UTILIZATION AND LOSS OF NITROGEN FROM AN ORGANIC SOIL PROFILE

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This is to certify that the

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ABSTRACT

UTILIZATION AND LOSS OF NITROGEN FROM AN ORGANIC SOIL PROFILE

By

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The utilization and loss of N from an organic soil profile was studied under field and greenhouse conditions. Seven field experiments were initiated to determine the effects of N fertilizer materials and application rates on plant tissue yield, NO_3^--N concentration, and uptake in lettuce, carrots and spinach grown on a Houghton muck soil. These effects were correlated to the different climatic conditions which affected the soil NO_3^--N content.

The effect of moisture (field capacity, 1/2-saturated and saturated) and temperature (10 C and ambient) on the leaching losses of organic-N and NO_3^- -N from a virgin muck soil was studied in the greenhouse under various soil management regimes.

The NO_3^-N concentration in plant tissue increased with increasing soil NO_3^-N concentration for all three crops. Soil NO_3^-N concentration was increased by fertilization. But increased rainfall accompanied by a high water table reduced the soil NO_3^-N levels especially at high fertilization rates. This was attributed to NO_3^-N leaching followed by denitrification. Maximum plant yield was obtained with addition of 180 Kg N/ha and in many cases higher levels suppressed yields. Fall grown spinach contained much more NO_3^-N than spring grown spinach.

In the greenhouse study, the soil NO_3^--N content was highest in the field capacity moisture treatment and in the ambient (28 C) temperature treatment, indicating the more favorable climatic conditions for the biological breakdown of organic matter to NH_4^+-N and its subsequent oxidation to NO_3^--N . Soil NH_4^+-N content was highest in the saturated moisture treatment and in the ambient temperature treatment, indicating the dependence of biological nitrification upon aerobic climatic conditions.

The leachates from the cores under the field capacity moisture treatment and 10 C temperature treatment contained the highest NO_3^--N content. This indicates that biological denitrification in the lower profile layers is decreasing the leaching losses of NO_3^--N from the soil cores which had the highest soil NO_3^--N content.

Adding inorganic-N fertilizer materials to the soil core surface and shortening the incubation period from 21 to 4 days greatly increased the leachate NO_3^--N content.

The cores under the 1/2-saturated moisture treatment and 10 C temperature treatment leached the highest total NO_3^--N . This is an indication that the increased soil NO_3^--N content of the upper layers and the increased water content of the lower layers of the 1/2-saturated profile causes an increased loss of total NO_3^--N .

The leachate organic-N content was highest from the saturated moisture treatment and ambient temperature treatment, indicating the incomplete breakdown of organic matter to NH_4^+ -N and reduced organic compounds. Additions of inorganic fertilizer nitrogen and shortening the incubation period did not change the organic-N content in the leachate.

UTILIZATION AND LOSS OF NITROGEN

FROM AN ORGANIC SOIL PROFILE

By

Jerry Burt Fitts

A THESIS

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To Betsy

I dedicate this thesis to my wife. Her love, encouragement and sacrifice made this work possible.

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INTRODUCTION

The element nitrogen (N) makes up approximately 0.03% of the earth's crust and atmosphere (Sisler, VanderWerf, and Davidson, 1961). Of this amount, 97.82% is found in fundamental rocks, 1.96% in the atmosphere, and 0.2% in ancient sedimentary rock. Negligible amounts are found in terrestrial humus, sea-bottom organic compounds, and living organisms (Stevenson, 1965).

Julius von Sacks (1860) was the first to recognize that N was necessary for plant growth. In a table of relative number of atoms of essential elements in alfalfa, N is at the top of the list of those elements that come from the soil or fertilizers, and is exceeded only by hydrogen (H), carbon (C), and oxygen (O), all of which the plant obtains from the atmosphere (Viets, 1961).

In soil-plant nutrition relations, N is unique. Too little N limits yield and reduces quality. Worldwide, more crops are deficient in N than any other nutrient. On the other hand, too much N can lower yield, reduce quality, cause lodging, and can make forage toxic to animals (Viets, 1965).

Recently, Kohl, Sheaver, and Commoner (1971) have presented evidence indicating that fertilizer N is a major pollutant in the nation's waterways, and have raised the question of the effect on public health concerning excess N, in the form of nitrate (NO_3) ,

in these waters. In at least one state, regulations on the rate and method of applying fertilizers have been proposed.¹

In Michigan, for 1969-71, a yearly average of approximately 110,169 metric tons of N was applied to agricultural crops. Fresh and processed vegetables and potatoes received an average of 112 and 168 kilograms of N per hectare (Kg N/ha) respectively.² In an extension study in 1971, it was found that fresh market celery grown on organic soils received from 168 to 269 Kg N/ha.³

This thesis reports the utilization and loss of N from an organic soil profile under different field and greenhouse conditions.

¹Controversy builds over fertilizer runoff. Chem. & Eng. News Jan. 10, 1972. p. 17-18.

²Data from the Crop Reporting Service and the Michigan Department of Agriculture.

³Fitts, J. B. The Nitrate Electrode: Its Use in Soil and Plant Tissue Tests. 1971.

LITERATURE REVIEW

Organic Soil-Forming Factors

An organic soil, sometimes referred to as peat or muck, is defined as a soil containing 20 to 30% or more organic matter, one foot or more in depth. It is formed under water or during cool, wet climatic conditions.

There are approximately 4.5 million acres of organic soils in Michigan, an area equal in size to the state of New Jersey. Less than 5% of this acreage is presently farmed, although many of these soils are suited for agricultural purposes and can be developed.

Climate, vegetation and parent material are the factors active in the development process, called paludification, of an organic soil.

Glaciation is the most important climatic agent in the paludification process. Three types of distribution patterns of organic soils can be attributed to glacial action.

(A) <u>Dendritic</u>.--This group includes organic soil formed in glacial beds, small depressions and low areas. An example is the St. Johns area in Clinton County, Michigan.

(B) <u>Lake Bed</u>.--Relatively large continuous areas were formed as a result of filling in of a shallow lake. The Gun Swamp area in Allegan County, Michigan is an example of this type.

(C) <u>Tilting of the Earth's Surface</u>.--An early outlet of the Grand River in the vicinity of Grand Rapids, Michigan was closed because of tilting of the land surface, and a new channel was developed to Grand Haven, Michigan. A large acreage of organic soils was formed in this abandoned channel.

The type of vegetation also plays an important role in paludification. The different plant associations which led to the organic soil formation can be divided into four groups.

(A) <u>Bogs</u>.--These peat soils are formed from acid tolerant plants from the genera including <u>Chamaedophne</u> (leatherleaf), <u>Vacinium</u>
(blueberry), <u>Ledum</u> (Labrador-tea), <u>Andromeda</u> (bog-rosemary), <u>Oxycoccus</u>
(cranberry), Ericaceae (heath), and <u>Sphagnum</u> (moss).

(B) <u>Marsh</u>.--This group includes: <u>Typha</u> (cattails), <u>Juncus</u> (rushs), <u>Phragmites</u> (reeds), <u>Carex</u> (sedges), and several genera of grasses.

(C) Forest or Swamp Peat.--Woody plants comprise this group. Included are: Larix (tamarack), Picea (spruce), Fraxinus (ash), Ulmus (elm), Acer (maple), Populus (poplar and aspen), Betula (birch), Almus (alder), Salix (willow) and others with an admixture of Calluna (heather), Oxycoccus (cranberry), Andromeda (bog-rosemary), Carex (sedge), and Phragmites (sedge).

(D) <u>Aquatic or Sedimentary Peat</u>.--This group includes organic matter from algae, <u>Potamogeton</u> (pondweed), <u>Nymphaea</u> (waterlily), and other water plantain (Davis and Lucas, 1959).

Water has been referred to as the parent material of organic soils. Weber (1903) pointed out that the mineral content of water

associated with paludification and plant associations determine the organic soil properties. Based on the water mineral content, three types of peat are recognized. (Table 1.)

1. Eutrophic. The water associated in this soil group is high in mineral nutrients, especially phosphorus (P), calcium (Ca), and magnesium (Mg). This permits plants to grow that require relatively high fertility levels.

2. Oligotrophic. This organic soil group formed under water that is low in plant nutrients, thus restricting the plants to those requiring low fertility.

3. Mesotrophic. This soil group is in between the eutrophic and oligotrophic groups.

The organic soils which are agriculturally developed in Michigan belong to the marsh plant association and the eutrophic water parent material. The classification of the organic soil which will be considered in this thesis is a Houghton muck. The 0-15 cm layer is fibrous material, being developed from marsh plant association. The 15-107 cm layer is finely fibrous and is a dark brownish yellow in color. The overall soil profile ranges from 3 to 6 m in depth, and the pH range is 6.0 to 6.4 (Davis and Lucas, 1959).

Organic Soil Decomposition

Decomposition of an organic deposit takes place even though the peat may be submerged for indefinite periods in water and is completely cut off from air. Under these conditions, the original cellulose softens and the ligning turn yellow. These changes are probably due

Constituent	Highmoor or Transition or ent Oligotrophic mesotrophic peats peats			
		mg/1		
Organic matter	249.2	73.7	170.5	
Ignition residue	23.8	73.2	141.9	
Nitrogen	3.6	2.5	2.7	
CaO	1.6	22.7	58.7	
MgO	0.6	3.9	9.2	
к ₂ 0	4.4	2.7	3.9	
Na ₂ 0	4.5	6.2	8.0	
P ₂ O ₅	0.6	1.0	1.3	
^{SO} 3	0.9	15.9	3.0	
Fe203-A1203	0.5	5.2	6.0	
sio ₂	9.1	13.2	4.4	
C1	12.3	6.6	11.3	

Table 1.--Chemical composition of peat waters.*

*Weber (1903).

to hydrolysis and decomposition by anaerobic organisms. This decomposition is termed "dy formation."

When the organic material is exposed to air, a different decomposition process, humification, takes place. The lignin, cellulose, other carbohydrates and proteins are oxidized at different rates to form humus. During humification the C:N ratio is reduced. When dy is formed, the C:N ratio is not significantly changed. Peats, which have been drained either naturally or artificially, show the humification process best. Maximum humification takes place at the surface with the maximum dy formation occurring below the permanent water table, with a transition zone lying in between (Davis and Lucas, 1959).

