THE UNIVERSITY OF MANITOBA

A KINETIC APPROACH TO THE DECOMPOSITION OF DAIRY MANURE IN A SCANTERBURY CLAY SOIL

by

Michael Brian Tokarz

A Thesis

Submitted to the Faculty of Graduate Studies

in partial fulfillment of the requirements

for the Degree of Master of Science

Department of Agricultural Engineering
University of Manitoba
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ABSTRACT

With the price of fertilizer rising, and with the trend of animal concentrations increasing in localized areas, there has been a renewed interest in the application of manure to soil. The rate of decomposition of manure in soil, which should provide the correct application rate of manure to soil such that no harmful effects result to plants, ground or surface waters, is currently unknown. This experiment was thus established to determine kinetic coefficients and reaction rates of dry and wet dairy manure in a Scanterbury clay soil.

Carbon mineralization in the diary manure followed a first-order kinetic equation in the form of $C = Ae^{-kt}$. The carbon evolved as CO_2-C after 64 days of incubation at 15^{o}C was 29.1, 15.2 and 8.9 percent of the original when dry manure was incubated and after 32 days of incubation at the same temperature was 50.5, 37.1 and 20.9 percent of the original when wet manure was incubated for the 112, 224 and 561 kg N ha^{-1} loadings, respectively. The turnover period required to remove 99.9 percent of the manure carbon was higher for the dry manure relative to the wet manure for the same loadings, ranging from 0.77 calendar years for the 112 kg N ha^{-1} of the latter to 13.5 calendar years for the 561 kg N ha^{-1} of the former when incubated at a temperature of 15^{o}C.

A lack of "smooth" nitrogen mineralization curves, required for kinetics, prevented a kinetic approach to

estimate nitrogen turnover rates. When dry dairy manure was added to soil, nitrification did not occur but when wet dairy manure was added to soil, nitrification occurred in the 112 and 224 kg N ha^{-1} loadings, but the amount nitrified was less than the control which had no manure added.

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CHAPTER 1

INTRODUCTION

1.1. Background

Nitrogen (N) is important in the life processes of all plants and animals. Organic and inorganic forms of N exist in the soil but plants are capable of utilizing only inorganic forms of N such as ammonium (NH_4) and nitrate (NO_3) for growth.

More than 95 percent of the N in the surface soil is organically combined (Bremner, 1965). Therefore, mineralization of the organic N in the soil must occur to provide plant-available N. Usually one to three percent of the organic N is mineralized throughout the growing season (Bremner, 1965). However, the amount of N made available by mineralization of soil organic matter is rarely sufficient to meet plant needs employing present day cropping practises. Consequently, fertilizers have been applied to supply the necessary N needs.

As a result of economic advantages of commercial fertilizers, other sources of N such as animal waste and sewage sludge have been used sparingly. Animal wastes have long been recognized as a beneficial source of nutrients for plants and recently there has been a renewed interest in the old method of land disposal of animal wastes. With a high

concentration of animals in localized areas, accumulation of manure may result. The "waste" may be spread on the land but, if abused, land disposal can create more problems than it solves. Manure disposal at moderate rates is a useful way to utilize the manure, but, when the applied rate greatly exceeds plant needs, it can pose a serious environmental hazard. For example, nitrate may be formed in excess of plant needs and, being a mobile ion, may percolate downward with the surface water resulting in nitrate contamination of ground water.

Methemoglobinemia or nitrate cynanosis can occur when infants consume substances high in nitrate nitrogen (NO_3-N). For example, water containing in excess of 10 mg l^{-1} of NO_3-N may result in nitrate reduction and nitrite (NO_2) substitution for oxygen (O_2) in the hemoglobin of the blood and subsequent suffocation and discoloring of the skin. Animals such as cattle, sheep, horses and swine can also be affected by waters and forages high in NO_2 or NO_3.

Ruminant animals are especially affected by high levels of NO_3 in forages. The intestinal bacteria of ruminants convert the NO_3 in forage to NO_2 resulting in methemoglobinema and finally death by asphyxiation (Sinclair and Jones, 1964; Wagner, 1971). Webber and Lane (1969) reported that livestock consuming forage in excess of 0.3 percent NO_3-N on a dry weight basis was sufficient to cause nitrate poisoning.

Buchanan (1971) stated that land application is the most economic and feasible method of animal manure disposal but, he noted, limitations to land application include air pollution, capabilities of the land and surface runoff. Surface runoff may not only occur from land application of heavily manured fields, but also from uncontrolled runoff and leaching from feed lots, and lagoons which may carry nutrients such as N to aquifers, streams, rivers and lakes (Schulte, 1975). Sawyer and McCarty (1967) noted that, after the addition of organic matter to a stream, the oxidation of inorganic N (nitrification) can deplete the dissolved oxygen level in streams resulting in fish kills. The nutrients from agricultural activity, for example, animal manure, contain the same essential nutrients for microbial growth and thus nutrients from agricultural runoff can hasten the eutrophication of lakes.

Plants cannot survive by utilizing N compounds alone but must also have access to other mineral nutrients and carbon. Some of the sixteen nutrients required for plant growth are phosphorous, potassium, sulphur, calcium, magnesium, iron, manganese, boron, copper, zinc, molybdenum and chlorine (Fehr et al, 1971). These elements can also be accumulated in the living organisms of the soil and then liberated upon death of the organism. The decomposition of all plant tissue and animal organisms after death does not always proceed com-

pletely to the final products of mineralization. Occasionally, new organic substances are formed. These substances, peat and humus for example, possess greater resistance to decomposition than the original material (Kononova et al, 1966).

The principal source of carbon (C) required by plants during photosynthesis is atmospheric carbon dioxide (CO_2). The most important source of replenishment of CO_2 in the atmosphere is the soil (Kononova et al, 1966). As a means of ensuring the production of CO_2 by the soil, Kononova et al (1966) stated that a systematic supplementation of its reserves (soils) can be achieved by the addition of fresh organic matter. Thus, the addition of animal manure to soil not only adds mineral nutrients to the soil but also replenishes the C supply.

1.2. Objectives

Carbon and nitrogen are important factors in determining the rate of decomposition of organic matter. The fate of applied N in the soil is especially complex due to the various paths such as nitrification, denitrification, mineralization, immobilization, fixation, volatilization, and plant uptake. Interest in the use of land for "waste" disposal indicates a need for more knowledge about interactions of soil and "waste". For instance, at what rate does

mineralization take place using a mixture of animal faeces and soil?

Information on C and N mineralization -- in particular reaction rates -- should provide an insight in adding the correct amount of manure to soil. Thus, the objectives of this project are to establish reaction rates and kinetic coefficients for the degradation of various amounts of manure, specifically dairy manure, in a clay soil. Dairy manure was selected because it represents approximately one-third[1] of the animal manure (dry weight basis) produced in the Red River Valley. Kinetic equations are to be used to describe the mineralization processes. Carbon dioxide (CO_2-C) and ammonia (NH_3) evolution are to be monitored and total Kjeldahl, ammonium (NH_4-N), nitrite (NO_2-N), and nitrate (NO_3-N) nitrogen are to be measured.

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[1] Calculations based on animal numbers in the Red River Valley in 1974 obtained from the Manitoba Agriculture Yearbook, 1974 published by the Manitoba Department of Agriculture.

CHAPTER 2

REVIEW OF LITERATURE

2.1. Nitrogen Mineralization

The term "nitrogen mineralization" has been employed to denote microbial transformation from the organic to the inorganic forms of N:NH_4-N, NO_2-N and NO_3-N. The decomposition of organic matter in soil is slow. This results in a large segment of the N in the decomposing phase. However, the addition of manure, which contains partially degraded plant material, to soil can cause the transformations to be rapid (Buckman and Brady, 1969) as evidenced by the ready decomposition of at least the soluble components (Brady, 1975). Brady (1975) also noted that manure, along with crop residues, is a primary means of replenishing soil organic matter. Furthermore, Loehr (1974) noted that the land (soil) remains the most appropriate point of disposal of animal waste. Since the soil is an important medium for manure disposal, the transformations that occur in soil will be discussed in the following sections. The same processes that occur in the soil can be applied to the decomposition of manure in soil.

2.2. Ammonification

Ammonification is the process whereby organic N is converted to NH_3. However, before ammonification can occur, a process known as aminization must occur. Through the aminization process, amino compounds such as proteoses, peptones, and amino acids are formed by the enzymatic hydrolysis of protein. Proteins and allied compounds largely constitute the N matter added to soil (Lyon et al, 1952). This transformation may be indicated as:

$$\text{Proteins and near proteins} + \text{Enzymatic digestion} \longrightarrow \text{complex amino compounds} + CO_2 + \text{energy} + \text{other products} \qquad (2.1)$$

These complex transformations are brought about by a large number of common heterotrophic organisms - bacteria, fungi and actinomycetes. The microbiology of protein breakdown in soil is inadequately understood (Alexander, 1967). Alexander stated that bacteria probably dominate in neutral or alkaline environments, but fungi and possibly actinomycetes may also contribute to the transformation. The key group in acid soils is the fungi. The organisms use energy from this type of digestion as well as utilize some N in the enzymatic process. As the protein is degraded, CO_2 is evolved.

Amino acids may then be (1) metabolized by microorganisms (immobilization); (2) transformed by microbial enzymes with the formation of NH_3 (ammonification); (3) adsorbed by clay minerals or incorporated in the humus

fraction; or (4) utilized by plants (Stevenson, 1964). Immobilization and ammonification are by far more important processes since little free amino acids can be found in the soil and higher plants rarely use amino N.

