soil in only the Almasippi Summerfallow plot. Identical percentages of the total soil organic nitrogen were mineralized in the seeded portions of the two Morden plots but a wide discrepancy occured between the two Almasippi plots.

These data imply that a strong, positive association exists between the quantity of organic nitrogen in a fallow soil and the net quantity mineralized during the summer. The relatively constant value obtained (between soils) when the quantity of nitrogen mineralized is

TABLE IX

PERCENT OF TOTAL ORGANIC NITROGEN MINERALIZED

Pounds of Nitrogen				Percent Mineralized		
0 - 6 inches	0 - 12 inches	0 - 24 inches		0 - 6 inches	0 - 12 inches	0 - 24 inches
Morden Summerfallow						
4,096	6,922	11,290	fallow	2.0	1.2	0.7
			seeded	0.7	0.4	0.2
Morden Stubble						
4,608	8,192	13,184	fallow	1.1	0.6	0.4
			seeded	0.7	0.4	0.2
Almasippi Summerfallow						
1,760	2,816	4,288	fallow	1.8	1.1	0.7
			seeded	1.7	1.0	0.7
Almasippi Stubble						
2,112	3,168	4,640	fallow	1.7	1.1	0.8
			seeded	1.0	0.7	0.5

calculated as a per cent of the total nitrogen to 12 and to 24 inches indicates that nitrogen in the 6-12 and the 12-24 inch depths may have contributed significantly to the total quantity of nitrogen mineralized.

The ratios of organic nitrogen in the 6 - 24 inch depth to that in the 0 - 6 inch depth are: 1.7, 1.8, 1.4 and 1.2 in the Morden Summerfallow, Morden Stubble, Almasippi Summerfallow, and Almasippi Stubble plots respectively. These values are relatively constant. A constant ratio of nitrogen in the 6 - 24 inch depth to that in the 0 - 6 inch depth would result in mineralization, as a per cent of the total nitrogen to 24 inches, being a constant (between soils) even if all the mineralization occured in the top six inches.

It is therefore not possible on the basis of these data to determine if significant quantitiies of the nitrogen in the 6 - 24 inch depth were mineralized.

b) Relationship Between Initial Soil Nitrate-Nitrogen Content and Quantity of Nitrogen Mineralized

In discussing the effect of initial NO_3^--N in the soil on yield, Ferguson[3] postulated that the quantity of NO_3-N in the soil at seeding time may be closely related to the quantity of nitrogen mineralized during the growing season. It is logical to assume that since NO_3-N produc-

---

[3] Data presented at the seventh annual Manitoba Soil Science Meeting, 1963.

TABLE X

INITIAL NITRATE-NITROGEN CONTENTS AND NET QUANTITIES OF NITROGEN MINERALIZED BETWEEN SEEDING AND HARVEST

PLOT	INITIAL NO_3^--N LB./AC. 0 - 24 inches	NET QUANTITY OF N MINERALIZED BY HARVEST fallow	seeded
Morden Summerfallow	117	82	24
Morden Stubble	33	49	25
Almasippi Summerfallow	27	23	26
Almasippi Stubble	9	21	16

tion is the result of mineralization, there should be a close relationship between the quantity of NO_3-N in the profile at seeding time and the quantity of nitrogen mineralized during the growing season.

Results of this experiment do not support this Hypothesis (Table X).

In the fallow portions of the plots the quantities of nitrogen mineralized have the same rank as the intial nitrate-nitrogen contents. With a greater population a meaningful correlation coefficient could be calculated. Identical quantities of nitrogen were mineralized in the seeded portions of three plots whereas their initial nitrate-nitrogen contents varied from 117 to 27 pounds per acre to two feet. These data indicate that there may not have been an association between the quantity of nitrate-nitrogen in the 0 - 24 inch depth at seeding time and the quantity of nitrogen mineralized in seeded or fallow soil during the growing season.

One may expect only a weak association between the initial nitrate-nitrogen content of the soil and the quantity of nitrogen mineralized for several reasons. The quantity of nitrate-nitrogen in the profile at seeding time is the net result of mineralization (and immobilization) during the previous summer and fall plus the quantities of nitrate-nitrogen stored from pervious years. A similar quantity may not be mineralized in following years. The quantity of nitrogen mineralized in cropped soil after fallowing may not be the same as that mineralized during the fallow year. Environmental variations and fluctuations in the mineralization-immobilization balance from year to year will alter the net quantities of nitrogen mineralized from one year to another.

c) Relationship Between Nitrogen Uptake and Nitrogen Mineralization

Only minor quantities of nitrogen were mineralized before July 6 in both the seeded and fallow portions of all four plots (Table VI). Nitrogen uptake by the crop started shortly after June 8. Before the main flush in net nitrogen mineralization started, a considerable quantity of nitrogen had been assimilated by the crop (fig. 5, 6, 7, and 8). Nitrogen uptake started four to six weeks prior to the main flush in mineralization. The per cent of maximum nitrogen uptake occuring before net nitrogen mineralization commenced varied from a low of 42.4 per cent in the Almasippi Summerfallow plot to a high of 64.5 per cent in the Morden Summerfallow plot.