Microorganisms play a very important role in the organic soil decomposition. Waksman (1942) has stated that microbes contribute to the chemical transformations leading to soil development and decomposition in at least three ways.

First during the early stages of plant residue decomposition, certain groups, mainly fungi and actinomycetes on the soil surface and bacteria below the surface, decompose cellulose, hemicellulose, and some proteins.

Second, after the initial decomposition, facultative and obligative anaerobic bacteria further decompose the celluloses, proteins, and other complexes. Major characteristics of this anaerobic decomposition are:

1. Incomplete breakdown of substrate into organic acids, and methane, H_2 and CO_2 gases with low energy yield.

Inefficient assimilation of substrate carbon, with only
to 5% being incorporated into microbial tissue compared to 20-40%
assimilation in the third group below.

3. Low N requirement leading to a net accumulation of ammonium (NH_{4}^{+}) in the soil (Patrick and Mikkelsen, 1971).

Thirdly, when an organic soil is drained and air is admitted, fungi, aerobic bacteria, and actinomycetes become active in the decomposition of the more resistant organic complexes, such as lignins. These organisms are responsible for the biological oxidation or subsidence of an organic soil. The final end products of decomposition include: CO_2 , H_2O , NO_3^- , sulfates (SO_4^-) , and other minerals.

Waksman and Stevens (1929) have reported that in high-lime peats (pH 4.6 or above), the aerobic bacteria population diminished rapidly with increasing soil depth. The anearobic bacteria population, however, increased with soil depth.

Soil N Accumulation

Soil N may be represented diagrammatically as two opposing processes, incoming and outgoing (Allison, 1965). Incoming processes that were important for soil N accumulation include: biological fixation, both symbiotic and non-symbiotic, of atmospheric N; atmospheric precipitation; and N leached from surrounding upland soils. Of these three processes, biological fixation of atmospheric N is probably the most important N source (Davis and Lucas, 1959; Stevenson, 1965).

Under a natural ecosystem, the soil N content approaches an equilibrium value. The magnitude of this value depends upon several

factors: climate, vegetation, topography, physical characteristics of the soil, and the activities of the microflora and microfauna organisms. Of these factors, the soil microorganisms are the most important in soil N accumulation. Soil N can be maintained at high levels only when microbial activity is arrested during some period of the year. Three factors (restricted drainage, low pH, and low temperatures) are most important in restricting microbial (decomposition) activities (Stevenson, 1965).

Using the formation of NO_3^-N as an indicator of organic soil decomposition, the effects of these three factors can be seen.

Waksman and Purvis (1932) in a laboratory study using 20 g soil samples having 67,200, and 300 percent moisture, obtained 22, 1.7, and 1.0 mg NO_3^-N respectively after a 20 day incubation period.

Work by Turk (1939) on two acid organic soils (pH 4.3 and 3.4) showed that the nitrifying capacity of both soils was increased about fourfold by liming.

Data from a Houghton muck soil showed that the NO_3^--N production is related to temperature. Using an 8 week incubation period, 31 and 136 ppm NO_3^--N were produced from 5 C and 22 C treatments, respectively. One non-biological factor does influence the net release of NO_3^--N from an organic soil. A low total N content, usually placed at 1.5% or less, gives the organic soil an unfavorable C:N ratio, such that all of the mineral N available is utilized by the microbial population, thus there is no net accumulation of NO_3^--N . The total N content of peat and muck soils varies between 0.3 and 4.0 percent. One organic deposit 3 m in depth has been reported to contain 224 tons (m) N/ha.

Based on a period of 5,000 years for development, this is an average yearly accumulation of 45 kg N/ha (Davis and Lucas, 1959).

Nitrogenous Compounds and Their Transformations

The N present in organic soils is practically all in organic forms, although measurable quantities of NO_3^--N are present in drained soils, and measurable quantities of NH_4^+ may be found in flooded soils (Davis and Lucas, 1959).

Based on the treatment of soils with hot acids, several investigators have reported that 20 to 40% of the total N in most surface soils is in the form of bound amino acids (Bremner, 1949; Cornfield, 1957; Keeney and Bremner, 1964; Kojima, 1947; Rendig, 1951; Sowden, 1956; Stevenson, 1954, 1956; Young and Mortensen, 1958). Another 5 to 10% is in the form of combined hexosamines (amino sugars), mainly glucosamine and galactosamine (Bremner, 1958; Bremner and Shaw, 1954; Keeney and Bremner, 1964; Sowden, 1959; Stevenson, 1957a, 1957b, 1957c). As indicated by the present evidence, these amino acids and amino sugars are not "free" in the soil, but their mode of linkage in soils has not been established (Bremner, 1965, 1967).

Purine and pyrimidine derivatives have been reported, but current evidence indicates that they account for less than 1.0% of the total N in surface soils (Adams, Bartholomew, and Clark, 1954; Anderson, 1958, 1961). Other organic compounds containing N, such as choline, creatinine, and allentoin, have been isolated, but there is no evidence to indicate that these compounds contribute significantly to the total soil N (Bremner, 1965). Identifiable (by acid hydrolysis) organic N forms account for only 25 to 50% of the total N in organic soils, leaving a substantial balance, including minor forms, of 45 to 70% unidentified (Black, 1968).

Two terms are commonly used to describe soil N transformations: N mineralization, and N immobilization. Mineralization refers to the change of organic-N to mineral-N forms without distinction among the several forms of mineral-N as end products. Immobilization refers to the change of mineral-N forms to organic-N forms (Black, 1968).

The first step in microbiological mineralization of organic-N is NH_4^+ formation. This transformation is carried out by many different organisms during organic matter decomposition, either under anaerobic or aerobic conditions.

The decomposition rate under anaerobic conditions proceeds at a much slower rate than under aerobic conditions. This results in a low N requirement in anaerobic metabolism, hence lower quantities of net N immobilization and a more rapid release of NH_4^+ to the organic soil (Acharya, 1935a, 1935b; Karim, 1948; Patrick and Mikkelsen, 1971).

Aerobic decomposition is much more rapid and is complete to the end products of CO_2 , H_2O , etc. It is carried on by bacteria, actinomycetes, and fungi, all of which are heterotrophic (requiring an organic source of C). The optimum moisture level for these organisms is between 50 and 75% of the soil water-holding capacity. The temperature range is from 2-60 C with a maximum activity occurring between 40-60 C. The total N and C content of the organic substrate is very critical in determining whether net N immobilization or mineralization will occur. With a natural material of approximately 40% C, the critical N content

is approximately 1.5%, thus giving a critical C:N ratio of 20-30:1. Thus, in substrates such as proteins with a C:N ratio below 20:1, net N mineralization takes place. In substrates, such as carbohydrates with a C:N ratio greater than 30:1, net N immobilization takes place (Alexander, 1961; Bartholomew, 1965; Black, 1968; Davis and Lucas, 1959).

Under aerobic conditions, further N transformations take place in the process of nitrification and NH_4^+ is oxidized to nitrite (NO_2^-) and then to NO_3^- by two specific bacteria genera, <u>Nitrosomonas</u> spp. and <u>Nitrobacter</u> spp., respectively. These organisms are autotrophic utilizing CO_2 as a C source, and carrying on the N transformations as a source of energy. The critical temperature values are from 5-40 C, with an optimum range between 30-35 C. Optimum moisture levels are between 1/2 to 2/3 soil water-holding capacity. The formation of NH_4^+ is not greatly affected by soil pH, but the maximum oxidation of NH_4^+ to NO_3^- occurs in a pH range of 7.0 to 9.0 (Alexander, 1961, 1965; Black, 1968).

Any change in the soil environment such as water-logging, which prevents O_2 from diffusing into the soil profile, will cause facultative anaerobic bacteria to use NO_3^- as an electron acceptor for metabolic processes instead of O_2^- . This process is termed denitrification and involves the biological reduction of NO_3^- to nitrous oxide (N_2^0) and then to N_2 gases. Both of these N forms are lost from the soil to the atmosphere by volatilization (Skerman, Lack, and Millis, 1951; Skerman and MacRae, 1957a, 1957b). The denitrifying bacteria have an optimum activity temperature in the range of 60-65 C (Broadbent and

Clark, 1965; Nommik, 1956) and are directly dependent on the soil moisture content. Several investigators have reported increased losses of N as a function of moisture content up to 450% of the water-holding capacity. Also, little N loss occurs if the moisture content is less than 60% of water-holding capacity, even when other conditions are very favorable for denitrification (Bremner and Shaw, 1958; Jannson and Clark, 1952; Nommik, 1956). The rate of denitrification is profoundly influenced by soil pH--being very slow in acid soils, and very rapid in soils of high pH (Bremner and Shaw, 1958; Jansson and Clark, 1952) with an optimum range between 5.5-8.0 (Alexander, 1961).

Several investigators (Allison, Carter, and Sterling, 1960; Broadbent, 1951; Broadbent and Stojanovic, 1952; Corbet and Wooldridge, 1940) have reported the occurrence of denitrification in aerated soils and in controlled atmospheres containing some 0_2 . An explanation of this phenomenon is that when the demand for biological 0_2 is high, the diffusion rate of 0_2 to the denitrifying bacteria may be limiting to the extent that the bacteria must utilize NO_3^- as an electron acceptor. This condition might occur when a readily decomposable substrate is being rapidly decomposed, and the micro-environment of the bacteria may essentially become anaerobic even though the soil is well aerated by ordinary standards (Broadbent and Clark, 1965). Skerman et al. (1951) have reported that an organism may use two alternative respiratory pathways at the same time, provided that conditions are satisfactory for both.

In recent years, several investigators (Allison, 1955, 1963; Clark, Beard, and Smith, 1960; Clark, 1962; Gerretsen and de Hoop,

1957; Smith and Clark, 1960; Soulides and Clark, 1958; Tyler and Broadbent, 1960; Wahhab and Uddin, 1955; Wijler and Delwiche, 1954) have reported evidence showing that biological or dissimilatory NO_3^- reduction does not adequately explain all the gaseous N loss from a soil. These additional losses appear to be greater in soils in which NO_2^- has been observed to accumulate following the addition of NH_3 or NH_3 releasing materials. Since the chemical reactions involve both NO_2^- and nitrous acid (HNO₂) Clark (1962) designated these losses as chemo-denitrification or NO_2^- dismutation. There is much disagreement as to the exact chemical pathways involved and the factors influencing these pathways. The proposed pathways have been extensively reviewed by Broadbent and Clark (1965). Since there is no reported accumulation of NO_2^- -N in an organic soil profile, chemo-denitrification is probably of no significance.