The same organisms responsible in aminization also promote ammonification (Lyon et al, 1952). The enzymatic process may be indicated as follows:

$$\underset{\text{amino combination}}{R\text{-}NH_2} + HOH \xrightarrow[\text{Hydrolysis}]{\text{Enzymatic}} ROH + NH_3 + \text{Energy} \qquad (2.2)$$

The NH_3 produced is converted rapidly to the NH_4^+ ion as shown below:

$$2NH_3 + H_2CO_3 \longrightarrow (NH_4)_2CO_3 \rightleftharpoons 2NH_4^+ + CO_3^= \qquad (2.3)$$

Once the N appears as NH_4^+, it can be synthesized by plants or soil microorganisms, fixed in the soil or can undergo oxidation to NO_2 and NO_3. Ammonification appears to proceed best in well drained, aerated soils with plenty of organic matter (Lyon et al, 1952). The process can take place, to some extent, under almost any condition, even anaerobic conditions, due to the great number of different organisms capable of ammonification.

2.3. Nitrification

The NO_3 ion is important for plant growth and is provided by a process known as nitrification. Alexander

(1967) defines nitrification as the biological conversion of N in organic or inorganic compounds from a reduced to a more oxidized state. By this definition NH_3 is oxidized to NO_2 and then NO_3. The rates of assimilation by plants for NH_4 and NO_3 are quite different due to the ion exchange capacity of the soil with NH_4 and so have a bearing upon the crop's nutrition. If the formation of NH_4 and NO_3 exceeds the assimilation rate of the plants, the NH_4 or NO_3 may percolate downward with the seeping water resulting in groundwater contamination.

2.3.1. Nitrifying Population

Two autotrophic genera are prominent in nitrification - *Nitrosomonas*, the ammonium oxidizer and *Nitrobacter*, the nitrite oxidizer. They are classified in *Nitrobacteriaceae*, one of the families of the order *Pseudomonadales*. Both genera are aerobes. Most of the ammonium oxidizers that have been isolated seem to be related or identical with *Nitrosomonas europaea*. This oxidizer is 0.9 to 1.0 by 1.1 to 1.8 micron in size, with a polar flagellum or occasionally one flagellum at each end of the cell (Alexander, 1967). Alexander (1967) referenced Breed et al, (1975) as stating that the *Nitrobacter winogradski* is a common nitrite oxidizer that is 0.6 to 0.8 by 1.0 to 1.2 micron in size, gram negative and a non-motile rod. Five other genera of nitrifyers are also recognized. These are the ammonium oxidizers - *Nitrosococcus, Nitrosospira, Nitrosogloca, and Nitrosocystis,*

and the nitrite oxidizer, *Nitrocystis*. The generation time of the NO_2 oxidizers is shorter than the NO_2 formers normally resulting in little accumulation of NO_2 in soils.

The *Nitrosomonas* and *Nitrobacter* populations are usually quite small and many soils, especially acidic ones, have fewer than 100 viable cells of one or both genera per gram. Rarely are populations in excess of 10^5 cells per gram in unfertilized soils. Addition of manure may cause the populations to rise and may reach values of 10^6 and 10^7 cells per gram (Alexander, 1965). The abundance of autotrophs declines with increasing soil acidity and depth and varies with cropping practice, soil treatment and season of the year.

2.3.2. Autotrophic Oxidation

The conversion of NH_4 to NO_2 and NO_2 to NO_3 are exothermic reactions that must take place under aerobic conditions. The oxidation of NH_4 to NO_2 is shown in the following equation.

$$NH_4 + 1\tfrac{1}{2}O_2 \longrightarrow NO_2^- + 2H^+ + H_2O \qquad (2.4)$$

The free energy from the oxidation process has been reported in the range of -65.2 to -84.0 kcal per mole of ammonium (Alexander, 1965). The nitrobacter oxidation reaction is shown in Equation (2.5).

$$NO_2^- + 1\frac{1}{2}O_2 \longrightarrow NO_3^- \qquad (2.5)$$

The free energy associated with this reaction is -17.5 to -20.0 kcal per mole (Alexander, 1965).

The above reactions require molecular oxygen which means that the process occurs most readily in well aerated soil. Also, H^+ ions are released in the first step of the nitrification process which acidifies the soil. As a rule, NO_2 oxidation proceeds most rapidly, ammonification most slowly with NH_4 oxidation in between.

2.3.3. Heterotrophic Microorganisms

Heterotrophic microorganisms such as bacteria, fungi and actinomycetes are also capable of nitrification. While the biochemical mechanisms of autotrophic and heterotrophic transformations are known to be quite dissimilar, the physiological or biochemical characteristics of the heterotrophs involved in nitrification are largely unknown (Alexander, 1965). This is because the heterotrophic microorganisms involved are difficult to isolate. However, the population of heterotrophs capable of some type of nitrogen oxidation is remarkably large (Alexander, 1965).

2.4. Immobilization

Immobilization denotes the process of the conversion

of inorganic N to the organic form during microbial synthesis. Micro-organisms, the same ones responsible for ammonification, use inorganic N (NH_4 or NO_3) in the synthesis of cell tissue resulting in the formation of organic N which is somewhat resistant to further biological degradation (Bartholomew, 1965).

Whenever mineralization occurs, immobilization runs counter to it. By measuring the quantity of N produced or lost, neither process is measured. Rather the net release or tie-up of N is indicated (Alexander, 1967). The carbon to nitrogen (C:N) ratio usually gives evidence of which process, mineralization or immobilization, predominates in the original material. If the C:N ratio is 30 to 1 or greater, net immobilization usually results from the initial decomposition stage (Tisdale et al, 1966). This occurs because all mineralized N will be reabsorbed by the micro-organisms for growth. For ratios between 20:1 to 30:1, there may be neither net immobilization nor release of mineral N while for a C:N ratio less than 20:1, mineral N is usually released in the first stages of decomposition (net mineralization).

When substances with a high C:N ratio are added to soil, the C is rapidly liberated and lost as CO_2. The N is retained mainly in the organic form as microbial tissue until the C:N ratio has become sufficiently reduced to allow accumulation of inorganic N. During the foregoing stages, the N of the original substrate may have been mineralized repeatedly by the successive decomposition and mineralization

of succedding generations of microbes. The above stage may be referred to as primary mineralization (Harmsen and Kolenbrander, 1965).

To estimate the N required to satisfy cell synthesis, data on the extent of carbon (C) assimilation and C:N ratios of the cell are required. As a rule, five to ten percent of substrate C is assimilated by bacteria, thirty to forty percent by fungi, and fifteen to thirty percent by actinomycetes (Alexander, 1967). Alexander also noted that Waksman (1924) approximated the C:N ratios of the cellular components of bacteria, fungi and actinomycetes to be 5:1, 10:1 and 5:1, respectively. Thus, the decomposition of a 100 units of substrate C require 1 to 2, 2 to 4, and 3 to 6 units of N for bacteria, fungi, and actinomycetes, respectively.

The ratio of C to N in the organic matter of the furrow slice of arable soils commonly ranges from 8:1 to 15:1 (Brady, 1975). Michalyna[1] (unpublished report) obtained the following C:N ratios: 8.9:1 for cultivated McTavish clay; 7.6:1 for cultivated Osborne clay; 8.5:1 for cultivated Scanterbury clay, and 10.:1 for cultivated Dencross clay.

Buckman and Brady (1969) stated that the addition of farm manure to soil may widen the C:N ratio, especially if the manure is strawy. Brady (1975) pointed out that strawy manures may have a C:N ratio as high as 100:1. The C:N ratio of dairy manure without bedding was 8.4:1 for cow faeces and 6.1:1 for calves (Loehr, 1974). Weber (1973) noted C:N ratios of 20:1 for livestock manure.

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[1] W. Michalyna, Department of Soil Science, University of Manitoba, unpublished Report of the Detailed Soil Survey of Glenlea Research Station, Glenlea, Manitoba.

2.5. Nitrogen Gas Losses in Soil

The liberation of gaseous N from soil is not always readily established, but three reactions have been proposed:

a) non-biological losses of ammonia;

b) chemical decomposition of nitrite to nitrogen oxides;

c) microbial denitrification leading to the liberation of nitrogen gas (N_2) and nitrous oxide (N_2O) (Alexander, 1967).

2.5.1. Ammonia Volatilization

Volatilization of free NH_3 is appreciable under certain circumstances and as much as one fourth of the NH_3^+ formed microbiologically may be lost as gaseous NH_3 (Alexander, 1967). Below pH 7.0, such losses are usually insignificant since NH_3 exists as the ammonium ion, NH_4. Ammonia volatilization can occur below pH 7.0 if there is sufficient alkalinity present. Above pH 8.0 NH_3 evolution is appreciable. During manure decomposition, at or near the soil surface, the pH rises during ammonification and gaseous NH_3 is released (Alexander, 1967).

2.5.2. Nitrite Decomposition

In acid environments, below pH 5.5, nitric oxide (NO) is formed from NO_2 decomposition. This process is chemical but depends on biological mass - nitrification or NO_3 reduc-

tion - to form NO_2. Appreciable losses can occur when NH_4 is oxidized at a pH initially below 5.5 or falling below 5.5 during nitrification.

2.5.3. Denitrification

The major mechanism of gaseous N removal is by microbiological denitrification but the exact mechanisms of denitrification are not known (Brady, 1975).