Before the main flush in nitrogen mineralization started, at least 50 per cent of maximum nitrogen uptake had occured. The potential for yield and nitrogen uptake by a cereal is determined at an early stage of development (81, 82, 83). A high dependence of crop yield and nitrogen uptake on the initial nitrate-nitrogen content of the soil would therefore result from the late start in net nitrogen mineralization. The potential dry matter yield and nitrogen uptake was probably determined prior to the main flush in nitrogen mineralization. Mineralized nitrogen was important only as a source of a portion of the nitrogen required to complete the growth and development of the crop.

Substantial net nitrogen mineralization occured during only a relatively brief period of time. The main flushes in nitrogen mineralization lasted for a period of two to four weeks (Fig. 5, 6, 7, and 8). This is probably due to the mineralization-immobilization relations existing in the soil system. The development of toxins in the soil as a result of microbial activity (27, 29) or a marked

TABLE XI

NITROGEN UPTAKE AS A PERCENT OF MAXIMUM AT START OF MAIN FLUSH IN MINERALIZATION

PLOT	DATE AT WHICH FLUSH IN MINERALIZATION STARTED	NITROGEN UPTAKE AS A PERCENT OF MAXIMUM
Morden Summerfallow	July 6	64.5
Morden Stubble	July 6	55.7
Almasippi Summerfallow	July 20	42.4
Almasippi Stubble	July 6	52.0
	Average = 54.9	

reduction in the supply of soluble organic matter to the ammonifiers due to the rapid microbial activity (18) may have curtailed the flush in net nitrogen mineralization. A combination of the above-mentioned factors may have interacted to produce the observed result.

Harmsen and VanSchreven (44) in reviewing nitrogen mineraliztion states that the main flush in nitrogen mineralization occurs in the early spring and supplies a considerable portion of the nitrogen required by the growing crop. The results of this investigation, and those of Ferguson (page 73), are contrary to those reviewed by Harmsen. Most of the research pertaining to nitrogen mineralization has been conducted in the more humid temperate regions and very little information regarding this process on the prairies is available. The different climatic conditions in these two regions may have caused the variation in results. Cooler spring temperatures on the prairies may result in an increased lag phase in net nitrogen mineralization. Further research is required to clarify this point.

d) Effect of a Growing Wheat Crop on the Quantity of Nitrogen Mineralized During the Growing Season

During the latter portion of the growing season smaller quantities of nitrogen were mineralized in the seeded than in the fallow portions of three plots (fig. 5, 6, and 8). In the Almasippi Summerfallow plot (fig. 7). the quantities of nitrogen mineralized in the seeded portions were almost equivalent on all dates (fig. 7). The net quantities of nitrogen mineralized in the seeded portions of the Morden Summerfallow, the Morden Stubble, and the Almasippi Summerfallow plots were almost identical (Table VI, page 71). Net nitrogen mineralization between seeding and the harvest sampling date was calculated on the basis of the quantity of nitrogen in the crop on the August 3 sampling date. During the period between August 3 and harvest the crop lost nitrogen. It was assumed that this nitrogen was not present in the soil in the form of