The Fate of NH₃ and NO₃ in an Organic Soil

Ammonia (NH_3) N is produced at varying rates from organic matter decomposition or it may be applied to the soil in the form of a chemical fertilizer. Once in the soil, it readily reacts with any proton donating substance to form the positively charged NH_4^+ ion (Mortland and Wolcott, 1965). Under conditions favorable for biological oxidation, NH_4^+ is quickly oxidized to NO_2^- and finally to NO_3^- . In the absence of O_2 , the mineralization of N from organic matter stops with the formation of NH_4^+ . Under anaerobic conditions, generally caused by waterlogging or flooding the organic soil, most of the NH_4^+ is adsorbed on the negatively charged organic complex with a small amount remaining in the soil solution. The NH_4^+ in soil solution is in a dynamic equilibrium with that adsorbed on the cation exchange complex. Any withdrawal from the soil solution, hence immobilization of N into organic forms, causes a movement of NH_4^+ from the exchange sites (Patrick and Mikkelsen, 1971). In actual practice, however, mineralization generally exceeds immobilization and there are often measurable amounts of NH_4^+ in flooded organic soils (Davis and Lucas, 1959).

Whereas NH_4^+ is insoluble in water and is quite stable in a water-logged soil, NO_3^- is soluble in water and is very unstable in a flooded soil. Because of the negative charge, there is little adsorption onto the organic complex and almost all of the NO_3^- -N is completely dissolved in the soil solution. Nitrate moves readily in the flooded soil: by diffusion, as a result of a concentration gradient in the soil solution; and by mass flow, as a result of hydraulic pressure (Patrick and Mikkelsen, 1971).

Nitrates may be applied to a flooded soil either by chemical fertilizers or by a biological nitrification process which occurs in the aerobic surface layer of the soil. Nitrate which remains in this aerobic layer is stable, but is readily lost from the soil profile if it is leached or diffused into the underlying anaerobic layer. The main mechanism of loss is by biological denitrification which produces N_2^0 or N_2 gas. Nitrates in flooded soils may be immobilized by incorporation into plant or microbial tissue. However, the requirement of facultative anaerobic bacteria for electron acceptors is usually much greater than any nutritional requirement for N. Therefore, very little NO_3^-N is immobilized (Patrick and Mikkelsen, 1971).

Unlike the flooded soil, NO_3^- in a drained or aerobic soil is quite stable. With heavy chemical fertilizer applications and/or a rapid biological mineralization process, NO_3^- may build up to extremely high concentrations in the soil. Extensive biological denitrification may also occur if anaerobic conditions prevail in the soil microenvironments. Because NO_3^- is completely soluble in water, it is quite mobile in the soil. It may be taken up (immobilized) by plant roots, or it may be leached out of the soil profile and into the drainage waters. Nitrate may also move upward in the soil profile and accumulate at the soil surface (Black, 1968). This is caused by the upward movement of water and its subsequent evaporation at or near the soil surface (Gardner, 1965).

Of particular interest is the leaching loss of NO_3^-N from the soil profile and into the drainage waters. The amount of NO_3^-N lost by leaching depends upon two factors: the amount of water percolating through the soil, and the NO_3^-N concentration in the water (Stewart, 1970). In 1945, NO_3^- in drinking water was first associated with a temporary but sometimes fatal blood disease, infantile methemoglobinemia, in infants (Winton, Tardiff, and McCabe, 1971). Based upon studies concerning this disease, the U.S. Public Health Service considers water containing more than 10 ppm NO_3^-N as unsafe for drinking purposes. However, Navone, Harmon, and Voyles (1963) have reported that several cities use water with considerably higher levels of NO_3^-N and have had no reported cases of infantile methemoglobinemia. In addition to the health hazard, NO_3^-N in streams and lakes stimulates algae growth when other nutrients are also available. MacKenthun (1965) has reported



that nuisance algae blooms occur in water when N levels in the water are as low as 0.3 ppm.

How much NO_3^-N is leached from an organic soil profile? Data by Erickson and Ellis (1971) obtained in 1969 from the Michigan State University Muck Experimental Farm shows a maximum NO_3^-N concentration of 2.8 ppm and a total of 109 cm of water/ha pumped from the Houghton muck soil. This gives a total loss of NO_3^-N of 18.7 Kg/ha. Unpublished data from 1972 has shown that as high as 25 ppm NO_3^-N may be obtained during the winter months.

Soil temperature and moisture content are the two most important environmental factors affecting leaching losses of N. In an Israeli study (Avnimelech, 1971) the NO_3^- produced in an organic soil reached an optimum at field capacity and increased markedly between 24 and 36 C. The values obtained at field capacity were 7 and 17 µg NO_3^-/g of soil per day at 24 and 36 C, respectively. These conditions increased NO_3^- in the soil to extremely high levels (1,000-10,000 µg NO_3^-/g soil) inducing salinity problems in crop production.

The leaching loss of NO_3^--N from an organic soil profile can be greatly reduced by controlling the water table and using submerged tile lines in the drainage system. This allows the biological denitrification process to take place, thus reducing the NO_3^--N concentration (Meek, Grass, and MacKenzie, 1969; Meek et al., 1970).

Plant uptake of N is almost exclusively in the NO_3^- form although some species, such as rice, and the soil microbial population prefer NH_4^+ -N. Since NO_3^- is soluble in water and is mobile in the soil solution, all of the NO_3^- -N in the soil profile is available for plant uptake (Black, 1968).

With large concentrations of NO_3^-N in the soil readily available for plant uptake, plants tend to "gorge" themselves resulting in soluble NO_3^-N being present in excess of assimilation needs (Cook and Millar, 1953). In many plants this accelerated uptake of NO_3^-N may be quite harmful. High concentrations of NO_3^-N in plant tissue have been reported to prolong vegetative growth and to delay flowering and fruiting or tubering. Another problem with an excessive accumulation of NO_3^-N in plant tissue is the injurious effects to animals and humans when such tissue is ingested. But the toxicity of a given amount of NO_3^-N varies tremendously, and an accurate diagnosis is often difficult.

The amount of NO_3^-N present in plants at any given time is dependent upon several factors:

- 1. The NO_3 content of a soil in the rooting zone of plants.
- 2. The plant species being grown and its stage of development.
- 3. Environmental conditions.
- 4. Herbicidial applications.

5. Effect of the method of harvesting and storing plant material. Excessive NO_3^-N accumulation occurs in the plant when:

- 1. There is a large amount of NO_3^- present in the soil.
- 2. Some factor interferes with the normal NO_3^- reduction processes in the plant. Several of these factors include:
 - a. Drought
 - b. Hail
 - c. Frost
 - d. Disease

- e. Herbicide applications, such as 2,4-D.
- f. Day length

These increases, however, are dependent upon the availability of NO_3^-N in the soil (Cantliffe, 1972; Hanway, et al., 1963).

Recently, particular concern has been directed towards the $NO_3 - N$ content in several vegetables in relation to the occurrence of methemoglobinemia in infants under 3 months of age (Phillips, 1968). Of the vegetables used in processed baby foods, spinach (Spinacea oleracea L.) probably accumulates the highest NO3-N concentrations. Spinach petioles which were purchased from several supermarkets once weekly for several months in 1970 in the Cornell University area contained NO_3^-N ranging from 7,500 to 22,500 ppm (dry weight basis), with an average of 12,900 ppm.⁴ Data by Barker, Peck, and MacDonald (1971) on the accumulation of NO_3^-N in spinach grown on two loam soils showed that the most efficient $NO_3 - N$ accumulation occurred with the chemical fertilizers being broadcast before planting as opposed to sidedressing the same amount of fertilizer one week before harvesting. There was no significant difference in the N carrier on the broadcast treatments, but KNO, form of N resulted in a higher accumulation of N than did NH₄NO₃ or urea forms. Further work by Barker and Maynard (1971a, 1971b) shows that low and high levels of N fertilization in sand cultures restricted spinach plant growth, with a lower critical level of 4000 ppm NO_3^-N necessary for 100% relative growth. In addition, the level of N fertilization and the length of plant exposure to this fertilization level are important factors in the NO_3 -N accumulation.

⁴Minotti, P. L. 1971. Unpublished data, Department of Vegetable Crops, Cornell University.

EXPERIMENTAL PROCEDURE

Field Experiments

In 1969, two field experiments were initiated to determine the effect of inorganic N fertilizer materials on the tissue yield, NO_3^--N concentration, and NO_3^--N uptake in several vegetable crops grown on a Houghton muck soil.

The first experiment was a randomized complete block design replicated eight times utilizing four application rates (0, 90, 180, and 360 Kg N/ha) of NH_4NO_3 . All plots received an initial broadcast application of 1,120 Kg/ha of an 0-10-30 +2% manganese (Mn) fertilizer prior to planting.

In one-half of the 0.01-ha plots (four replications) carrots (<u>Daucus carota</u> L. var. 'Gold Pak') were planted on May 28. The N treatments were applied by hand in two equal applications on July 2 and July 30. The carrots were sampled, 10 roots/plot, on July 30 and September 15, and the samples were frozen for NO_3^-N analysis. Yields were determined on September 15.

In the other half of the experiment, head lettuce (Lactuca sativa L. var. 'Fulton') was planted on May 28 and the N treatments were applied by hand in one application on July 2. The head lettuce was sampled, 10 heads/plot, on July 28. The heads were cut into quarters with one quarter/head being frozen for NO_3^-N analysis. Due to heavy damage by rain, hail, and bottom rot disease, yields were not determined.

Following the head lettuce crop, the N treatments were again applied on the same area in one application. The land was plowed to a depth of 15 cm, and spinach (<u>Spinacea oleracea</u> L. var. Hybrid No. 7, Northrup, King and Co.) was planted on August 6. The spinach was sampled, 10 leaves/plot, on September 19, and the samples frozen for NO_3^-N analysis. Yields were determined on September 19.

After freezing, all crops were stored in a freezer at -17.8 C. The samples were analyzed for NO_3^-N on a wet weight basis using a modified NO_3^- reductase enzyme procedure developed by Lowe and Hamilton (1967). The wet weight values were then converted to a dry weight basis. The NO_3^-N uptake was calculated by multiplying the dry weight yields by the NO_3^-N concentrations.