Brady (1975) showed the general trend of the reactions to be represented as:

$$2HNO_3 \xrightarrow{-2(0)} 2HNO_2 \xrightarrow{-2(0)} N_2O \xrightarrow{-(0)} N_2 \xrightarrow{-(0)} 2NO \qquad (2.6)$$

Nitrates Nitrites Nitrous oxide Elemental Nitrogen Nitric Oxide

Under field conditions, N_2O is the dominant product (Brady, 1975). In acid surroundings, nitrite decomposes according to the following reaction:

$$3HNO_2 \longrightarrow 2NO + 4NO_3 + H_2O \qquad (2.7)$$

The NO, which depends on the decomposition of nitrate to nitrite, may be reduced to N_2 by microflora or oxidized in air to nitrogen dioxide.

Denitrification is accomplished by facultative anaerobic bacteria capable of using NO_3 instead of O_2 as a hydrogen acceptor under O_2 limiting conditions (Broadbent and Clark, 1965). Many of the micro-organisms responsible

for denitrification are capable of other transformations, for example, ammonification, and do not depend strictly on anaerobic conditions in order to survive. The denitrifying bacteria can grow aerobically without NO_3 and anaerobically in its presence. The active species of common facultative bacteria are largely limited to the genera *Pseudomonas*, *Achromobacter*, *Bacillus* and *Micrococcus* (Alexander, 1967). Alexander also noted that the *Pseudomonas* and *Achromobacter* are the dominant genera in soil and that the *Bacillus* strains, though numerous, are of less importance.

Under conditions where a readily decomposible substrate (organic matter) is undergoing rapid decomposition and where the oxygen diffusion rate to the bacteria is slow, the bacteria can utilize NO_3 as a hydrogen acceptor. The above process occurs more readily in fine-textured soils than in sandy ones. Broadbent and Clark (1965) noted that in Nommik's experiment (1965), in which he studied different sizes of soil aggregate, denitrification decreased with increasing particle size. Also, small pores which are filled with water aid in developing micro-environment anaerobic conditions. Broadbent and Clark (1965) quoted Bremner and Shaw (1958) as stating that there is little loss of N_2 gas if the moisture content is less than 60 percent of the water holding capacity.

Since denitrification is very rapid, the process can remove a significant quantity of NO_3. Soil pH influences the denitrification rate which is usually very slow in acid soils

and very rapid in high pH soils (Bremner and Shaw, 1958). Denitrification is optimum in the temperature range of 60-65^{o}C. A readily decomposable source of organic C must be available to induce rapid denitrification. Bremner and Shaw (1958) obtained a general relation between rate of denitrification and organic matter or total C content in soil. However, Stanford et al (1975) found that extractable glucose - C provided a more reliable basis for prediction than total organic C.

2.6. Carbon Mineralization

Carbon is the common constituent of all organic matter. The carbon in organic matter decomposition serves two functions, providing energy for micro-organism growth as well as supplying C for the formation of new cells.

The conversion of organic C to inorganic C is referred to as carbon mineralization. The principal reaction in the decay is the oxidation of C compounds to CO_2 and H_2O. As much as 50 percent of the C in compounds attacked by heterotrophic decay organisms (bacteria, fungi and actinomycetes) may be retained as reconstituted structural and protoplasmic tissue (Hausenbuiller, 1972). On the other hand, autotrophic bacteria, such as nitrifiers, obtain their C mostly from CO_2. The CO_2 gas in the soil escapes to the atmosphere where it can be assimilated by plants through the photosynthesis process. Fungi release less CO_2 than other microbial groups

because the fungi are more efficient in their metabolism (Alexander, 1967).

Under optimum conditions as much as 112 kg of CO_2 per hectare per day (100 lb CO_2 $acre^{-1}$ day^{-1}) may be evolved from the soil. Approximately 9 to 14 kg (20 to 30 lb) are probably more common (Buckman and Brady, 1969). Lesser amounts of CO_2 react with the soil to produce carbonic acid (H_2CO_3) as well as the carbonates and bicarbonates of calcium, potassium, magnesium and other bases. These salts are soluble and may be lost in drainage or can be utilized by higher plants.

Carbon mineralization is most rapid in neutral or slightly alkaline soils. The decay organisms function most effectively between approximately 27°C to 38°C (80°F-100°F) and the rate of decay decreases until freezing point is reached. Carbon content of the soil is important in deciding whether mineralization or immobilization governs as reflected previously in the C:N ratio of the soil.

2.7. Nitrogen Availability in Soil

The need for a satisfactory index of the availability of N in soil was recognized for a long time in order to predict the amount of fertilizer N required to produce a desired crop yield. However, there is little information on N availability from organic wastes deposited in soil. Some investigators who have made efforts in this direction

include Floate and Torrance (1972), Mathers and Stewart (1970), and Finstein (1972). Many of the biologic methods proposed to determine N availability have been reviewed by Harmsen and Van Schreven (1955), Bremner (1965), and Daknke and Vasey (1973). A summary of these reviews follows.

2.7.1. Field, Greenhouse and Laboratory Experiments

Field and greenhouse trials have been used to predict N availability in soils for plant growth, but these tests are expensive and time-consuming. Field experiments are subject to uncontrollable external influences such as climatic conditions, variations between seasons, influence of the crop and treatments of previous years (Harmsen and Van Schreven, 1955). In greenhouse experiments, the external conditions are more easily standardized. However, laboratory studies have been considered most suitable for assessing N availability in soils in spite of the fact that laboratory tests may not correlate to vegetative tests. Tchan (1959) pointed out that a lack of correlation between laboratory and vegetative tests may not necessarily be a reflection of the value of the laboratory tests as a measure of nutrient availability. Laboratory trials are performed on soils that have limitations and must be taken into account in interpreting the data. For instance, laboratory studies are not affected by crop cover as well as root range and root pattern of the crop.

2.7.2. Biologic Incubation Techniques

The biologic laboratory methods used to determine nitrogen availability are:

Type 1: The measurement of microbial growth;

Type 2: The estimation of CO_2 produced by incubation;

Type 3: The estimation of mineral N formed by incubation where conditions promote mineralization.

2.7.2.1. Type 1 Microbial Growth

The microbial methods used to assess plant nutrient availability in soil are unsatisfactory (Tchan, 1959) and have aroused little interest. Tchan stated that some problems included: (1) pH adjustment may be different from actual soil conditions; (2) addition of organic matter is necessary to promote growth of test organisms. Other organisms may compete with the test organism and even suppress the test organism; (3) sterilizing the soil may release nutrients from tissues of living cells when killed and represent soil conditions for only that particular circumstance; and (4) the test organisms may not have the same uptake rate or growth rate as higher plants and so the test organisms may not be able to simulate higher plant life.

2.7.2.2. Type 2 CO_2 Production

The methods used for estimating CO_2 production have been described as "indirect procedures" (Harmsen and Van Schreven, 1955). The amount of CO_2 produced will be proportional to the amount of mineral N initially present plus the amount made available during incubation. Bremner (1965) noted that Cornfield (1961) used this procedure and considered that the main advantage of this method was that the soil doesn't have to be extracted to determine mineral N since it is related to CO_2 evolved. Methods to monitor CO_2 evolution have differed from investigator to investigator with three types of aeration techniques used: (1) no air flow; (2) continuous air flow and; (3) intermittent air flow.

In the first type, soil samples are incubated in stoppered flasks containing an alkali to absorb CO_2. Problems with this system can occur. If microbial activity is high, the supply of O_2 can become limiting. By opening the flask frequently the O_2 supply can be replenished but may not be effective if the O_2 added is less than the constant high level of O_2 required. Carbon dioxide may be lost with prolonged exposure to the atmosphere. Nevertheless, this procedure has been used by researchers such as Floate and Torrance (1970) and Finstein (1972).

In the continuous air flow method, a stream of CO_2-free air which passes over the soil flushes the soil-evolved CO_2 into a separate alkali container. This method is best suited for experiments where CO_2 is rapidly evolved since the formed CO_2 is removed almost as fast as it is produced and O_2 is replenished rapidly. Pressure or suction may be used to provide the air flow. The soil is usually maintained at "field capacity" due to the ease of maintaining a high relative humidity in the air stream. Mathers and Stewart (1970) used this procedure in their experiment.

Since the respiration of many organisms is different in the presence or absence of CO_2, and since CO_2 is normally present in soil, intermittent air flow has an advantage over continuous air flow because some CO_2 will always be present in the soil atmosphere (Stotzky, 1965). However, toxic conditions may occur if O_2 levels become too low or CO_2 levels become too high.

2.7.2.3. Type 3 Mineral N

The third method, estimation of mineral N under aerobic conditions, has been considered the most satisfactory method of assessing the availability of N to plants (Harmsen and Van Schreven, 1955 and Bremner, 1965) because the organisms responsible for mineralization in the field are the same ones found in the incubation experiments. Results of such experiments can give an indication of the potential of soils

to supply available N under more controlled conditions than can those measuring the amount of N that will become available under field conditions. Incubation experiments can provide an accurate index of soil N availability to plants (Bremner, 1965; Daknke and Vasey, 1973).