FIG. 5 NITROGEN UPTAKE AND MINERALIZATION

ALMASIPPI STUBBLE

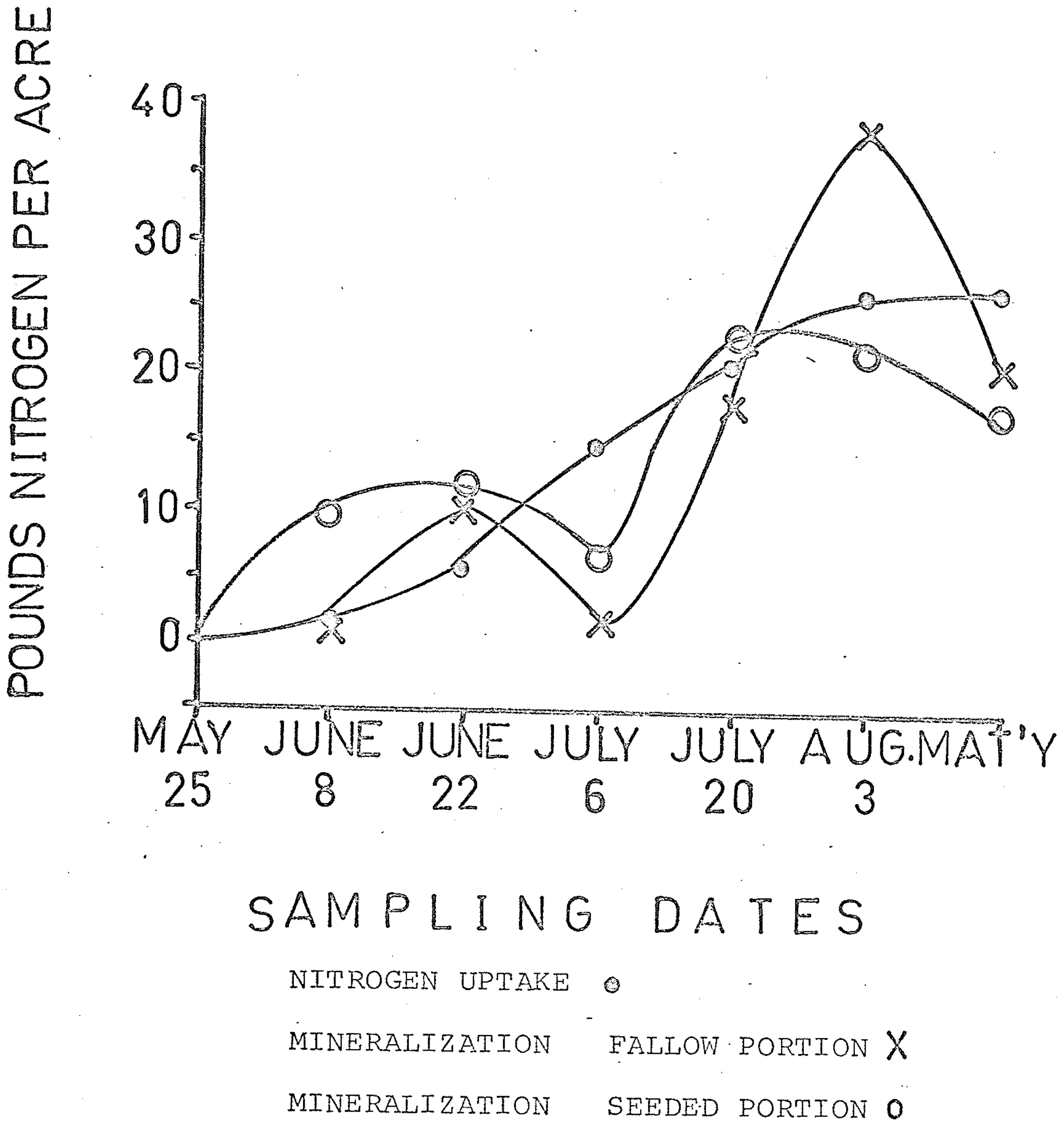
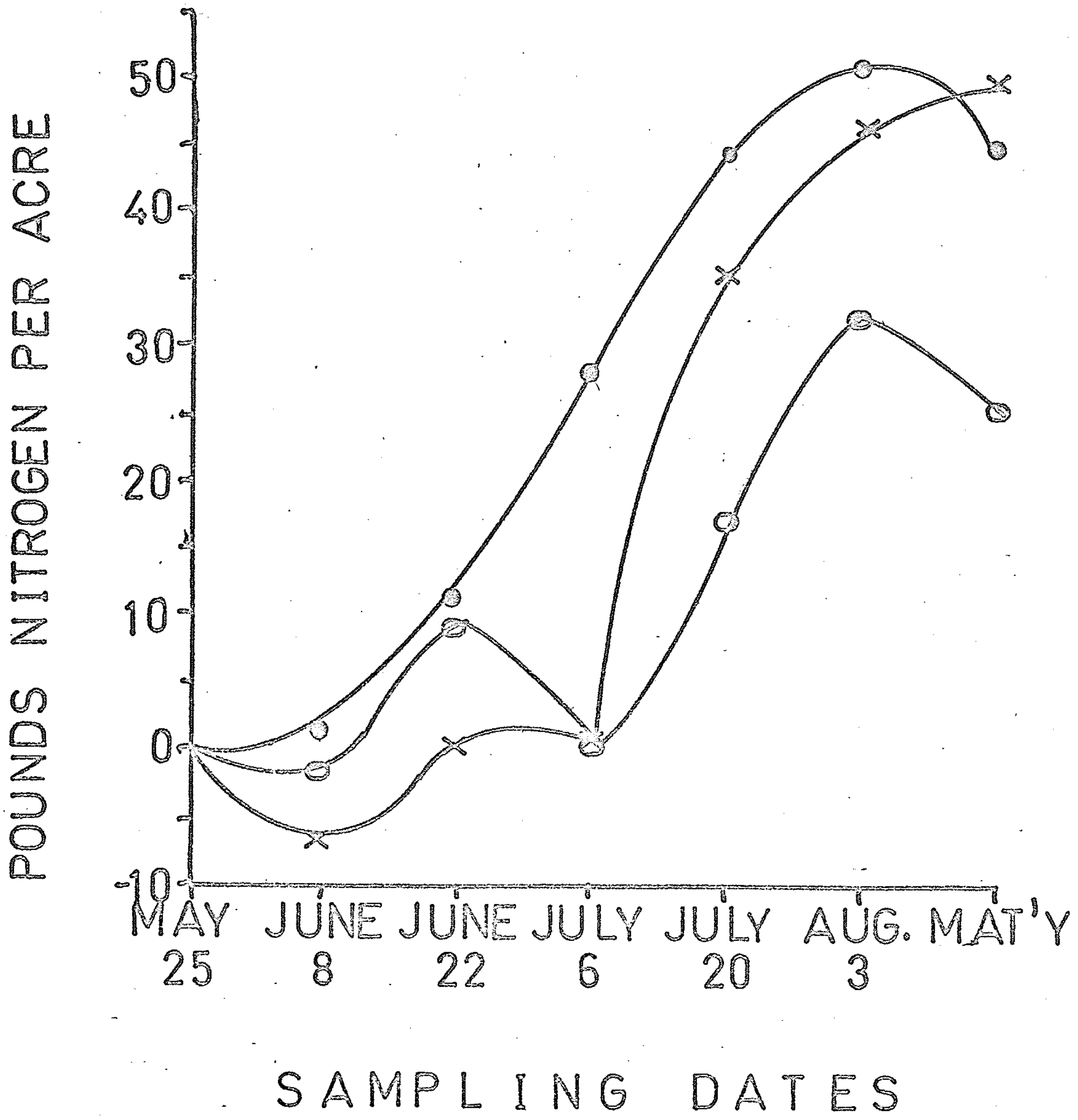