The second experiment in 1969 was a randomized complete block design replicated six times utilizing two N carriers, NH_4NO_3 and urea, and five application rates, 0, 56, 112, 224, and 448 Kg N/ha. All plots received a broadcast application of 1,120 Kg/ha of an 0-10-30 + 2% Mn fertilizer prior to planting. The N treatments were applied by hand in a single application to the 0.001-ha plots. Spinach (<u>Spinacea</u> <u>oleracea</u> L. var. Hybrid No. 7, Northrup, King and Co.) was planted on August 6. The spinach was sampled, 10 leaves/plot, and frozen for NO_3^-N analysis on September 19. Yields were also determined on September 19. The NO_3^-N analysis was the same as in the first experiment.

In 1970, the first experiment was continued to determine the effect of repeated high inorganic N fertilizer applications on the soil and tissue NO_3^--N concentration, yield, and NO_3^--N uptake. Soil

samples were taken at 15, 30, 46, and 61 cm depths on July 30, August 13, and September 10 and analyzed for NO_3^-N . The same basic fertilizer and N treatments were applied to the plots prior to planting. Because of its high NO_3^-N accumulation, spinach (<u>Spinacea oleracea</u> L. var. Hybrid No. 7, Northrup, King and Co.) was planted on August 4 in all replications. This seeding did not result in a satisfactory crop stand. Two replantings did not improve the stands. The spinach was sampled, 10 leaves/plot on September 13 and analyzed for NO_3^-N . The NO_3^-N analysis in both the muck soil and spinach tissue was carried out potentiometrically using the NO_3^- ion specific electrode procedure of Myers and Paul (1968) and Paul and Carlson (1968).

In 1971, the first experiment was again continued. Soil samples were taken at 15, 30, 46, and 61 cm depths on May 17, July 1, July 31, and September 7, and analyzed for NO_3^-N . The same basic fertilizer and N treatments were applied to the plots prior to planting. Spinach (<u>Spinacea oleracea</u> L. var. 'Nore Green', Northrup, King and Co.) was planted on May 14 in 8 replications. The spinach was sampled, 10 plants/ plot, for NO_3^-N analysis on June 24 and July 12. Yields were determined on July 7.

The N treatments were again applied and the plots plowed to a depth of 15 cm prior to the second planting. Spinach (<u>Spinacea</u> <u>oleracea</u> L. var. Hybrid No. 7, Northrup, King and Co.) was planted on August 5, using only 4 replications. The spinach was sampled, 10 leaves/ plot, for NO_3^-N analysis on September 1, 14, and 30. Yields were determined on October 10. The NO_3^-N analysis was by the same procedure used in 1970.
Greenhouse Experiments

In the fall of 1970, a 2 X 3 X 5 factorial experiment replicated four times was initiated in the greenhouse to study the leaching losses of organic-N and NO_3 -N from a virgin muck soil. Twenty-four core samples were obtained by driving cylindrical galvanized containers (35.6 cm in diameter and 76.2 cm in length) into a virgin muck. The containers with the soil cores were then moved to the greenhouse where caps were fitted and sealed on the bottom of each container. After capping, the cores were placed upright and flooded with distilled water for two-week incubation periods. At the completion of each twoweek period, the cores were drained by means of a plastic hose which was attached approximately 3 cm above the bottom of the core container. The cores were flooded again and leachate from each core for each twoweek period was subsequently analyzed for organic-N and NO_3^--N using the semi-microkjeldahl procedure of Bremmer (1965a) and the NO_3^- ion specific electrode procedure of Myers and Paul (1968), respectively. The two-week incubation periods were continued until the NO_3^-N concentration in all 24 leachates stabilized. After this stabilization (check) period, three moisture treatments (field capacity, one-half saturated, and saturated) and two temperature treatments (ambient and 10 C) were initiated. The moisture level in the containers that corresponded to field capacity was determined during the stabilization period by observing the water level in the plastic hose upon draining the cores. The saturated level was maintained at the surface of the soil core. The one-half saturated level was the difference between the field capacity and the saturated level. The core containers maintained under the ambient temperature treatment were randomized on a greenhouse

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bench. The containers under the 10 C treatment were randomized in two water baths which were constantly kept at 10 C.

In addition to the stabilization (check) incubation period, the following regimes were initiated: covering the containers with polyethylene sheets to prevent evaporation, removing the polyethylene sheets to allow evaporation, broadcasting the equivalent of 112 Kg N/ha as NH_4NO_3 on the core surface, and shortening the incubation period to 4 days. The second, third, and fourth regimes consisted of four threeweek incubation periods. After each three-week period, the cores were drained, the amount of the leachate recorded, and the moisture levels re-established. A sample of the leachate was collected and analyzed for organic-N and NO_3 -N as previously described. Prior to broadcasting the 112 Kg N/ha, the cores were sampled at three depths (15, 30 and 46 cm) for NO_3^-N and NH_4^+-N content. The NH_4^+-N analysis was accomplished by the semi-microkjeldahl method as previously described for organic-N but without the digestion period. The NO3-N was carried out potentiometrically using the NO_3^- ion specific electrode previously described. The oxidation-reduction potentials using the method of Brown (1935) and the 0_2 diffusion rates using the procedure of Lemon and Erickson (1955) were also determined prior to the surface application of 112 Kg N/ha.

RESULTS AND DISCUSSION

Field Experiments

Yield and NO_3^-N content of the 1969 carrot crop are shown in Figure 1. The NO_3^-N content of plants sampled July 30 was increased from 380 to 780 ppm N by the addition of 90 Kg N/ha five weeks after planting (1/2 of the 180 Kg N/ha rate). Plant NO_3^-N content decreased to 570 ppm N as a result of adding 180 Kg N/ha (1/2 of the 360 Kg N/ha rate). The NO_3^-N content of plants grown without added N was similar at both sampling times. But the NO_3^-N content of plants grown on the 90 and 180 Kg N/ha treatments decreased even though the last half of the fertilizer N was applied just two weeks prior to sampling.

Carrot yield from the 180 Kg N/ha rate (280 Kg/ha) was significantly greater than the yield from the 0 and 360 Kg N/ha rates (226 and 222 Kg/ha respectively), but there was no statistical significance between the yield from the 90 and 180 Kg N/ha rates. These data show the growth effects of limited and excessive N on crop yield where maximum yield was obtained from the 180 Kg N/ha rate.

The highest N application rate resulted in a significant increase in carrot NO_3^-N uptake over the 0 and 90 Kg N/ha rates. There was no statistical difference in the NO_3^-N uptake between the 180 and 360 Kg N/ha rates.



Figure 1. Carrot NO₃-N content and yield as affected by N application rates (1969).

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The lettuce NO_3^-N content is summarized in Figure 2. Although not statistically significant, the reduction in NO_3^-N content at the highest N application rate, which was apparent in the first carrot sampling, is also evident here.

Since the lettuce and carrot studies were conducted concurrently with N fertilizer being applied July 2 in both experiments, a possible explanation for their effects may be found in the climatic data, Figure 5. The rainfall for July, 1969 greatly exceeded the water loss by evaporation; consequently, the N applied July 2 was subject to leaching. The heavy rainfall also raised the water table to 45 cm in the plots in early July. Thus the NO_3^--N which leached to the ground water table was subject to denitrification. The rate of denitrification is known to increase with increasing soil NO_3^--N contents (Wijler and Delwiche, 1954). Thus the highest N application rate could, under these climatic conditions, result in less total NO_3^--N in the soil after accelerated denitrification has occurred than for lower N application rates which stimulated denitrification to a lesser extent.

During August of 1969 the water loss by evaporation greatly exceeded water gain by rainfall. The water table was lowered to 76 cm in early August and remained there the entire month. The second half of the fertilizer applied to the carrots on July 30 was not, therefore, subjected either leaching loss or to intensive denitrification. Hence, the plant NO_3^--N contents determined on September 15 showed an increase which was directly related to increased N fertilizer application.

Figur





N Rates, Kg/ha

Figure 2. Head lettuce and spinach leaf NO₃-N content as affected by N application rates.

Figure 3 shows the data from the first spinach experiment in 1969. This experiment followed the lettuce with the N being applied to the lettuce residue on August 3. The field was subsequently plowed to a 15 cm depth. The plant NO_3^--N content at harvest of the 360 Kg N/ha rate was significantly increased over the NO_3^--N content of the 180, 90, and 0 Kg N/ha rates. The reduced plant NO_3^--N content of the highest N application rate, evident in the carrot and lettuce experiments was not present in this data. This is agreement with the data of the second carrot sampling.

The yields, however, showed a "leveling off" trend as the curve approached the 360 Kg N/ha rate. But the plant yields of the 360 Kg N/ha rate were significantly increased over the 90 and 0 Kg N/ha rates. The difference between the 180 and 360 Kg N/ha rates were not significant.

The NO_3^-N content of 27,700 ppm for the 360 Kg N/ha rate is quite high. The use of this rate of N fertilization does not appear to be justified since it only produced a 5 percent yield increase over the 180 Kg N/ha rate while at the same time a 48 percent NO_3^-N content increase occurred. The use of 180 Kg N/ha increased the yield 36 percent over the check with only a 38 percent NO_3^-N content increase.

The data from the second spinach experiment conducted in 1969 are summarized in Figure 4. There were no differences in yields, plant NO_3^-N contents, and NO_3^-N uptake as related to NH_4NO_3 and urea fertilizer sources; therefore, each point in Figure 4 is an average of both N sources. Differences were noted in yields, NO_3^-N content, and NO_3^-N uptake as related to N rates.



Figure 3. Leaf NO₃-N content and yield of spinach as affected by N rates (1969).



Figure 4. Leaf NO₃-N content and yield of spinach as affected by N rates (1969).



----- Rainfall

---- Evaporation



Figure 5. Water loss and gain and mean temperature for 1969.

At harvest, the spinach NO_3^-N content on the 448 Kg N/ha rate was significantly increased over the 224, 112, 56, and 0 Kg N/ha treatments, and the plants on the 224 Kg N/ha rate contained significantly more NO_3^-N than those on the 56 and 0 Kg N/ha rates. Again, the high NO_3^-N content (32,300 ppm) of the highest N application rate appears excessive. And as noted previously, the lack of yield response does not justify the use of the highest N increment.