2.7.3. Environmental Variables

Many factors affect the release and uptake of N during a growing season. Such factors include soil physical properties and soil profile characteristics such as water level, length of growing season, weather during a growing season, pH, microbial activity, nutrient interactions, previous cropping practises, pests and disease, plant population, residual fertilizer effects, availability of subsoil nitrogen and the root range and root pattern of the crop (Bremner, 1965). In laboratory studies, most of these variables must be controlled to elucidate the effect of one or more of the above factors. To accomplish this, numerous mineralization procedures have been used to estimate mineral N. Noticeable variations in procedure include differences in nutrient supply, quantity of soil, particle size, the use of physical or chemical amendments, pH, temperature, water level, aeration technique, incubation period and type of incubation vessel. Many of the attempts have been made to make the conditions of incubation such as moisture content, aeration and temperature favourable for nitrification.

Consequently, comparisons between laboratory results and what might be expected in the field are difficult to make since the nitrifying bacteria in a particular soil are a result of adjustment to the climate and a particular soil environment.

2.7.3.1. Temperature

Temperature stimulates microbial activity with optimum activity for NH_3 oxidation being most often accepted between 30 and 35°C. However, Daknke and Vasey (1973) stated that Mahendrappa et al (1966) noted that soils from the northern states of the U.S.A. nitrified best between 20 and 25°C while, in southern states, nitrification was best at 35°C. Both ammonification and nitrification are limited at low temperature with most investigators agreeing that nitrification is more retarded than ammonification at low temperatures. Below the optimum temperature of 25 to 35°C, nitrification decreases gradually following an asymptotic curve and practically ceases near the freezing point. Harmsen and Kolenbrander (1965) noted that Tyler et al (1959) reported vigorous nitrification at temperatures as low as 3°C while Gerretsen (1942) and Anderson (1960) obtained considerable nitrification only above 6 or 7°C. Topnik (1976)[1] verified Alexander's (1965) statement which stated that there is little reason to doubt that there may be a slow nitrification below 2°C. Topnik[1] obtained 18% nitrification at 0°C using extended aeration of human sewage. The difference in microbial preferences for temperature is

[1] B. Topnik, Department of Civil Engineering, University of Manitoba, personal communication, August, 1976.

probably related to the soil type and climate since the microorganisms in a particular soil are a result of acclimation to the soil and the climate.

2.7.3.2. Aeration and Soil Moisture

Aeration and soil moisture are interrelated. Bremner (1965) suggests that aeration is not a serious problem if the amount of water present is not significantly in excess of the amount required for optimal nitrification and that the aeration method doesn't result in a significant loss of water. The optimal water level for nitrification depends on soil texture and organic matter in the soil and is a function of the water retaining properties of the soil. Naturally, therefore, there is a lack of agreement about the optimum moisture content for nitrification. Published data vary between 40 percent water holding capacity to more than field capacity. Discrepancies that cause this disagreement are due to variations in other factors and the broad, flat curve near the optimum moisture content.

Penkava[1] stated that the method of determining field capacity has varied from researcher to researcher with variation in the method of saturation and length of drainage. Alexander (1967) noted that the optimum moisture content for ammonification generally falls between 50 and 75 percent of the water holding capacity of the soil.

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[1] F. Penkava, Department of Agricultural Engineering, University of Manitoba, personal communication, September, 1976.

2.7.3.3. pH

The rate of nitrification is closely related to soil pH. The optimum reaction in soil for many of the ammonium oxidizers is above neutrality while that for nitrite oxidizers is close to a neutral pH. The Nitrosomonas thrive in a pH range of 7 to 9 while Nitrobacter strains are detectable in a pH range of 5 to 10. Complete agreement, however, has not been reached about the optimum and limiting pH values for nitrification.

2.7.3.4. Nutrient Supply

Rarely would any nutrient other than the energy substrate (CO_2) be limiting for an active population of nitrifying organisms. As a rule, the slowest step in mineralization is ammonification which, in turn, affects the substrate concentration for NH_4 oxidation and NO_2 oxidation.

2.7.3.5. Soil

The type and particle size of the soil affects mineralization. The type of clay mineral influences the extent of nitrification with montmorillonite permitting the greatest oxidation followed by illite. Clay-fixed NH_4 is nitrified slowly in vermiculite soils. Most researchers

have used air-dried surface soils (0 - 15.2 cm) for incubation experiments. The size of samples used for incubation experiments has varied with most samples being less than 50 g since better soil aeration is possible with small samples.

For incubation experiments Bremner (1965) states that the soil should be ground to pass a 2-mm sieve in order to standardize soil particle size. He noted that grinding the soil increases the accessibility of organic matter to microbes and thus increased mineralization could be expected.

The soil may be amended by adding sand or vermiculate to improve the physical condition of the soil. Keeney and Bremner (1967) felt that by mixing quartz sand (three times the soil weight) with soil, the amount of water required for maximum mineralization would be practically the same for all soils (6 ml of H_2O per 10 g of soil). Therefore, preliminary analysis for determining water requirements would be eliminated.

2.7.4. Incubation Vessels

Although the number of devices employed as incubation vessels is large, the basic types of aeration devices are similar to the ones mentioned for the Type 2 method (Section 2.7.2.2.). Recently, jars sealed with a semi-permeable membrane have been used. The semi-permeable membrane allows O_2 and CO_2 to transfer but prevents moisture from passing through. However, Ryan et al (1973) stated that this method was

inadequate at high loading rates since aeration is slow and anaerobic conditions may exist.

Most studies on soil N mineralization within the past 20 years have been short-term, motivated primarily for a rapid and reliable method of assessing soil N availability. Such studies used incubation times of a practical minimum of about 7 to 14 days. The results from the mineral N released in short-term incubations often appear to reflect relative N supplying capacities of the soil.

2.8. Kinetics

Reaction kinetics are concerned with the determination and interpretation of the velocities or rates of reactions. The former relates to the direction and extent of reaction and the latter to the rate of reaction (Weber and Canale, 1972). Essentially all research on biological processes should include kinetic descriptions of the process. Without such descriptions one cannot evaluate accurately or scientifically the effect of a particular variable or environmental factor (Pearson, 1968). Furthermore, it is only by means of kinetic descriptions of processes that waste treatment technology can be taken out of the "black box" and put on a sound technological basis (Pearson, 1968). It is in this vein that the research project described in subsequent chapters has been established.

Early studies of N mineralization have plotted N mineralization curves against time for various loading rates, temperatures, etc., but seldom have they provided a rational or consistent basis for estimating N-supplying capacities of the soil. Describing the process of mineralization using kinetic equations can provide a means of showing the quantitative effect of the different parameters (Hadas and Kafkafi, 1974). However, few mineralization experiments using kinetic equations have been performed.

Information using kinetic equations for the degradation of organic waste in soil is especially scarce. If the soil is to be utilized effectively as a treatment device for organic residues, as it appears it will be in the future, kinetic information must be developed to enable rational design of such a process.

Loehr (1974) noted that Monod (1950) applied Michaelis-Menton equations which explained enzymatic reactions to microbial growth systems. Monod (1950) assumed a relationship between a specific growth rate for pure cultures and a limiting substrate production.

The specific growth rate is defined as

$$\mu = \frac{\hat{\mu}\, S}{K_S + X} \qquad (2.8)$$

where μ = rate of growth (quantity of cells produced per unit time per quantity of existing cells) corresponding to a substrate concentration, S;

S = substrate concentrations, S (mass per volume), of limiting nutrient in the system;

$\hat{\mu}$ = maximum rate which prevails when S is large (quantity of cells produced per unit time per quantity of existing cells);

K_s = constant (mass per volume).

This relationship was derived empirically and has been found to fit a large number of experimental absorption, transport and enzymatic data related to the microbial metabolism of organic matter (Loehr, 1974).

Hadas and Kafkafi (1974) applied the Michaelis-Menton equation for the mineralization of ureaform. The Michaelis-Menton equation explained the enzyme-substrate interaction. The rate equation for ureaform was

$$\frac{-d(UF)}{dt} = \frac{k_1(E_1)o}{K_m}(UF) \qquad (2.9)$$

where (UF) = concentration of ureaform, ppm at time t;

$(E_1)o$ = concentration of enzyme E_1 (ppm);

K_m = Michaelis constant (ppm);

k_1 = rate constant of urea production ($days^{-1}$).

Nitrification rates may be taken as proportional to the growth of nitrifiers provided populations are small compared to the carrying capacity of the environment and provided that substrate concentrations are high enough to yield maximum specific growth rates (McLaren, 1971).

McLaren (1970) derived an equation to describe nitrification for urea and soil in terms of time and depth of a soil column. For NH_4 or NO_2 oxidation, the rate of change may be given as

$$\frac{-d(S)}{dt} = \frac{Adm}{dt} + \alpha m + \frac{k^{11}\beta m(S)}{km + (S)} \qquad (2.10)$$

where S = substrate concentration, (ppm);

m = microbial biomass, (g);

t = time (day);

km = saturation constant, (ppm N);

k^{11} = specific rate constant (day^{-1});

α = proportionality constant (N oxidized per unit weight per unit time, t for maintenance, ppm g^{-1} day^{-1});

A = proportionality constant (reciprocal of growth yield: equal to N oxidized per unit weight of biomass synthesized, ppm day^{-1});

β = proportionality constant (amount of enzyme per unit biomass involved in waste metabolism, ppm).

Stanford and Smith (1972) used the following first-order equation to evaluate N mineralization potential.

$$\log (N_o - N_t) = \log N_o - \frac{kt}{2.303} \qquad (2.11)$$

where N_o = N mineralization potential (ppm);

N_t = cumulative amount of N mineralized, ppm, during a specific period of incubation, t, (weeks);

k = a rate constant (weeks^{-1}).