FIG. 6 NITROGEN UPTAKE AND MINERALIZATION

MORDEN STUBBLE



NITROGEN UPTAKE ●

MINERALIZATION FALLOW PORTION X

MINERALIZATION SEEDED PORTION O

FIG. 7 NITROGEN UPTAKE AND MINERALIZATION

ALMASIPPI SUMMERFALLOW

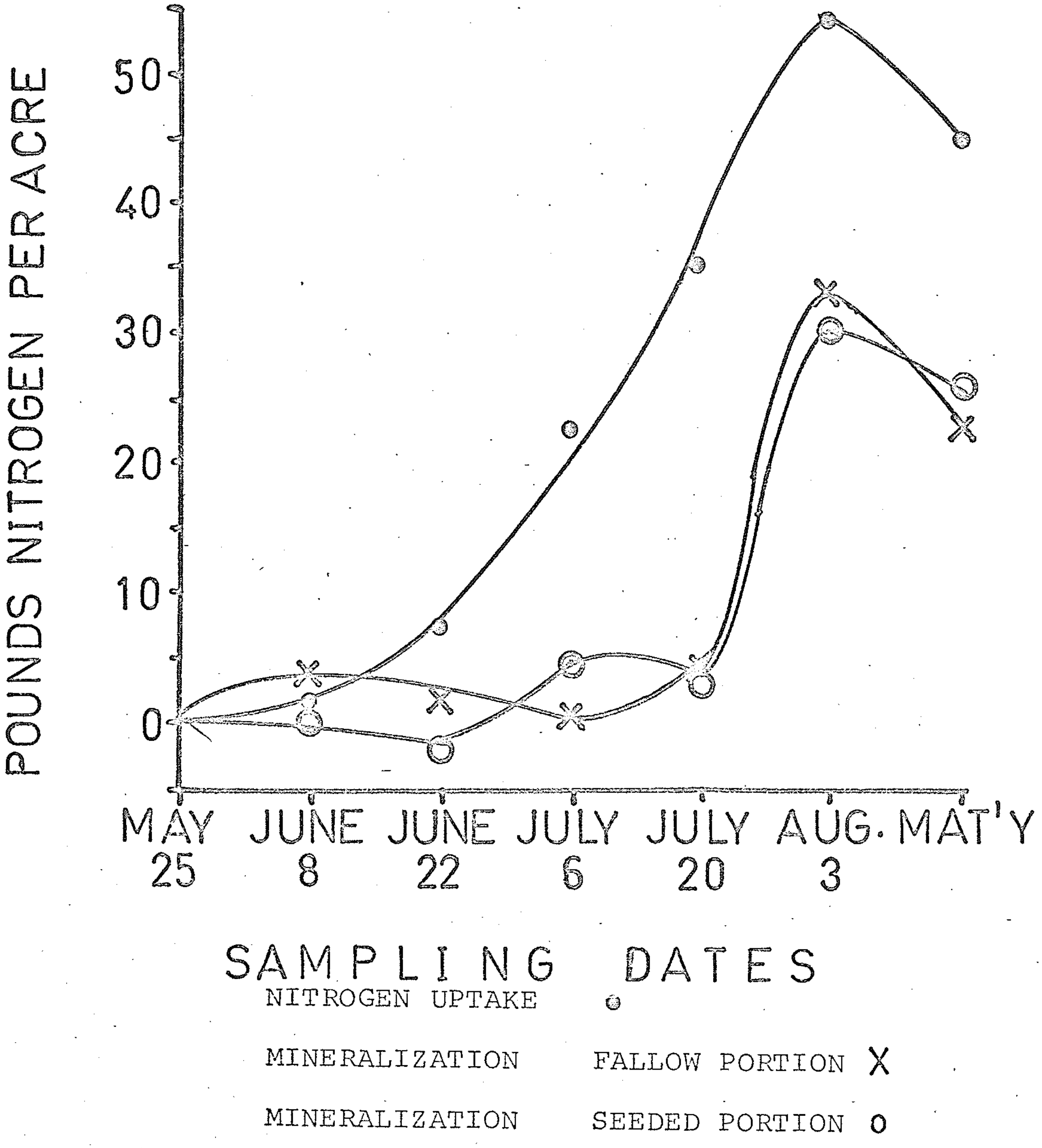
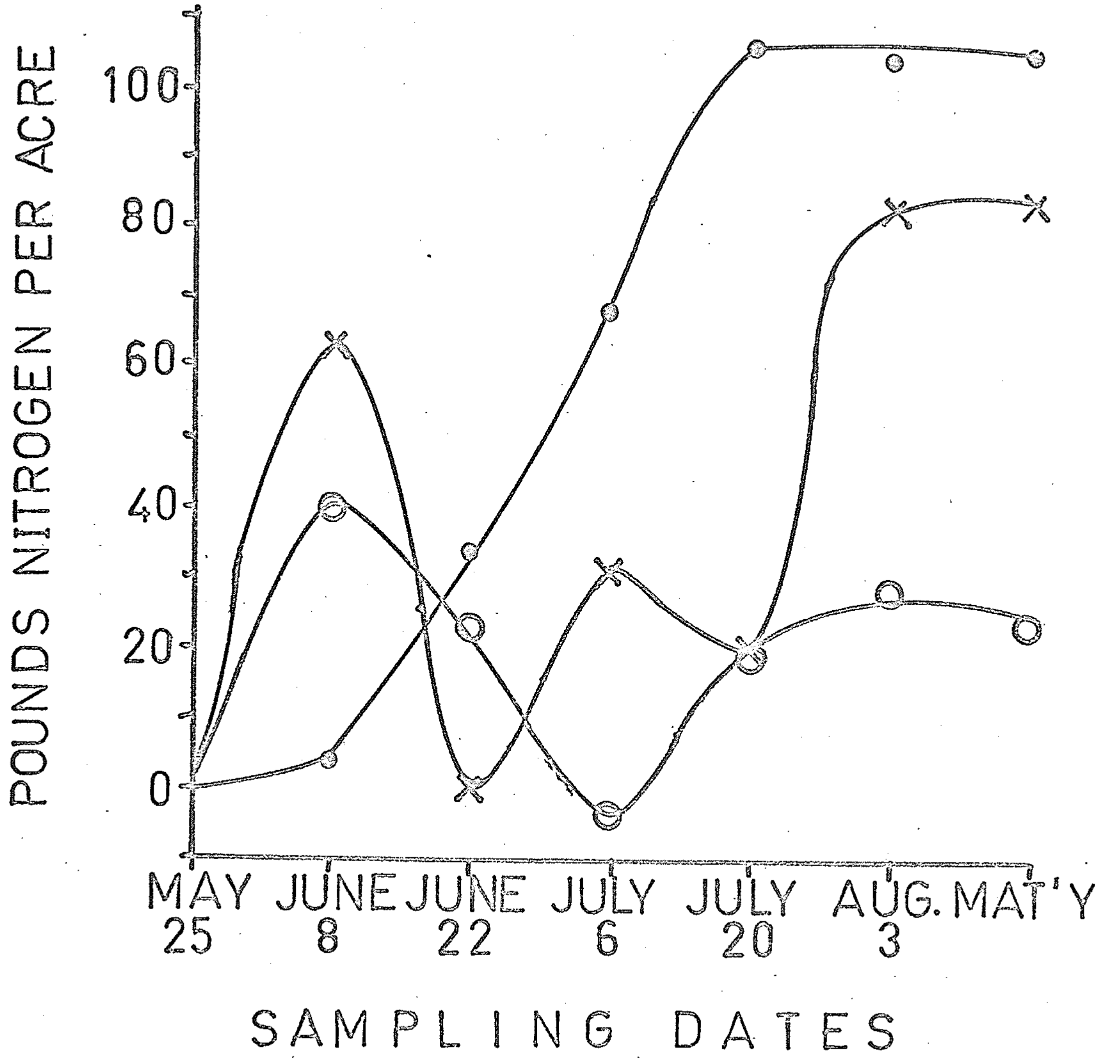