Plant yields on the 448 and 224 Kg N/ha were significantly increased over the yields of the 56 and 0 Kg N/ha rates. The yield curve follows a normal growth curve in that the yields increase linearly as N rates increase up to the 224 Kg N/ha rate. Beyond the 224 Kg N/ha rate, yields were not increased by increased N application rates.

The plant uptake of NO_3^-N was increased from 70 to 130 Kg/ha by the addition of 448 Kg N/ha. Thus, 13 percent of the added N was in the harvested crop in the NO_3^- form. It is expected that additional N would be present as amino acids and proteins.

In 1970, a spinach experiment was established with the same treatments and on the same location as the first spinach experiment grown in 1969. The N treatments were applied at planting but a poor stand necessitated replanting. The plant NO_3^--N contents shown in Figure 2 for this experiment were from the later re-planting of spinach even though no additional fertilizer was added. All plant NO_3^--N contents were lower in 1970 than in 1969 (for example, the high content in 1970 was 520 ppm as compared to 27,700 ppm for 1969). The 1970 lower values likely occurred because of the length of time between fertilizer N

application and crop production. The plant NO_3^-N content of the 180 Kg N/ha rate was significantly increased over the contents of the 90 and 0 Kg N/ha rates. But again lower NO_3^--N content was observed at the highest N application rate. The reduced plant NO_3^--N content may again be attributed to increased rainfall which caused an increased leaching and denitrification loss from the soil profile (see Figure 6).

The soil NO_3 -N data of this experiment support the theory of increased leaching and denitrification losses of soil NO_3 -N as related to increased rainfall (see Figures 7 and 8). In all 1970 sampling dates, the soil NO_3^-N content of the 360 and 180 Kg N/ha rates were increased significantly over the 90 and 0 Kg N/ha rates, and, in the last sampling, the 90 Kg N/ha rate had significantly higher soil NO_3^-N than the 0 Kg N/ha rate. The soil NO_3^-N curves of the 360, 180, and 90 Kg N/ha rates continued to increase linearly with each sampling date, whereas the 0 Kg N/ha rate began to "level off" with the last sampling. Since the O Kg N/ha treatment had received no fertilizer N, this "leveling off" may be attributed to either a decreased biological nitrification or an increased biological denitrification (or a combination of both processes) caused by an increased rainfall which raised the water table in the organic soil profile from below 76 cm up to less than 51 cm below the soil surface. By the last sampling date, little difference occurred in the soil NO_3^-N content between the 180 and 360 Kg N/ha rates. On the first sampling date, prior to the fertilizer N applications, the 0-15 cm depth had a slightly higher soil NO_3 -N content than other depths. Two weeks later, however, the 30-46 and 46-61 cm depths had slightly higher soil NO_3^-N content than the 0-15 cm depth.



Figure 6. Water loss and gain and mean temperature for 1970.



Figure 7. Soil NO₃-N content as related to sampling date and application rates.



Figure 8. Soil NO₃-N content as related to sampling date and soil depth.

This increased soil NO_3^-N content in the lower soil depths is attributed to the increased rainfall which leached the NO_3^-N out of the upper profile layers and away from the root zone. At the last sampling date, however, the soil NO_3^-N content of the 46-61 cm depth remained the same as that on the second sampling. This stabilization effect was attributed again to the increased rainfall and water table which caused an increased biological denitrification due to the anaerobic conditions. The soil NO_3^-N content of the 30-46, 0-15, and 15-30 cm depths continued to increase linearly with the third sampling, but in the same order as with the second sampling. The soil profile NO_3^-N content of each sampling date was: July 30, 598 ppm; August 13, 865 ppm; and September 10, 1055 ppm.

A spinach experiment was conducted in Spring and Fall of 1971, where the treatments utilized in 1969 and 1970, were again repeated in the same locations. Therefore, by Fall of 1971 the individual treatments had received four times the stated application rate applied over a three year period. The data for the 1971 experiments are given in Figures 9 and 10. The NO_3^-N content of the Spring 1971 plant tissues are quite low in comparison to the 1969 results. This may be attributed to the cool and dry spring (see Figure 13), insufficient irrigation water during May, differences in spring and fall spinach varieties, and greater day length in the spring. The fact that the stem tissue $NO_3 - N$ content was considerably higher than the leaf tissue NO_3^-N content, suggest that the latter two factors may be the most important. Both the leaf and stem tissue contents of the 360 Kg N/ha rate were significantly greater than those from the 180, 90, and 0 Kg N/ha rates.



Figure 9. Leaf and stem NO_3 -N and yields of spinach as affected by N rates (spring 1971).



Figure 10. Leaf NO₃-N content, sampled at different dates, and yield of spinach as affected by N rates (fall 1971).

Regarding NO_3^-N concentration of the stem, the 180 Kg N/ha rate had significantly increased plant content over the 90 and 0 Kg N/ha rates, and the content of plants from the 90 Kg N/ha rate were significantly increased over the content from the 0 Kg N/ha rate.

The plant yields from this experiment were not statistically different, although there was a decreasing trend from the 0 Kg N/ha rate to the 360 Kg N/ha rate. The NO_3^- -N uptake from the 360 and 180 Kg N/ha rates was significantly increased over the 0 Kg N/ha rate uptake. There were no statistical differences in the plant uptake from the 360, 180, and 90 Kg N/ha rates.

The NO_3^-N contents of the fall (late) spinach crop are more "mormal" than the previous two crops (see Figure 10). The first sampling date had a higher tissue NO_3^-N content than did the last two samplings. Although tissue contents of plants from the first sampling were not statistically different, there was an increased plant tissue content up to the 180 Kg N/ha rate. The tissue contents of the 90, 180, and 360 Kg N/ha rates "leveled off" in the last two samplings, with the contents of the 90 and 360 Kg N/ha rates being significantly increased over the content of the 0 Kg N/ha rate in the last sampling. Large amounts of irrigation water (see Figure 13) were applied to the experiment. What effect this had on tissue NO_3^-N content is not known. The plant yields were not significantly different, although the 360 Kg N/ha rate yield was slightly decreased. The plant NO_3^-N uptake differences between treatments were not significant.

Soil NO_3^-N content for 1971 is summarized in Figures 11 and 12. The soil NO_3^-N content, as related to both depth and N application



Figure 11. Soil NO₃-N content as related to sampling dates and N rates.



Figure 12. Soil NO₃-N content as related to sampling dates and soil depth.



rates, was quite low at the May sampling. In May, the soil profile NO3-N content was 271 ppm, whereas; the September 1970 was 1055 ppm. These observations are in agreement with the increased NO_3^-N content in the drainage water from the MSU Muck Experiment Farm in the late winter and early spring as opposed to the NO_3^-N content of the summer drainage water. These observations indicate that some of the NO₃-N present in the soil in late fall is leached out of the profile and that together with denitrification reduces NO_3^-N levels over winter. With the May sampling, the soil NO_3^-N content of the 360 and 180 Kg N/ha rates is significantly increased over the content of both the 90 and 0 Kg N/ha rates, and the content of the 90 Kg N/ha rate is significantly increased over the content of the 0 Kg N/ha rate. The soil NO_3^-N content of the 46-61 cm depth is significantly greater than the content of both the 0-15, and 15-30 cm depths, and the content of the 46-61 cm depth is significantly increased over the content of the 0-15 cm depth. Once again these data indicate the leaching losses of soil NO_3^-N from the upper layers of the profile into the lower layers.

In the July 1 samples, the soil NO_3^-N content from all N application rates and soil depths increased dramatically. The soil NO_3^-N content was significantly increased when related to increased N application rates, with the increases being in the order 0 < 90 < 180 < 360 Kg N/ha. The soil NO_3^-N content as related to depth was not significantly different. However, there was an increased amount of soil NO_3^-N in the 30-46 cm depth, indicating that biological denitrification was still not an important factor in total soil NO_3^-N content. The July 31 samples showed a decrease in soil NO_3^-N content in all N application rates and with all soil depths. This decrease may be attributed to an increased rainfall and decreased evaporation (Figure 13). The biggest decrease occurred with the 360 Kg N/ha rate and at the 30-46 and 46-61 cm depths (where anaerobic conditions are most likely to occur). These data indicate that biological dentrification is playing an important role in the total soil NO_3^-N content of a profile. The soil NO_3^-N contents are still significantly different in the same order as in the July 1 sampling, except that no significant difference occurred between the 90 and 0 Kg N/ha treatment. The soil NO_3^-N content of the 0-15 cm depth was significantly greater than the soil NO_3^-N content of all the lower depths. No statistical differences were noted between the soil NO_3^-N content of the lower depths, although a decreasing trend is noted with increasing depth.

The September 7 samples showed a large increase in soil NO_3^-N content as related to increased N applications, and at all soil depths, reflected the additional fertilizer applications on August 5. These increases were the largest with the 360 Kg N/ha application rate, and the 15-30 cm soil depth, and may be attributed to an increase in evaporation and a decrease in rainfall. Considerable irrigation water was applied to the experiment during August. What effect the added water had on soil NO_3^-N content is not known. There were significant differences in soil NO_3^-N content in all N application rates, with the content increasing in the order 0 < 90 < 180 < 360 Kg N/ha. The area receiving the 360 Kg N/ha rate had an average soil NO_3^-N content of 780 ppm. Soil NO_3^-N content of the 0-15 cm depth was significantly

greater than the content of both the 30-46 and 46-61 cm depths. The soil NO_3^-N content of the 15-30 cm depth was also significantly increased over the content of the 46-61 cm depth.

For each 1971 sampling date, the soil profile NO_3^-N contents were: May 17, 271 ppm; July 1, 1452 ppm; July 31, 811 ppm; and September 7, 1792 ppm. These values represent an overall increase in soil NO_3^-N content from 1970, which may be attributed to the decrease in rainfall. Soil NO_3^-N content increased with increasing N application and decreased with increasing soil depth.