This equation was used for long-term incubations (greater than 8 weeks). Stanford, Carter and Smith (1974) conducted short-term incubations (2 weeks) and concluded that estimates of N_o were similar to those derived after extensive periods of incubation.

Stanford et al (1975) studied the denitrification in soil and found the rate equation to be first-order:

$$(NO_3\text{-}N)_r = (NO_3\text{-}N)_i e^{-kt}$$

where

$(NO_3\text{-}N)_r$ = NO_3-N remaining at time t (percent);

$(NO_3\text{-}N)_i$ = NO_3-N at beginning of incubation (equal to 100 percent);

k = rate constant (hours^{-1});

t = time (hours).

In 1936, Millar et al (1936) used a second-order equation to describe CO_2 evolution:

$$y = Ft^m;$$

where y = amount of CO_2 produced in time t (mg CO_2);

t = time (days);

F = amount of CO_2 at the beginning of the experiment (mg CO_2);

m = measure of the retardation in the rate of CO_2 evolution during the phase of decrease, dimensionless.

The same second-order equation, used by Millar et al (1936), also fit CO_2 evolution data of Pal and Broadbent (1975). Pal and Broadbent added C-labelled rice straw to soil.

CHAPTER 3

METHODS AND PROCEDURES

3.1. Incubation Apparatus

Four incubation chambers - one chamber for the control and the remainder for manure-soil treatments - were used to perform two incubation experiments. The incubation experiments were conducted - one in July of 1975 and the other in June of 1976. The former will be referred to as Incubation I while the latter will be referred to as Incubation II.

One of the four apparata used for the incubation experiment is shown in Figure 3.1. A schematic of the apparatus can be found in Figure 3.2. A Parker Masterflex pump unit, model 7568, was used to move air through the system. Atmospheric air was forced by means of the Masterflex pump into a 500-ml Erlenmeyer flask containing 450 ml of 5N NaOH to absorb CO_2. The CO_2-free air was moved through a second 500-ml Erlenmeyer flask containing 450 ml of 36N H_2SO_4 to remove NH_3. A flask containing distilled water followed the NH_3 scrubbers to prevent any NH_3 or CO_2 evolved from the soil from diffusing back into the scrubbers. Also, the distilled water produced a high relative humidity in the incubation chamber which prevented moisture loss from the soil. After passing through the scrubbers, the air entered into a sealed incubation chamber. This air aerated the soil.

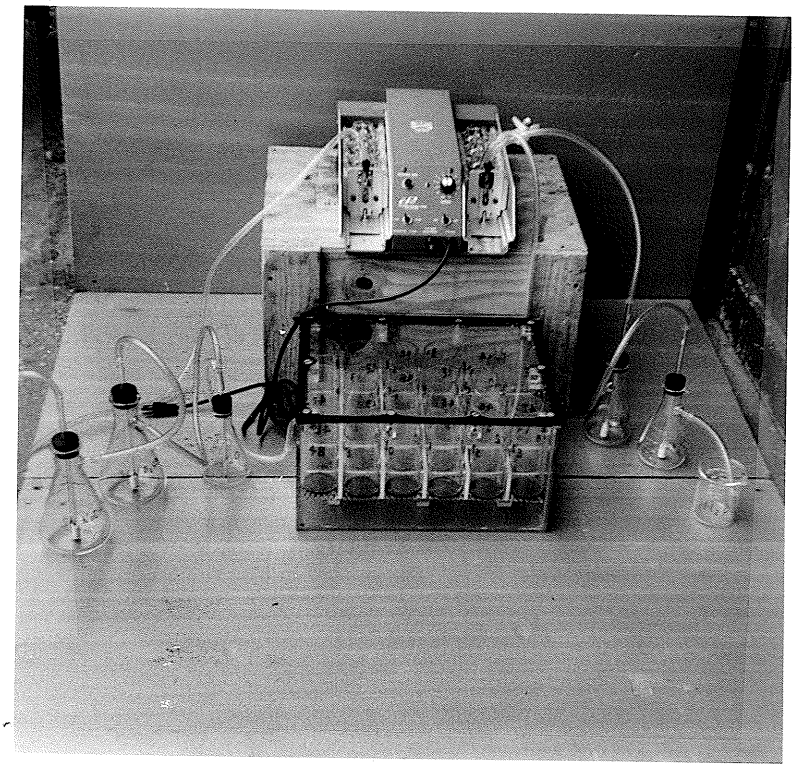

FIGURE 3.1. One of the Four Apparata used for the Incubation Experiment.

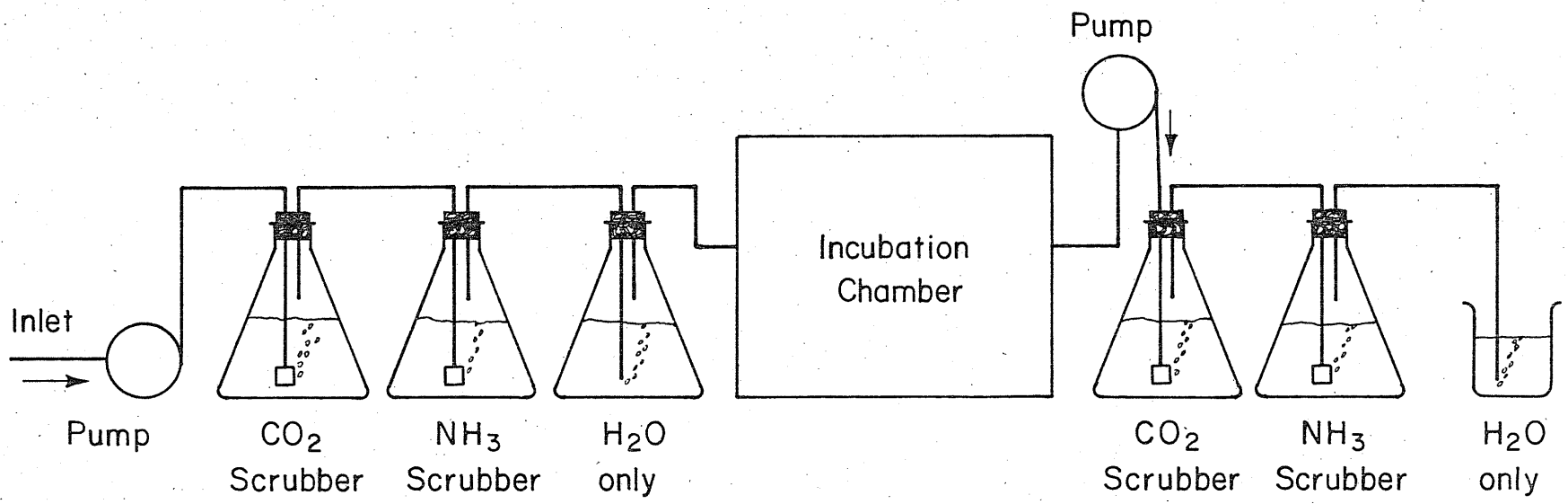

Fig. 3.2. Schematic of the Carbon Dioxide and Ammonia Scrubbing System.

The incubation chamber, constructed from 6-mm plexiglass, measured 20.3 by 30.5 by 38.1 cm and contained twenty-four plastic tubes used to hold the soil and soil-manure mixtures. Each tube had an inside diameter of 5.1 cm and a length of 10.2 cm. Fixed to each tube bottom was a number 40-mesh brass screen. Glass wool was placed on top of the screen to prevent fine soil particles from passing through the screen.

Air from the incubation chamber was forced through a second set of CO_2 and NH_3 scrubbers by a second Masterflex pump to remove CO_2 and NH_3 produced by the soil microorganisms. Next to the second set of scrubbers was a 300-ml beaker containing distilled water to prevent atmospheric NH_3 and CO_2 from diffusing back into the scrubbers.

Cylindrical fritted glass diffusers were used in the CO_2 and NH_3 scrubbers to increase the contact surface area of the air with the scrubbing solution. Preliminary testing showed that 98% of the CO_2 could be absorbed in the CO_2 scrubber following the incubation chamber while essentially complete CO_2 removal occurred in the CO_2 scrubber preceding the chamber. Since H_2SO_4 is used in the Kjeldahl analysis to absorb NH_3, this chemical was assumed to be an efficient scrubber of NH_3.

The two Masterflex pumps were run simultaneously in series each pumping 40 ml per minute of air (Appendix A). A manometer connected to the chamber showed that the pressure oscillated from 20.3 cm (8 in) of water to a vacuum of 20.3 cm (8 in) of water using the two-pump system. Such oscillations were considered small and were ignored.

3.2. Soil Description

A Scanterbury clay soil of the Red River Association was used in this experiment. The Red River Association is one of the major soils found in the Red River Valley[1]. Bergson (1975) noted that the Scanterbury soil is subject to waterlogging and has a slow permeability. Michalyna[2] stated that this clay is moderately drained with the surface 17.8 cm being a very dark grey clay. This soil is friable when moist and slightly hard when dry. Appendix B shows the analysis of a cultivated surface Scanterbury clay.