FIG. 8 NITROGEN UPTAKE AND MINERALIZATION

MORDEN SUMMERFALLOW



NITROGEN UPTAKE ●

MINERALIZATION FALLOW PORTION X

MINERALIZATION SEEDED PORTION O

nitrate or ammonium-nitrogen and that using the August 3 value for the quantity of nitrogen in the crop was therefore justified. Even with this method of calculating net nitrogen mineralization at harvest the value obtained was considerably less than that obtained on August 3 in the seeded portions of the plots.

During the early stages of plant growth an increase in net nitrogen mineralization in the seeded relative to the fallow portions of the plots occured at the Morden Stubble and the Almasippi Stubble sites. The crops on the previously fallowed sites had no consistent effect on net mineralization during the early stages of growth (Table VI: fig. 5, 6, 7 and 8).

The reduced net quantity of nitrogen mineralized by harvest in the seeded relative to fallowed soil is important in determining fertilizer requirements in predicting yield. The similarity in the quantities of nitrogen mineralized in the cropped portions of the plots will also have implications in soil testing and in determining fertilizer requirements. On the basis of only one year's results and only four plots it is not possible to establish limits for the probable quantities of nitrogen mineralized in soil seeded to wheat.

Reduced net nitrogen mineralization during the growing season in seeded relative to fallow soil has been reported by several workers (16, 28, 40, 44, 74, 77, 80).

Results reported herein are in accordance with those in the literature. The reduction in mineralization due to a crop has generally been attributed to increased immobilization resulting from microbial degradation of sloughed off root material (16, 40).

Denitrification may also be a factor due to reduced oxygen tension in the root zone (44, 67). Theron (73) has also postulated that perennial grass roots inhibit nitrification. The latter two possibilities probably do not contribute significantly to the observed reduction in net nitrogen mineralization (9, 16, 17, 32).

The quantity of nitrogen immobilized (calculated as the quantity of nitrogen mineralized in the fallow portions of the plots minus that in the seeded portions) would be expected to be proportional to the quantity of root material in the soil and hence to the crop yield. With the Morden soil, immobilization was 2.42 times greater in the seeded portion of the Summerfallow plot than in the seeded portion of the Stubble plot while yield was 1.74 times as great. These values are similar and indicate that in this soil immobilization in the seeded portions of the plots may have been proportional to yield. In the Almasippi soil the respective ratios were 0.60 and 1.69 indicating that no proportionality existed between yield and the quantity of nitrogen immoblized. Future investigations into introgen mineralization under field conditions should examine this aspect of net nitrogen mineralization closely. If the reduction in net nitrogen mineralization due to crop growth can be shown to be proportional to yield, and a proportionality constant developed, it will facilitate predictions of fertilizer requirements.

During the first two to four weeks the wheat crop caused an apparent increase in net nitrogen mineralization in the Morden Stubble and the Almsaippi Stubble plots. The crop had no apparent effect on net nitrogen mineralization on the previously fallowed sites.

Although these values may not be statistically significant, they do exhibit a definite trend and do warrant some discussion. An increase in net nitrogen mineralization during (and resulting from) the early stages of plant growth would have important implications in the nitrogen nutrition of the crop. Goring and Clark (40) detected increased net nitrogen mineralization during the first four to five weeks of growth.

This effect may be due to:

a) A stimulation of nitrogen mineralizing organisms during the early stages of plant growth.

b) A reduction in nitrogen immobilization due to the removal of mineral nitrogen from the soil by the crop.

Substantial quantities of carbonaceous residues had been incorporated into the soil of the Morden Stubble

Leaf 89 missing in page numbering.

growing crop reduces nitrogen immobilization. As plants mature, the C:N ratio of sloughed-off root material increases. During the latter stages of plant development nitrogen uptake by a crop is reduced thus leaving the mineral nitrogen vulnerable to assimilation by the heterotrophic microflora. Nitrogen immobilization therefore increases as the plants mature until by harvest the net quantity of nitrogen mineralized in the cropped soil is very much less than in fallow soil.