Greenhouse Experiments

Moisture Treatments

The leachates from the field capacity treatment of the evaporation, 112 Kg N/ha and four-day incubation regimes contained significantly greater amounts of NO_3^-N than leachates from the 1/2 saturated and saturated treatments under similar regimes. Differences were small until evaporation from the column surface was allowed, and they became very large only after N fertilization (see Figure 14). For example, during the four-day regime the soil NO_3^-N content in the field capacity treatment was 7.6 ppm as compared to 0.3 ppm in the saturated treatment. The average difference was 2.68 ppm soil NO_3^-N for the field capacity as compared to 0.36 ppm for the saturated treatment (see Table 5).

The leachate from the saturated treatment of the evaporation and the 112 Kg N/ha regimes contained significantly more organic-N than the field capacity treatment, and, in the 112 Kg N/ha regime, the organic-N contant of the saturated treatment was also significantly greater than the 1/2-saturated treatment (see Figure 15).



Figure 14. NO₃-N content of water leached through a muck profile as affected by regimes and moisture treatment.



Figure 15. Organic-N content of water leached through a muck profile as affected by regimes and moisture treatment.

The soil NH_4^+ -N content of the saturated treatment was significantly greater than the 1/2-saturated and field capacity treatments at the 0-15 and 15-30 cm depths. There was no significant difference in soil NH_4^+ -N content at the 30-46 cm depth, although the contents still followed the order of saturated > 1/2-saturated > field capacity (see Figure 16).

The soil NO_3^-N content of the field capacity treatment was significantly higher than the 1/2-saturated and saturated regimes at the 0-15, 15-30, and 30-46 cm depths (Figure 18). The soil NO_3^-N content of the 1/2-saturated treatment was also increased significantly over the saturated treatment at the 0-15 cm depth (see Figure 17).

The oxidation-reduction (redox) potentials and the 0_2 diffusion rates (see Table 2) are also significantly different when related to the moisture treatment. At the soil surface, the field capacity and 1/2-saturated treatments had significantly increased potentials over the saturated treatment indicating oxidizing conditions were present at the soil surface. The 0_2 diffusion rate of the field capacity treatment was significantly increased over the 1/2-saturated and saturated treatment at the soil surface. The 1/2-saturated treatment 0_2 diffusion rate was also significantly increased over the saturated treatment.

From these data, the moisture regimes have both an indirect and direct effect upon the soil and leachate NO_3^-N content, and upon the leachate organic-N content. The indirect effect is probably the more important of the two. By controlling the water table in the soil, the O_2 diffusion through the soil profile is controlled, thus creating either aerobic (O_2 present) or anaerobic (O_2 deficient) conditions.





Figure 16. Soil NH₄⁺-N content as affected by soil depth and moisture treatment.



Figure 17. Soil NO -N content as affected by soil depth and moisture treatment.



Figure 18. NO -N leached from a muck profile as affected by regime and moisture treatment.

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	Redox potential	02 diffusion
	mv	$gm \times 10^8/cm^2/min$
Temperature		
Ambient	324	30.2
10°C	315	28.5
LSD (0.05)	n.s.	n.s.
Moisture		
Field Cap.	412	43.4
1/2 Sat.	414	33.1
Sat.	131	11.7
LSD (0.05)	60	9.8

Table 2.--Redox potential and O₂ diffusion rates at the surface of a muck soil as affected by temperature and moisture levels after evaporation regime.
Under aerobic conditions, the heterotrophic organisms break down the organic-N compounds to NH_4^+ , which is quickly biologically oxidized to NO_3^- -N, the final oxidation product. This process accounts for the increased soil and leachate NO_3^- -N content of the field capacity treatment.

The breakdown of organic-N compounds under anaerobic conditions to NH_4^+ proceeds at a much slower rate. This process is less efficient than under aerobic conditions, and not all of the organic-N is converted to NH_4^+ . Because of the reduced metabolism, the microbes do not utilize all of the NH_4^+ produced, and thus, a net increase in the NH_4^+ content in waterlogged or saturated organic soils results. This process accounts for the increased soil NH_4^+ -N and increased leachate organic-N contents in the saturated treatment.

The 0-15 cm depth of the 1/2-saturated treatment also had an increased soil NO_3^-N content, but the leachate NO_3^-N content did not significantly increase above the saturated treatment in the noevaporation and evaporation regimes, and was significantly decreased below the field capacity treatment in the N treatment and reduced incubation regimes. This is an indication that much of the soil NO_3^-N that is leached out of the 0-15 cm depth of the 1/2-saturated treatment is biologically reduced (denitrified) to N_2O and N_2 gases upon reaching the anaerobic conditions at the water table.

The direct effect of the moisture treatment is twofold. When surface evaporation is prohibited, moisture movement upward and loss from the soil profile is greatly restricted. Without the upward movement of water, the soil moisture content of the field capacity treatment

and upper layers of the 1/2-saturated treatment is probably reduced below field capacity, and hence may be the limiting factor for microbial population. If this is the case, then the oxidation of organic-N to NO_3^-N would be reduced and the leachate content of NO_3^-N would also be reduced. Another factor is the evaporation of water from the soil surface. If evaporation is restricted, then the need to replenish the water lost from the profile is decreased. Thus, there is also a decrease in the volume of water that is leaching the profile. If this is the case, then there would be less NO_3^-N leached from the profile.

The total NO_3^-N and organic-N leached from the soil profile is dependent upon the N content in the leachate and the leachate volume. The average volumes for the moisture treatments were: field capacity 0.4 l; 1/2-saturated 1.6 l; and saturated 3.0 l. There were no statistical differences in the $NO_3 - N$ leached as related to moisture treatment except in the four day incubation regime where the $NO_3 - N$ leached in the 1/2-saturated treatment was significantly increased over the losses of the field capacity and the saturated treatments (see Figure 18). These data indicate that, in relation to moisture treatment, minimum amounts of NO3-N are lost from an organic soil profile if conditions permit denitrification to occur. The 1/2-saturated treatment allowed for $NO_3 - N$ production in the surface one half of the profile and a sufficient volume of water to allow for the loss of much of the NO_3 -N if denitrification does not occur. Although the field capacity treatment allows for production of NO_3^-N in the profile, the total volume of water loss is only one-fourth of that from the 1/2saturated treatment; thus, less total NO3-N is lost. The quantity of

 NO_3^-N which leached from the profiles was greater during the short, 4 day incubation period because this did not allow sufficient time for denitrification.

The leaching losses of organic-N were significantly affected by moisture treatment. The amount of organic-N leached from the saturated treatment was increased significantly over that leached from the 1/2saturated and field capacity treatments in all regimes, except in the stabilization period. The amount of organic-N leached from the 1/2saturated treatment was significantly increased over that leached from the saturated and field capacity treatments in the stabilization regime, and was significantly increased over that leached from the field capacity treatment in all other regimes (see Figure 19). These data again indicate the differences between the anaerobic and aerobic conditions and the biological processes occurring under these different conditions.

Temperature Treatments

The 10 C treatment leachates of the no-evaporation regime contained significantly greater amounts of NO_3^-N than the ambient (28 C mean) treatment in the no-evaporation (5% level) and the evaporation (10% level) regimes. This trend continued in the 112 Kg N/ha and shortened incubation regimes (see Figure 20).

The ambient treatment leachates of the stabilization and noevaporation regimes contained significantly greater organic-N amounts than the 10 C treatment. This trend continued with the evaporation and 112 Kg N/ha regimes. The leachate from both temperature treatments



Figure 19. Organic-N leached from a muck profile as affected by regime and moisture treatment.



Figure 20. NO -N content of water leached through a muck profile as affected by regime and temperature treatment.

contain the same amounts of organic-N in the four day incubation regime (see Figure 21).

The soil NH_4^+ -N content of the ambient temperature treatment was significantly higher than the content of the 10 C treatment at the 15-30 cm depth. The same trend was observed at the 0-15 and 30-46 cm depths (see Figure 23).

The soil NO_3^-N content (see Figure 22) of the ambient temperature treatment was significantly higher than that of the 10 C treatment at the 0-15 cm depth. This trend continued at the 15-30 cm depth, but the soil NO_3^-N content at the 30-46 cm depth was the same for both temperatures.

There were no differences noted in the oxidation-reduction potentials and 0_2 diffusion rates as related to the temperature treatments.

From these data the temperature treatment directly effects the soil microorganisms. The increased soil NH_4^+ -N and NO_3^- -N content of the ambient treatment is due to the increased heterotrophic microbial activity under both aerobic and anaerobic conditions, which continually work to break down and oxidize the organic soil. The soil NH_4^+ -N content of the saturated, ambient treatment interaction was increased at both the 0-15 and 15-30 cm soil depths, and the soil NO_3^- -N content of the ambient, field capacity treatment interaction was also greater at 0-15, 15-30, and 30-46 cm soil depths than any other temperature and moisture interactions.

The increased NO_3^-N content of the leachate with the 10 C treatment is an indication that the increased soil NO_3^-N in the ambient



Figure 21. Organic-N content of water leached through a muck profile as affected by regimes and temperature treatment.



Figure 22. Soil NO -N content as affected by soil depth and temperature treatment.





Figure 23. Soil NH₄⁺-N content as affected by soil depth and temperature treatment.

temperature treatment is being denitrified to N_2^0 and N_2^0 gases. Again, the heterotrophic bacteria responsible for denitrification in an anaerobic environment are mesophilic microbes, and have a greater population in the higher temperature treatment.

The increased organic-N content of the leachate with the ambient temperature treatment is again due to the increased microbial activity. Because there is no reducing organism, this differs from the NO_3^--N data.

The total leaching losses of NO_3^--N from the soil profiles are not significantly different, although the losses of the 10 C treatment are slightly greater than the ambient treatment (see Figure 24). The total leaching losses of organic-N from the ambient treatment, however, are significantly increased over the losses of the 10 C treatment in the stabilization, no-evaporation, and evaporation regimes. This increased loss from the ambient treatment is continued through the 112 Kg N/ha regime. But there is no difference in the organic-N losses during the four-day incubation regime (see Figure 25).

The greater loss of NO_3^--N at the lower temperature is in agreement with field observations. Since the temperature of the soil profile in the field is less than 10 C during the winter months denitrification would be expected to progress slowly. Even in the summer months the temperature at the water table will not greatly exceed 10 C suggesting that denitrification will require a few days for completion. The microbial breakdown of organic matter and subsequent oxidation to NO_3^- would proceed rapidly at the high temperatures at the surface. Should heavy water be applied at this point NO_3^- could be leached from the profile so rapidly that denitrification could not occur.