3.3. Soil Amendment

3.3.1. Incubation I

The soil was obtained from a ploughed fallow field at Glenlea Research Station, Glenlea, Manitoba in October, 1974. The soil was air-dried, and stored in flour sacks. Prior to Incubation I, the soil was ground to pass a 2-mm sieve. The excess ground soil was stored in a plastic container for use in Incubation II. Dairy manure was obtained from the Brockville Dairy, Winnipeg, Manitoba. The dairy manure samples, with the straw bedding, were gathered while the manure was being loaded onto a manure spreader to represent the actual mixture that a farmer would spread on a field. Since literature on manure-drying procedures for incubation studies is

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1 W. Michalyna, Department of Soil Science, University of Manitoba, personal communication in October, 1974.

2 W. Michalyna, Department of Soil Science, University of Manitoba, unpublished report of the Detailed Soil Survey of Glenlea Research Station, Glenlea, Manitoba.

lacking the composite initially was air-dried. However, this procedure proved too slow. Subsequently, the manure was oven-dried at 103°C for 24 hours to remove the moisture. After drying, the straw-manure mixture was ground with a "hand-operated" meat grinder. Visual inspection showed that the manure was finely ground, the straw was relatively long and slender with the larger particles being slightly more than 2 mm long.

Equivalent manure rates of 112, 224 and 561 kg N ha^{-1} (100, 200 and 500 lb N $acre^{-1}$, respectively) plus a control was chosen for the incubation trial. The manure loading rate was based on the N content of the dry dairy manure (dry basis) since dry manure was used in the incubation trial. For purposes of establishing loading rates the manure that could be ploughed under in a field was assumed to be mixed with the top 15.2 cm (6 in) of soil. The dry bulk density of a Scanterbury clay soil sample was measured to be 0.80 g cm^{-3}. The weight of a hectare of soil that is 15.2 cm (6 in) deep would be 1 222 300 kg (2,689,000 lb).

The total dry weight of soil plus manure or soil alone in each tube was 120 g. Knowing the manure loading rate, bulk density, total dry weight of soil or soil manure mixture in each tube and N content of the dry manure, the amount of dry manure required for each loading rate in Incubation I was determined (Table 3.1). The equations

developed to determine the dry manure loading rate are shown in Appendix C1. Appendix C2 shows an example of the method used to calculate the 561 kg N ha^{-1} loading rate for the dry manure.

Once the required amount of manure was added to the soil, the amount of water required to bring the soil-manure mixture and soil alone to field capacity (Israelsen and Hansen, 1962) was determined (Table 3.1). Field capacity was chosen as it was thought to represent the maximum amount of water available to the microorganisms (Stanford and Epstein, 1974).

After the field capacity was obtained, the next step was to estimate the strength of the NaOH and H_2SO_4 necessary to effectively scrub the CO_2 and NH_3 from the air leaving the incubation chamber. To achieve this, a "trial run" was conducted at 15°C with a dry manure loading rate of 561 kg N ha^{-1} since maximum CO_2 and NH_3 evolution was expected at this loading rate. The amount of CO_2 and NH_3 produced per day were measured using 250 ml of 2.5N NaOH and 250 ml of 1.0N H_2SO_4 respectively. From the absorption results, 250 ml of 1.0N NaOH and 250 ml of 0.5N H_2SO_4 appeared to be sufficient to scrub the CO_2 and NH_3, respectively.

An incubation temperature of 15°C (59°F) was used as it represented the average soil temperature at the 10-cm (4-in) soil depth for the months of May, June, July, and August at the Glenlea Research Station for the years 1970 to

Table 3.1. Sample Preparation for Incubation

Incubation I

Manure Loading Rate, kg N ha^{-1}	Soil Added g	Dry Manure Added, g [1]	Moisture Content (a) Field Capacity % weight
0	120.0	--	60
112	116.1	3.9	65
224	112.5	7.5	72
561	102.9	17.1	82

[1] Based on TKN value of 0.277% for the dry dairy manure, dry basis.

Incubation II

Manure Loading Rate, kg N ha^{-1}	Soil Added g	Wet Manure Added, g [2]	Dry Matter in Wet Manure, g [3]	Moisture Content (a) Field Capacity % weight
0	120.0	--	--	62.5
112	119.4	3.3	0.58	64.0
224	118.9	6.5	1.14	67.0
561	117.2	16.1	2.83	79.2

[2] Based on the TKN value of 0.335% for the wet dairy manure, wet basis.

[3] Moisture content of the wet dairy manure was 82.4%.

1974[1]. These four months were selected because the majority of crop growth occurs during this period. A walk-in environmental control chamber housed the experimental apparatus for Incubation I.

3.3.2. Incubation II

The soil amendment procedure of Incubation II was basically the same as Incubation I with some modifications. Dairy manure that was used for Incubation II was also obtained from Brockville dairy but the procedure for gathering and preparing the manure for incubation was quite different from Incubation I. Dairy manure "paddies" which contained no urine or straw were gathered in a five-gallon pail one week prior to starting the incubation trial. The manure was stored in a refrigerator at 2°C. No drying or grinding of the manure was performed.

The equivalent manure loading rates of Incubation I was also used for Incubation II but, in this incubation, the manure loading rate was based on the N content of wet dairy manure (wet basis) since wet manure was used in this incubation trial. Since wet manure was used, the moisture content had to be determined. Because the same soil was used for both incubations, the same bulk density was also used. The total dry weight of soil or soil plus manure in each tube was the same as Incubation I. For this incubation, the manure loading rate, bulk density, total dry weight of

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[1] Temperature soil data from Glenlea Research Station.

soil or soil manure mixtures, and N content as well as the moisture content of the wet manure had to be known in order to calculate the amount of wet manure required for each loading rate (Table 3.1). The equations developed to determine the wet manure loading rate are shown in Appendix C1. Appendix C3 shows an example of the method used to calculate the 561 kg N ha^{-1} loading rate for wet manure.

The moisture level chosen was field capacity as in Incubation I. The concentrations of the NaOH and H_2SO_4 scrubbers used in Incubation I were the same for Incubation II. However, on day 4 of Incubation II the NH_3 scrubbing solution was changed to 0.05 N H_2SO_4 from 0.5 N H_2SO_4 since a low level of NH_3 would be more measurable in a low concentration of H_2SO_4.

An incubation temperature of 15^{o}C was also used for Incubation II but a Fisher model 300 low-temperature incubator was utilized to maintain this temperature.

3.4. Analytical Procedures

3.4.1. Sampling

3.4.1.1. Incubation I

In Incubation I, three sample tubes were removed from each chamber on day 0, 1, 2, 4, 6, 8, 16, 32, and 64, respectively. The initial soil-manure mixtures or soil alone at day 0 did not have any water added. The removed sample tubes were weighed to check for excessive water loss.

The soil was then spread to form a thin soil layer and allowed to air dry. The dried samples were stored in small plastic bags before analysis.

3.4.1.2. Incubation II

In Incubation II, the sampling time was slightly modified from Incubation I such that three samples tubes were removed from each incubation chamber on day 0, 1, 2, 4, 8, 16, and 32, respectively.

3.4.2. Chemical

3.4.2.1. Scrubbing System

At the same time that the soil and soil-manure mixtures were sampled, the amounts of CO_2 and NH_3 collected in the second set of scrubbers, for both incubations, were measured. Fresh NaOH and H_2SO_4 replaced the spent solutions. Appendix D1 outlines the procedure required to calculate the amount of CO_2 and NH_3 absorbed in its respective scrubbing solution. The data obtained were to be used to plot CO_2-C and NH_3-N evolution curves. The percentage of the CO_2-C evolved as a direct result of the C added in the manure was calculated as shown in Appendix D2.

3.4.2.2. Total Kjeldahl Nitrogen

The dried and wet dairy manure samples were analyzed for total Kjeldahl nitrogen (TKN) according to the procedures stated in Standard Methods, Section 135. In Incubation I, all control samples and the soil-manure mixtures after incubation were analyzed for TKN with no provision to include NO_2-N and NO_3-N by the Kjeldahl-Gunning method (Jackson, 1958). In Incubation II, the TKN of the control and the soil-manure mixtures for day 0 were analyzed by the Kjeldahl-Gunning method. Appendix D3 shows the analysis procedures for the Kjeldahl-Gunning method.

3.4.2.3. Extractable Ammonium, NH_4^+

Ammonium nitrogen was determined on all control samples and soil-manure mixtures using the procedure of Bremner (1965). Appendix D4 outlines the procedure.

3.4.2.4. Extractable Nitrite (NO_2) and Nitrate (NO_3)

Extractable NO_2-N and NO_3-N was determined on all control and soil-manure mixtures at the Manitoba Provincial Soils Testing Laboratory using a Technicon Auto Analyzer. Appendix D5 shows the preparatory steps required before using the auto analyzer.

3.4.2.5. Organic Carbon

The organic carbon of the soil, manure, and soil-manure mixtures was measured at the Manitoba Provincial Soils Testing Laboratory using a modified Walkey-Black method similar to the Walkey-Black method outlined by Allison (1965). Appendix D6 shows the preparatory steps required before titrating the solution.

3.4.2.6. pH Measurement

For Incubation I, the pH of the soil and soil-manure mixtures was analyzed on samples obtained for day 0, 8, 32, and 64, respectively, using the procedure (1:1 soil to water ratio) of Jackson (1958). Incubation II utilized the same analytical procedure as Incubation I but analyzed the soil and soil-manure mixtures for samples obtained on day 0, 8, 16, and 32, respectively. Appendix D7 outlines the analytical procedure.

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Kinetics

Hedlin and Cho (1974) stated that the addition of manure to farmland is important as a means of maintaining soil fertility. They also noted that the maximum acceptable quantity is not known at present. Furthermore, they say that we need to study methods of increasing fertilizer efficiency, the fate of nutrients in soil, their chemistry and transport, and recycling of plant nutrients within a soil profile. Buchanan (1974) expressed a need for research on manure utilization such as application rate and groundwater contamination.