3.2.5. Rooting Depth and Zone of Nitrogen Mineralization

It was felt that by comparing the soil moisture contents at the various depths in cropped and fallow soil an indication of the rooting depth and feeding zone of the crop could be obtained. In the Almasippi soil, however, the moisture contents of the seeded and fallow portions of the plots were similar to each other throughout the entire summer (See Appendix I Tables III and IV) thus making it impossible to accurately determine the feeding zone of the crop. Data and discullion on rooting depth will therefore be restricted to the Morden soil.

In both Morden plots measureable moisture removal from the top six inches occured within four weeks (Table XII and XIII). Within six weeks (July 6) significant moisture removal had occured to a depth of two feet in both plots. In the Morden Stubble plot, root penetration could not be definitely proven to exceed two feet. In the Morden Summerfallow plot, significant root penetration (moisture removal) had occured to a depth of three feet by July 20 (eight weeks) and to four feet by August 3 (ten weeks). The restricted root penetration in the Morden Stubble plot probably resulted from a reduced demand for moisture in this plot relative to the previously fallowed soil. Crop grwoth on the fallowed soil was more profuse and therefore required more moisture than on the Stubble plot. Structural peculaiarities probably do not account for the restricted rooting in this plot because its texture was the same as that of the fallowed soil and during sampling no structural variations were observed between the two plots.

These data indicate that for at least the first four to six weeks the top one to two feet of soil were most important in the nutrition of the growing crop. These data apply to only one soil type and one set of environmental conditions. Root penetration in different soils and under different environmental conditions may follow a somewhat different pattern. Notwithstanding these limitations, the top one to two feet of soil are probably very important in plant nutrition.

Data on the quantities of nitrate-nitrogen at the various depths (fig. 9, 10, 11, 12) also support this conclusion. In the fallow portions of the plots the first major increase in nitrate-nitrogen content occured in the top two feet and was generally followed by an accumulation in the third and fourth foot levels two to four weeks later. In the seeded portions of the plots the only major nitrate-nitrogen accumulation occured in the top two feet and no appreciable accumualtion occured in the third and fourth foot levels.

Tracing the movement of nitrogen in the profile without isotopic nitrogen was practically impossible. Further examination of fig. 9, 10, 11, and 12 indicates that the second foot depth is probably not as important as the first in nitrogen mineralization. Most nitrate-nitrogen accumulation in the fallow portion of the plots appears to occur in the top foot prior to any build up in the second foot. Whether the build up in the second foot is due to leaching or to delayed mineralization in that region cannot be established on the basis of data presented herein. Sampling error in the top two feet may have been great enough to cause the observed differences between the first and second foot depths. It is therefore not possible to reach any definite conclusions concerning the differences in nitrate-nitrogen accumulation in either of the top two one foot depths.

It appears that root penetration did not exceed 12 to 18 inches in the first month of growth. This places special significance on the quantity of mineral nitrogen

TABLE XII

SOIL MOISTURE CONTENT ON FIVE DATES IN THE SEEDED AND FALLOW PORTIONS OF THE MORDEN STUBBLE PLOT

(Percent Oven Dry Weight)

		DEPTH (INCHES)					L.S.D.	
DATE	TREAT.	0 - 6	6 - 12	12 - 24	24 - 36	36 - 48	p = .95	p = .99
June 22	fallow	24.8	*23.2	23.4	28.2	32.1	2.27	2.76
	seeded	18.6	22.2	22.0	24.5	29.0		
July 6	fallow	25.5	23.4	22.3	26.3	30.0	2.26	2.72
	seeded	17.2	15.0	18,4	25,3	29.0		
July 20	fallow	24.8	22.9	21.7	25.3	28.9	3.22	3.88
	seeded	8.8	12.4	15.7	22.2	26.8		
Aug. 3	fallow	19.1	20.7	12.7	24.8	28.5	2.43	2.92
	seeded	8.2	11.9	16.1	23.9	28.8		
Maturity	fallow	25.8	23.2	22.3	26.4	29.2	2.16	2.59
	seeded	14.7	14.2	18.4	26.8	29.3		

*Significant difference (at 99 per cent level) between seeded and fallow portions of the plot for depths to left of line

TABLE XIII

SOIL MOISTURE CONTENT ON FIVE DATES IN THE SEEDED AND FALLOW PORTIONS OF THE MORDEN SUMMERFALLOW PLOT

(Percent Oven Dry Weight)

		DEPTH (INCHES)					L.S.D.	
DATE	TREAT.	0 - 6	6 - 12	12 - 24	24 - 36	36 - 48	p = .95	p = .99
June 22	fallow	23.2	*20.3	23.9	24.3	26.7	4.41	6.09
	seeded	14.4	19.4	22.3	24.9	27.4		
July 6	fallow	24.2	18.9	23.3	23.6	26.1	3.72	4.47
	seeded	18.1	13.4	14.8	22.7	24.1		
July 20	fallow	26.6	19.9	20.9	24.5	25.3	4.40	5.29
	seeded	8.7	10.3	11.5	17.2	22.8		
August 3	fallow	18.6	21.2	21.9	23.5	25.7	3.95	4.76
	seeded	9.2	8.8	10.9	15.0	15.4		
Maturity	fallow	20.8	17.4	20.4	21.7	26.5	3.87	4.66
	seeded	12.2	9.2	12.4	16.4	23.4		

*Significant difference (at 99 per cent level) between seeded and fallow portions of the plot for depth to left of line.