Figure 24. NO₃-N leached from a muck profile as affected by regime and temperature treatment.



Figure 25. Organic-N leached from a muck profile as affected by regime and temperature treatment.

Regimes

Comparing the average leachate NO_3^-N content of the stabilization, no-evaporation, and evaporation regimes, the NO_3^-N content of evaporation regime is significantly greater than the content of the first two regimes. The average NO_3^-N content of the leachates from the first three regimes for the 10 C treatment is significantly greater than the content of the ambient temperature. The average leachate NO_3^-N content of field capacity treatment was also significantly greater than that of the 1/2-saturated and saturated average contents (see Table 3). The average leachate NO_3^-N content of the evaporation regime, 10 C, field capacity treatment, was significantly greater than the contents of the other regime treatments.

The average leachate organic-N content of the stabilization, no-evaporation, regimes are also significantly less than the evaporation regime. The three regime average leachate organic-N content of the ambient treatment is significantly increased over that at the 10 C. The average organic-N content from the 1/2-saturated and saturated treatments was significantly greater than that at the field capacity treatment. There were no significant differences in the interactions involving the temperature treatments. The average organic-N content of the leachate under the evaporation regime of the saturated treatment was significantly greater than all regime treatment contents, with the exception of the contents under the evaporation regime of the 1/2saturated treatment.

During the first three regimes, the average leaching loss of NO_3^-N during the 10 C treatment was significantly greater than the loss of the ambient treatment. There was no significant difference

	NO ₃ -N	Organic-N
		ppm
Temperature		
Ambient	0.065	3.17
10 C	0.277	2.46
LSD (0.05)	0.179	0.32
Moisture		
Field Cap.	0.354	2.48
1/2 Sat.	0.089	2.97
Sat.	0.070	2.98
LSD (0.05)	0.220	0.39
Regime		
No Treat.	0.095	2.54
No Evap.	0.073	2.58
Evap.	0.344	3.33
LSD (0.05)	0.220	0.39

Table 3.--Nitrogen content of H_2O leached through a muck profile as affected by temperature, moisture level, and regime prior to N fertilization.

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in NO_3^-N loss as related to the moisture treatment. The average NO_3^-N loss from the stabilization regime was significantly greater than the loss from the no evaporation regime. The average NO_3^-N loss from the evaporation regime was slightly greater than the loss from the no-evaporation regimes. There were no significant interaction differences noted between the three regimes and six treatments (see Table 4).

The average organic-N leaching loss from the ambient temperature treatment during the first three regimes was significantly greater than that from the 10 C treatment. The average organic-N leaching loss from the saturated treatment during the three regimes was significantly greater than either the 1/2-saturated or the field capacity treatment, and the 1/2-saturated treatment loss was significantly greater than the loss at field capacity. The stabilization regime average organic-N loss is significantly greater than the average loss of the other two regimes, and the organic-N loss of the evaporation regime is significantly increased over the loss of the no-evaporation regime. The organic-N leaching loss from the ambient temperature treatment of the stabilization regime was greater than the loss from any other regime temperature treatment. The average organic-N loss from the evaporation regime of the saturated treatment was significantly greater than the average loss from all other regime treatments.

These data indicate that significant leaching losses of NO_3^--N and organic-N can occur in the development of a virgin organic soil. The increased losses from the stabilization regime are due to the increased leachate volume. The increased losses during the evaporation regime, over the no-evaporation regime, are due to the increased NO_3^--N

	N0 ₃ -N	Organic-N
	mg	
Temperature		
Ambient	0.132	6.70
10 C	0.242	4.98
LSD (0.05)	0.089	0.80
Moisture		
Field Cap.	0.183	2.85
1/2 Sat.	0.183	6.05
Sat.	0.194	8.63
LSD (0.05)	n.s.	0.99
Regime		
No Treat.	0.266	7.05
No Evap.	0.096	4.56
Evap.	0.198	5.92
LSD (0.05)	0.109	0.99

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Table 4.--Nitrogen leached from a muck profile as affected by temperature, moisture level, and regime prior to N fertilization.



and organic-N contents of the leachate. The role of biological denitrification is again shown in the NO_3^-N loss as related to the temperature treatments.

The 112 Kg N/ha addition and the reduced incubation regimes resulted in an increased leachate NO_3^-N content and leaching loss; whereas the organic-N content and loss remained the same as it was during the evaporation regime. Summarizing the average leachate $NO_3 - N$ contents for all five regimes and six treatments (see Table 5), the 10 C treatment was significantly (10% level) greater than that from the ambient temperature treatment. The $NO_3 - N$ loss from the field capacity treatment was significantly greater that from either the 1/2saturated or saturated treatments, and the 1/2-saturated treatment lost significantly more than the saturated treatment. The loss at 10 C under the field capacity treatment was slightly greater than all other treatments. The losses during the short incubation regime were significantly greater than those from all other regimes, and 112 Kg N/ha regime lost significantly more than the first three regimes. Losses under the short incubation regime and the 10 C treatment were slightly increased over the other regime and temperature treatments. The loss under the short incubation regime at field capacity was significantly greater than the other regime and moisture treatments. The losses under the short incubation regime at 1/2-saturation, and the 112 Kg N/ha regime at field capacity are significantly greater than the remaining regime and moisture treatments. There were no interaction differences between the five regimes and six treatments.

	NO ₃ -N		Organic-N
		ppm	
Temperature			
Ambient	1.29		3.38
10 C	1.83		2.85
LSD (0.10)	0.52	LSD (0.05)	0.32
Moisture			
Field Cap.	2.68		2.83
1/2 Sat.	1.64		3.12
Sat.	0.36		3.39
LSD (0.05)	0.76		0.40
Regime			
Stabilization	0.10		2.54
No Evaporation	0.07		2.58
Evaporation	0.34		3.33
112 Kg N/ha	2.70		3.56
4 Day Incub.	4.59		3.57
LSD (0.05)	0.98		0.51

Table 5.--Nitrogen content of H_2O leached through a muck profile as affected by temperature, moisture level, and regime.

Summarizing the average organic-N content of leachates from all five regimes and six treatments, the loss from ambient temperature treatment was significantly greater than the 10 C treatment. The losses from the saturated treatment were significantly greater than that from the field capacity treatment. The losses from ambient temperature saturated moisture treatment were increased, but not significantly over all other temperature and moisture interactions. The organic-N removed from the short incubation, 112 Kg N/ha, and evaporation regimes was significantly greater than that from the no-evaporation and stabilization regimes. There were no interaction differences between the five regimes and six treatments.

Considering the average NO_3^--N leaching loss during the entire experiment, the losses from the 10 C treatment were greater than from the ambient treatment; the losses from the 1/2-saturated treatment were significantly greater than from either the field capacity or saturated treatments (see Table 6). The losses from the 10 C, 1/2-saturated treatment interaction were also significantly greater than all other temperature and moisture interaction treatments. The loss from the short incubation regime was significantly greater than the other five regimes, and the loss from the 112 Kg N/ha regime was significantly greater than the loss from the first three regimes. The losses from the short incubation regime with the 10 C treatment were increased, over all other temperature and regime interactions. The losses from the short incubation regime at 1/2-saturation were significantly greater than from all other moisture and regime interactions.

	NO ₃ -N	Organic-N
	mg	
Temperature		
Ambient	1.37	6.60
10 C	1.86	5.38
LSD (0.05)	n.s.	0.67
Moisture		
Field Cap.	1.01	2.23
1/2 Sat.	2.75	5.82
Sat.	1.07	9.92
LSD (0.05)	0.87	0.82
Regime		
Stabilization	0.27	7.05
No Evaporation	0.10	4.56
Evaporation	0.20	5.92
112 Kg N/ha	2.93	6.37
4 Day Incub.	4.58	6.05
LSD (0.05)	1.12	1.06

Table 6.--Nitrogen leached from a muck profile as affected by temperature, moisture level, and regime. The losses from the short incubation regime at 10 C and 1/2-saturation were greater than those from all other regimes and treatments.

The losses from the ambient temperature treatment were significantly greater than those from the 10 C treatment. Organic N losses from saturated treatment were significantly greater than losses from either the 1/2-saturated or the field capacity treatments, and the losses from the 1/2-saturated treatment were significantly greater than those from the field capacity treatment. Losses from the ambient temperature and the saturated moisture level were greater than all other treatments. The no evaporation regime losses were significantly less than the other four regimes, and the stabilization regime losses were significantly greater than those from the evaporation regime. The losses from the stabilization regime at ambient temperature were significantly greater (10% level) than losses from the other regime and temperature treatments. The losses from the evaporation, 112 Kg N/ha, and short incubation regimes under the saturated moisture treatment were significantly greater than the losses from all other regime and moisture treatments. There were no interactions between the five regimes and six treatments.

SUMMARY AND CONCLUSIONS

Field Experiments

- 1. Plant NO_3^{-N} concentration is increased with increasing soil NO_3^{-N} concentration.
- 2. Soil NO₃-N concentration is related to fertilizer N applied and to soil moisture conditions. Increased rainfall and a high water table caused an increased leaching loss of NO₃-N from the upper soil layers, and an increased biological denitrification loss in the lower soil layers.
- 3. Plant yields increased with increasing soil N application rates up to 180 Kg N/ha. However, yields were suppressed at 360 Kg N/ha or higher N rates.
- 4. Fall spinach accumulated much more NO_3^-N in the tissue than did spring spinach.

Greenhouse Experiments

- Soil NH⁺₄-N concentration reached a maximum under saturated soil moisture conditions with ambient (28 C) temperature. This may be attributed to the occurrence of biological ammonification and the suppression of biological nitrification under anaerobic conditions.
- 2. Maximum soil NO_3^-N accumulation occurred at field moisture capacity and ambient (28 C) temperature indicating the high rate of biological ammonification and nitrification under aerobic conditions.