Statements such as the above have led to research projects such as this one to investigate more fully the area of animal manure management. This project was established to determine the decomposition rate of manure in soil by monitoring C and N transformations. Stanford, et al (1973) pointed out that, while N mineralization has long been recognized, the quantitative relationships have not been elucidated. Hadas and Kafkafi (1974) stated, as noted in the literature review, that the use of kinetic equations can describe the process of mineralization.

To obtain the kinetic equations, "smooth" C and N mineralization curves are required to produce kinetic coefficients. Therefore, a favourable environment to promote mineralization must prevail, otherwise, a kinetic approach is futile. Millar, et al (1936) brought out the fact that the reaction of the soil, the amount of moisture, the temperature, the aeration and kind of species of microorganisms are all important factors in the rate of decomposition of any organic matter in soil. If kinetic equations cannot be applied to a set of data, the experiment is not necessarily a failure but may simply be reflecting the interactions in soil that probably occur in the field under similar environmental conditions.

Few researchers, if any, have attempted to obtain kinetic information using manure in soil. In fact, only in recent years has there been any great interest in using the kinetic approach to any medium. For example, the kinetics of biological growth in sewage treatment plants are only now becoming understood.

In this experiment, the carbon mineralization study was successful and reliable kinetic coefficients were obtained. However, the nitrogen mineralization study by itself did not produce useful kinetic data. A great deal was learned, however, and the remainder of this chapter is devoted to a discussion of the carbon and nitrogen mineralization studies.

4.2. Moisture Loss during Incubation

Table 4.1 shows the moisture loss from the soil in the sample tubes for Incubation I and II after incubation times of 64 and 32 days, respectively. The control in both incubations yielded the highest moisture loss, however, the loss was less than 5 g of water (8.0 percent, Table 4.1). Such low losses were not considered large enough to warrant water addition.

4.3. Soil pH

The pH of the soil and soil-manure mixtures of Incubations I and II can be found in Table 4.2. The dried manure in Incubation I had a higher pH (8.6) than the wet manure in Incubation II (pH 7.9). Furthermore, the pH of the control in Incubation I at day 0 was 0.5 units higher than in Incubation II. Both soils were gathered at the same time but the soil in Incubation II was stored 1-yr longer prior to use. Bremner (1965) noted that many workers have found that mineralizable N values increase with time during storage of air-dried samples. Storage probably decreased the pH as the length of storage increased.

In both incubations, the addition of manure to the soil increased the pH as the N loading rate increased. As time progressed during incubation, the pH of the soil-manure mixtures rose similiar to the phenomena noted in section 2.5.1.

Table 4.1. Moisture Loss during Incubation.

Manure Loading Rate, kg N ha^{-1}	Moisture loss, %	
	Incubation I (after 64 days)	Incubation II (after 32 days)
0	8.0	7.3
112	1.6	1.3
224	1.2	4.4
561	<1.0	4.7

Table 4.2. pH of the Soil or Soil-Manure Mixtures during Incubation.

Incubation I

Manure Loading Rate, kg N ha^{-1}	Time, Days			
	0	8	32	64
	pH			
0	7.3	7.2	7.2	7.3
112	7.2	7.5	7.7	7.5
224	7.4	7.8	8.1	7.7
561	7.7	8.1	8.3	8.0
Manure, dry	8.6			

Incubation II

Manure Loading Rate, kg N ha^{-1}	Time, days			
	0	8	16	32
	pH			
0	6.8	6.9	7.1	7.2
112	6.7	7.1	7.2	7.3
224	6.8	7.1	7.3	7.4
561	7.1	7.1	7.5	7.5
Manure, wet	7.9			

Floate and Torrance (1970), Olsen, et al (1970) and Finstein (1972) also noted a similar pH rise when faecal material was added to soil. Incubation I, however, showed a decrease in pH at day 64 for all soil amended with manure. Buckman and Brady (1969) noted that, as time progresses, the CO_2 produced by microbial activity in the soil combines with water to form carbonic acid (a weak acid) and lowers the pH. The pH decrease could also be due to the acidifying process of nitrification (Olsen et al, 1970). The pH of the control in Incubation I was fairly constant ranging from 7.2 to 7.3 whereas the pH of the control in Incubation II rose from a pH of 6.8 to a pH of 7.2.

4.4. Carbon Mineralization

4.4.1. C:N Ratio

Measured and calculated C:N ratios of the soil, soil-manure mixture and manure can be found in Table 4.3. The procedure employed to obtain the calculated TKN, organic C and C:N ratio can be found in Appendix C.4.

In Incubation I the measured C:N ratio increased as the loading rate increased because of the high C:N ratio of the dry dairy manure. The calculated C:N ratio had the same increasing trend but had higher values. Differences in the C:N ratios occurred because of differences in TKN's.

The calculated TKN values in Incubation I were considerably lower than the measured TKN values. Since both the control and dry dairy manure had a TKN of 0.28 percent,

Table 4.3 C:N Ratios

Incubation I

Manure Loading Rate, kg N ha^{-1}	TKN		Organic C		C:N Ratio	
	Measured[1]	Calculated[2]	Measured[1]	Calculated[2]	Measured	Calculated
	%		%			
0	0.28		3.93		14.0:1	
112	0.32	0.28	4.91	4.93	15.3:1	17.6:1
224	0.36	0.28	5.90	5.85	16.4:1	20.9:1
561	0.49	0.28	8.58	8.31	17.5:1	29.7:1
dairy manure	0.28 d.b.		34.70 d.b.		124:1	

[1] Based on dry weight of soil, soil-manure mixture or manure.

[2] Calculated from measured values of control and dairy manure based on the amount of soil and manure added for each loading rate.

Incubation II

Manure Loading Rate, kg N ha^{-1}	TKN		Organic C		C:N Ratio	
	Measured[1]	Calculated[2]	Measured[1]	Calculated[2]	Measured	Calculated
	%		%			
0	0.26		3.69		14.2:1	
112	0.29	0.264	3.76	3.83	13.0:1	14.5
224	0.29	.272	3.79	3.97	13.1:1	14.6
561	0.35	.296	3.85	4.39	12.5:1	14.8
dairy manure[3]	0.34 w.b.		5.85 w.b.		17.2:1[5]	
dairy manure[4]	1.90 d.b.		33.23 d.b.		17.5:1	

[1] Based on dry weight of soil or soil manure mixtures.

[2] Calculated from measured values of control and dry dairy manure based on the amount of dry soil and dry weight of manure added for each loading rate.

[3] Calculations based on wet weight of manure.

[4] Calculations based on dry weight of manure assuming no loss of N or C.

[5] Lower C:N ratio than dry manure due to round off.

no matter how much manure was added the TKN should not have increased to the levels indicated by the measured values. Difficulty in obtaining a consistent end-point during titration (manual) could have caused high TKN values for the measured results (Table 4.3). Alternatively, the procedure to obtain the TKN of the manure (Standard Methods) was different than the procedure for the TKN of the soil (Kjeldahl-Gunning), therefore differences in measured TKN's between the two procedures may have occurred.

The calculated values for organic C as compared to the measured values of organic C were quite similar for Incubation I (Table 4.3). The 0.58 factor used to determine organic C for the soil and manure may be different for this particular soil or manure but was the best available estimate. A different factor would cause organic C to change and in turn, the C:N ratio to change.

The measured C:N ratio differed considerably from the calculated C:N ratio due mainly to differences in the TKN values. However, both C:N ratios (measured and calculated) did have the same increasing trend as loading rates increased (Table 4.3).

The calculated TKN in Incubation II also varied from the measured but the differences were not as great as in Incubation I. The analytical procedure for determining the TKN of the soil (Kjeldahl-Gunning) of Incubation II used an automatic rather than manual titration to maintain a more constant end-point.

The calculated values for organic C of Incubation II did not vary more than 0.5 percent from the measured results (Table 4.3). Again, the 0.58 factor was used to determine the organic C as it was the best available estimate as discussed previously.

In Incubation II, the calculated C:N ratio had a slight increasing trend whereas the measured C:N ratio decreased. A slight increase in C:N ratio would have been expected since the C:N ratio of the manure was 17.5:1 which was greater than the soil alone (14.2:1). Once again, the TKN procedures probably did not measure the same amount of TKN due to variation in technique, error in analysis or both.

When comparing Incubation I to Incubation II, the controls had approximately the same C:N ratio as expected since both were from the same soil with no manure added. Also, the organic C content of the manure-amended soil in Incubation II was lower than in Incubation I due to the fact that less dry manure (Table 3.1) was added in Incubation II than in Incubation I which in turn, resulted in less C being added to the soil.

The C:N ratio of fresh, wet dairy manure (17.5:1) was considerably higher than the dried dairy manure containing straw bedding (124:1). A combination of effects probably yielded the high C:N ratio (124:1) of the dry manure in Incubation I. First, drying the manure in preparation for incubation probably caused NH_3 volatilization while the

organic C was retained. Second, the manure was mixed with straw bedding which contained a considerable amount of C. However, since faeces is partially digested plant material, the straw that was used as bedding probably did not change the C content of the manure significantly. Third, because a strawy material would be low in N, mixing it with manure would have resulted in even a lower N content for the manure-straw mixture than would occur with air-dried, fresh manure. The first and third reasons probably contributed most to the fact that the N content of the dry manure was 0.28 per cent (d.b.).