FIG.9a NITRATE-NITROGEN DISTRIBUTIONS
MORDEN SUMMERFALLOW FALLOW PORTION

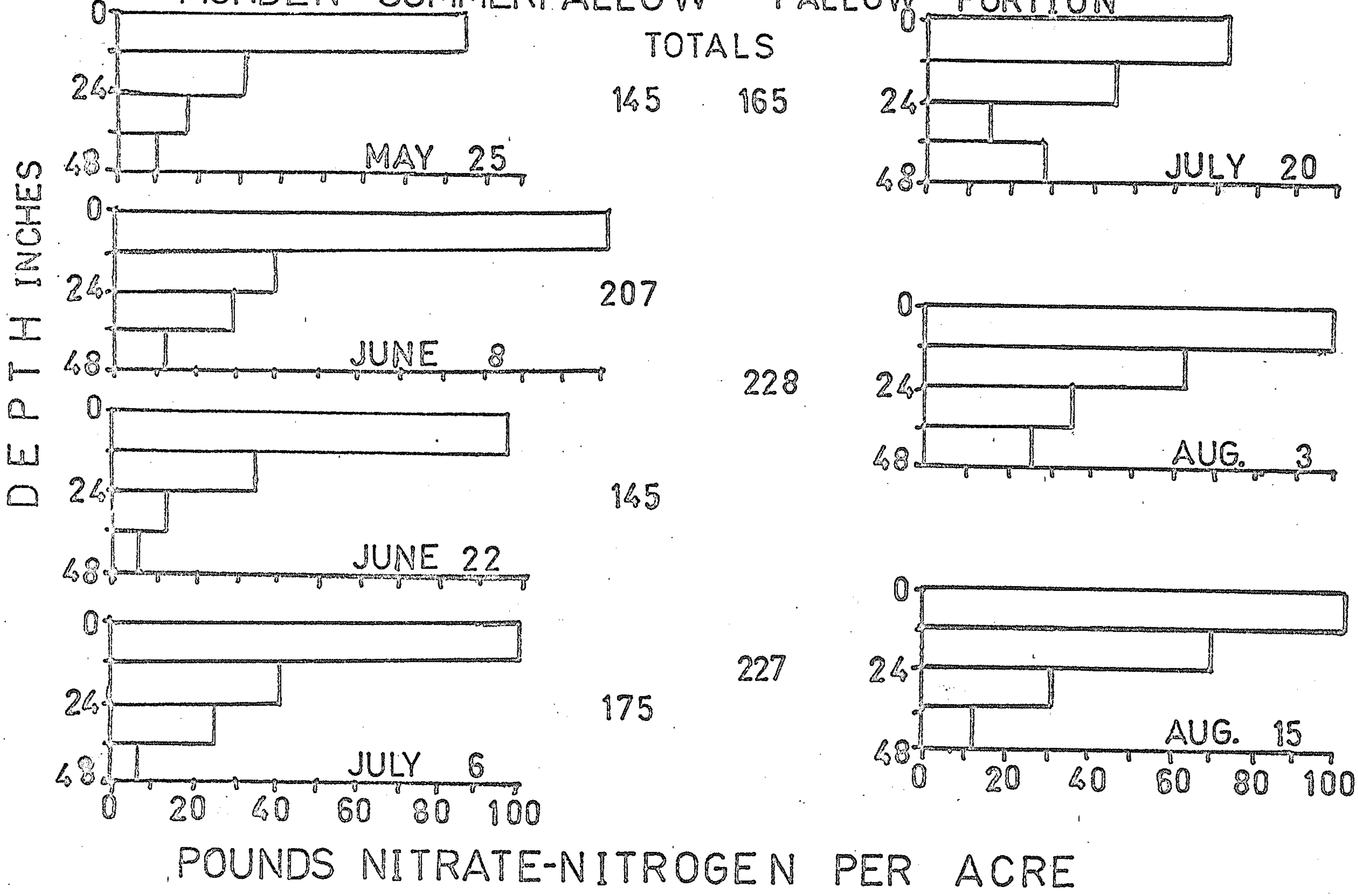


FIG.9b NITRATE-NITROGEN DISTRIBUTIONS MORDEN SUMMERFALLOW SEEDED PORTION