- 3. The greatest leachate NO_3^-N concentration was present with the field moisture capacity and 10 C treatment indicating that significant dentrification occurred in the leachate water from the pro-files highest in soil NO_3^-N content.
- 4. Maximum leachate organic-N content was present in the saturated moisture and ambient temperature (28 C) treatment. This coincides with the increased soil NH_4^+ -N content of the same treatment.
- 5. Prior to fertilizer N application, the evaporation regime had increased leachate NO_3^-N and organic N contents over the noevaporation and stabilization regimes, indicating the necessity for water movement in the profile for increased leaching loss of N to occur.
- 6. Application of 112 Kg N/ha greatly increased the leachate NO_3^--N content over the evaporation regime indicating the important contribution of fertilizer N towards NO_3^--N content of the leachate. The N application did not increase organic N content of the leachate leachate over the content in the evaporation regime.
- 7. Reducing the incubation time following the N application also increased the NO_3^--N content of the leachate above that from the 112 Kg N/ha regime. Reducing the incubation time reduces the loss of NO_3^--N by biological denitrification. The reduced incubation period did not increase the organic-N content of the leachate over the evaporation regime.
- 8. The 1/2-saturated and 10 C treatments resulted in the highest amount of leachable NO_3^-N . The reduced incubation and 112 Kg N/ha regimes resulted in the highest total NO_3^-N losses, respectively.

This high total value reflects the NO_3^-N concentration and volume of leachate.

9. The saturated and ambient temperature (28 C) treatments caused the highest leaching of total organic-N. The stabilization regime lost the highest total organic-N. Again, this reflects the leachate organic-N concentrations and volumes.

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APPENDIX

N Rate	Tissue NO ₃ -N		Yield
Kg/ha	ppm X	ppm X 10 ⁻²	
	Jul. 30	S ep. 15	
0	3.7	3.7	2.3
90	4.0	3.4	2.7
180	7.8	5.1	2.8
360	5.7	8.3	2.2
LSD (0.05)	2.7	3.0	0.4

Table 1A.--Carrot yield and tissue NO_3^-N content as affected by N rate and sampling date (1969).

Average of 4 Reps.

Analysis of Varience

		F Statistic	
Source	Tissue NO ₃ -N		Yield
	Jul. 30	Sep. 15	
N Rates	4.4*	5.0**	5.2**
Reps	1.1	0.8	1.7

**Significant at 0.01 level.

*Significant at 0.05 level.


N Rate	Tissue NO ₃ -N	
Kg/ha	$ppm \times 10^{-3}$	
0	6.5	
90	8.9	
180	9.8	
360	6.8	
LSD (0.05)	n.s.	

Table 2A.--Lettuce tissue NO_3^-N content as affected by N rate (1969).

Average of 4 Reps.

Analysis of Varience

F Statistic		
Source	Tissue NO ₃ -N	
N Rates	0.7	
Reps	0.6	

N Rate	Tissue NO ₃ -N	Yield
Kg/ha	$ppm \times 10^{-3}$	$Kg/ha \times 10^{-3}$
0	13.5	3.2
90	18.7	3.9
180	18.7	4.4
360	27.7	4.6
LSD (0.05)	6.9	0.4

Table 3A. Spinach yield and tissue NO_3^-N content as affected by N rate (1969).

Average of 4 Reps.

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Analysis of Varience

F Statistic

Source	Tissue NO ₃ -N	Yield
N Rates	6.3**	17.3**
Reps	0.3	1.9

****Significance** at 0.01 level.

N Rate	Tissue NO ₃ -N	Yield
Kg/ha	ppm X 10 ⁻³	Kg/ha X 10 ⁻³
0	20.7	3.4
56	19.1	3.6
112	24.6	3.7
224	27.1	4.1
448	32.3	4.1
LSD (0.05)	4.7	0.4
N Carriers		
NH4NO3	24.8	3.8
Urea	24.7	3.7
LSD (0.05)	n.s.	n.s.

Table 4A.--Spinach yield and tissue NO_3^-N content as affected by N rates and carriers (1969).

Average of 6 Reps.

Analysis of Varience

F Statistic

Source	Tissue NO ₃ -N	Yield
Rates (A)	10.1**	4.1**
Carriers (B)	0.0	0.7
АХВ	1.0	0.1
Reps	2.5*	6.5**
**Significance at 0.0)1 level.	

N Rates	Tissue NO ₃ -N	Soil NO3-N			
Kg/ha	ppm X 10 ⁻²		ppm		
		Ju1. 30	Aug. 13	S ep. 10	
0	2.3	125	171	184	
90	3.5	123	180	241	
180	5.2	164	250	313	
360	4.0	186	264	317	
LSD (0.05)	1.4	25	38	40	
Soil Depth					
0-15		159	214	272	
15-30		151	187	269	
30-46		151	235	283	
46-61		138	229	230	
LSD (0.05)		n.s.	n.s.	n.s.	

Table 5A.--Spinach tissue NO_3 -N content and soil NO_3 -N content as affected by N rates, sampling dates, and soil depths (1970).

Average of 8 Reps.

Analysis of Varience

F Statistic

Source	Tissue NO ₃ -N	Soil NO ₃ -N			
		Jul. 30	Aug. 13	Sep. 10	
Rates (A)	5.8**	12.5**	12.2**	19.7**	
Depth (B)		1.0	2.5	2.5	
АХВ		1.2	0.5	1.4	
Reps	5.1**	15.5**	6.5**	9.0**	
**Significance	at 0.01 level.				

N I Kg L! D 1 3 ł N D A R (**

Table

N Rates	Tissue NO ₃ -N			Yield	Soil	N0 ₃ -N
Kg/ha	Р	pm X 10 ⁻²		Kg/ha X 10^{-2}	P	pm
	Jun. 24	Jul. 12 (stem)	Jul. 12 (leaf)		May 17	Jul. 1
0	1.0	2.6	0.4	9.2	44	139
90	1.3	6.2	0.4	8.0	59	264
180	1.3	8.3	0.7	8.5	86	463
360	1.6	10.5	1.3	6.0	82	586
LSD (0.05)	0.5	2.0	0.4	n.s.	11	60
Depth						
0-15					57	350
15-30					65	361
30-46					79	389
46-61					71	353
LSD (0.05)					11	n.s.

Table 6A.--Spinach tissue NO₃-N content, yield, and soil NO₃-N content as affected by N rates, sampling dates, and soil depths (Spring 1971).

Average of 8 Reps.

Analysis of Varience

				F Statistic		
Source	Ti	ssue NO_3^{-}	N	Yield	Soil NO3-N	
	Jun. 24	Jul. 12 (stem)	Jul. 12 (leaf)		May 17	Jul. 1
N Rate (A)	3.2*	26.0**	8.4**	0.8	23.6**	87.3**
Depth (B)					5.3**	0.7
АХВ					0.9	0.2
Reps	3.1*	1.6	2.7*	1.62	16.5**	7.7**
**Significanc	e at 0.0	l level.				
*Significanc	e at 0.0	5 level.				

N Rates	Tissue NO ₃ -N			Yield	Soil	NO ₃ -N
Kg/ha	F	opm X 10 ⁻³		$Kg/ha \times 10^{-3}$	P	pm
	Sep. 1	Sep. 14	S ep. 3 0		Jul. 31	Sep. 7
0	6.7	0.5	0.9	2.6	87	159
90	7.0	2.5	2.9	2.5	112	318
180	9.3	3.3	1.9	2.8	264	534
360	8.2	2.6	2.8	2.2	348	781
LSD (0.05)	n.s.	n.s.	1.5	n.s.	66	97
Depth						
0-15					310	554
15-30					202	486
30-46					158	414
46-61					140	338
LSD (0.05)					66	97

Table 7A.--Spinach tissue NO_3 -N content, yield, and soil NO_3 -N content as affected by N rates, sampling dates, and soil depths (Fall 1971).

Average of 4 Reps.

Analysis of Varience						
			F	Statistic		
Source	Ti	ssue NO_3^-	N	Yield	Soil N	0 ₃ -N
	Sep. 1	Sep. 14	Sep. 30		Jul. 31	Sep. 7
Rates (A)	0.9	1.1	3 .9 *	0.7	28.4**	63.2**
Depth (B)					10.7**	7.5**
АХВ					1.4	2.2*
Reps	1.6	1.5	1.9	0.5	9.1**	2.0
**Significan	ce at 0.0	l level.				
*Significand	ce at 0.0	5 level.				

Temperature	Soil NO ₃ -N	Soil NH ⁺ ₄ -N
	ppm	
Ambient	34.4	49.7
10 C	18.2	33.0
LSD (0.05)	11.9	11.4
Moisture		
Field Capacity	55.7	22.7
1/2-Saturated	20.8	30.8
Saturated	2.4	70.5
LSD (0.05)	14.6	14.0
Depth		
0-15	41.3	39.0
15-30	19.2	42.3
30-46	18.4	42.7
LSD (0.05)	14.6	n.s.

Table 8A.--Soil NO₃-N and NH₄-N contents from greenhouse experiment, prior to applying N fertilizer, as related to temperature, moisture, and soil depth.

Analysis of Varience

	F Statistic	
Source	N0 ₃ -N	NH ⁺ ₄ -N
Temperature (A)	7.4**	8.6**
Moisture (B)	27.8**	26.9**
AXB	12.1**	2.4
Depth (C)	6.4**	0.2
AXC	2.2	0.8
BXC	1.8	2.7*
AXBXC	1.1	1.6
Reps	1.0	2.4
** Significance at 0.01 level	•	

Table 9A.--Analysis of varience for Table 2.

F Statistic

Source	Redox Potential	0 ₂ Diffusion
Temperature (A)	0.1	0.2
Moisture (B)	66.6**	24.6**
АХВ	0.9	0.1
Reps	1.3	0.2

******Significance at 0.01 level.

*Significance at 0.05 level.

Table 10A.--Analysis of variance for Table 3.

F Statistic

Source	NO ₃ -N	Organic-N
Temperature (A)	5.7*	19.5**
Moisture (B)	4.3*	4.2*
АХВ	3.5*	2.1
Regimes (C)	3.8*	10.3**
A X C	3.5*	1.1
ВХС	3.8**	2.8
АХВХС	3.4*	0.4
Reps	0.9	3.7*

**Significance at 0.01 level.
*Significance at 0.05 level.

Table 11A.--Analysis of variance for Table 5.

F Statistic

Source	N0 ₃ -N	Organic-N
Temperature (A)	2.9	10.8**
Moisture (B)	18.4**	3.9*
AXB	0.9	1.0
Regime (C)	33.4**	8.1**
AXC	0.5	1.3
ВХС	6.9**	1.4
АХВХС	0.7	0.3
Reps	4.4**	4.7**

****Significance at 0.01 level.**