The low C:N ratio (17.5:1) in the wet manure occurred because no drying procedure was utilized in which N could be removed. Furthermore, the wet manure did not contain bedding. The C content of the wet manure (33.2 per cent, d.b.), however, did not vary too much from the strawy dried manure of Incubation I (34.7 per cent, d.b.). These C contents were slightly less than the value (37.7 per cent, organic C in cow faeces) reported by Loehr (1974) and slightly higher than the value (32 per cent organic C in beef faeces) obtained by Mathers and Stewart (1970).

4.4.2. Carbon Evolution

The cumulative CO_2-C curves (Figure 4.1 and 4.2) from the manure-amended soil for Incubations I and II illustrate increased CO_2-C production relative to the control due to

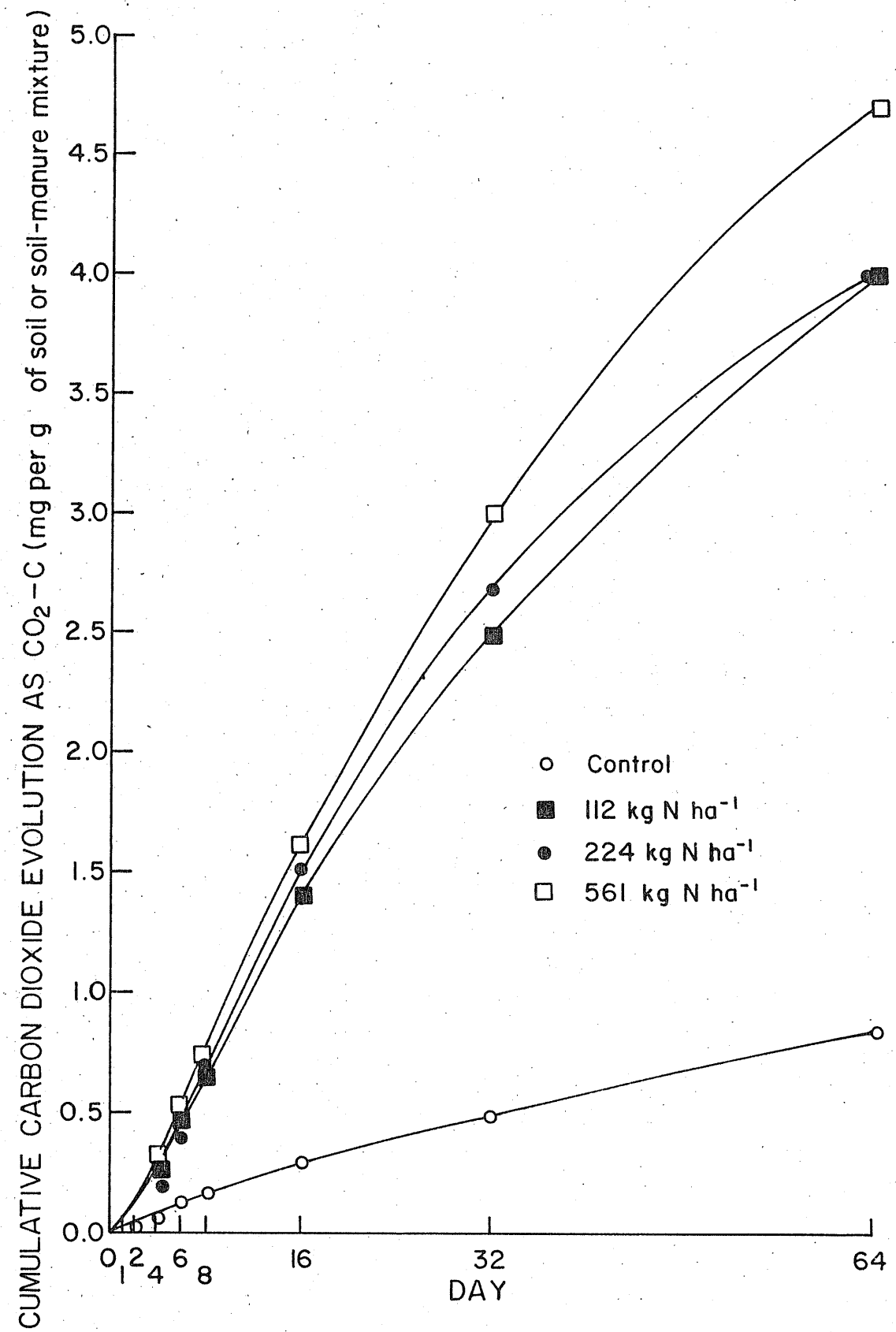

Fig. 4.1. Cumulative Carbon Dioxide Evolved in Incubation I

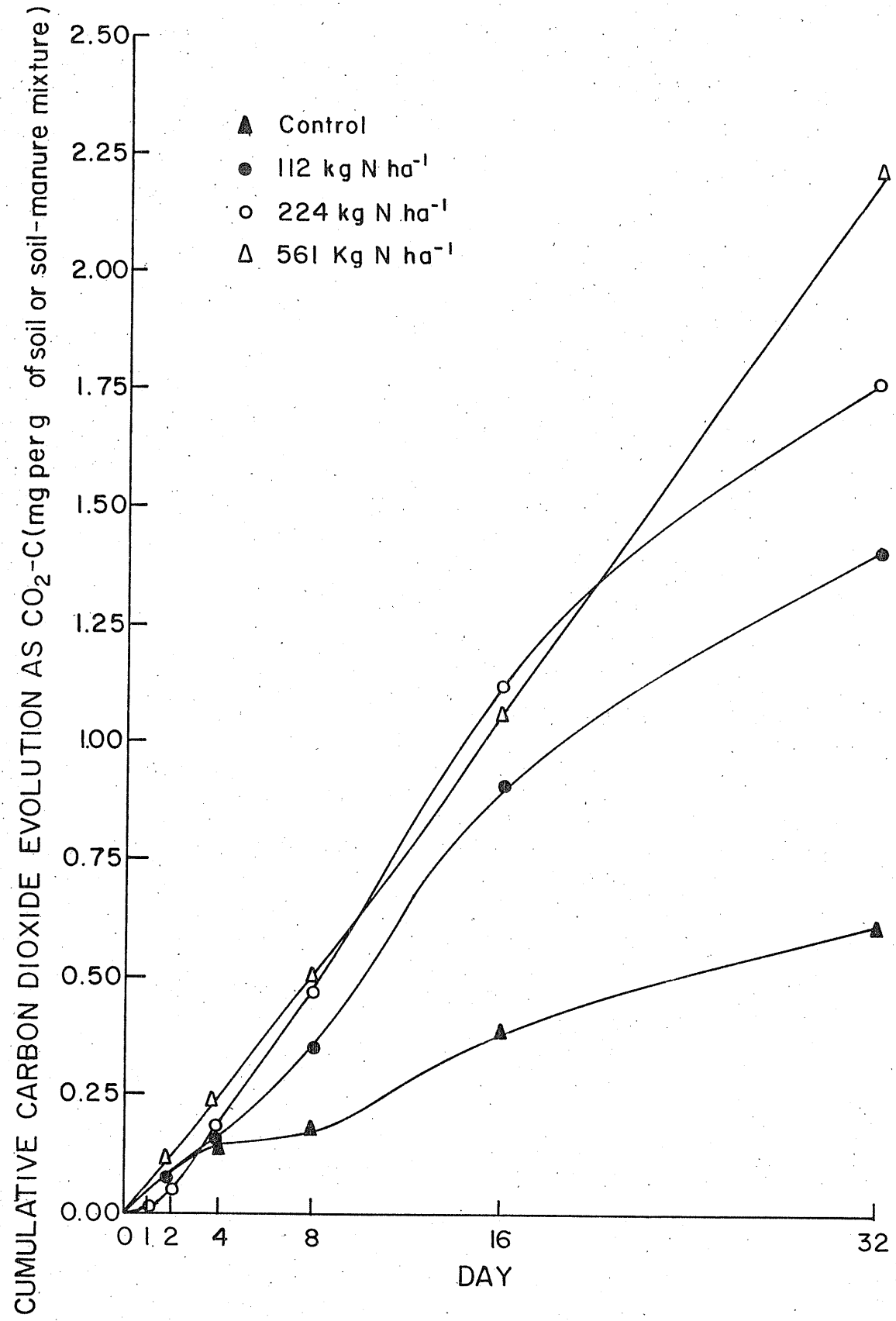

Fig. 4.2. Cumulative Carbon Dioxide Evolved in Incubation II

addition of dairy manure. The manure added to the soil increased microbial activity resulting in more CO_2-C being evolved than from the control. By the end of Incubation I, a slight increase in CO_2-C production for the 561 kg N ha^{-1} loading rate had occurred relative to the 112 and 224 kg N ha^{-1} loading rates. At day 16 in Incubation I, the cumulative CO_2-C for the various manure loading rates did not vary much (1.41 to 1.63 mg CO_2-C per g of soil-manure mixture). Similarly, in Incubation II, after 16 days of incubation, the cumulative CO_2-C for the soil-manure mixtures were much the same (0.90 to 1.06 mg CO_2-C per g of soil-manure mixture). Comparing the controls, Incubation II produced slightly more CO_2-C than Incubation I after 32 days of incubation (Figure 4.1 and 4.2). The difference, however, in CO_2-C production was small. Similar CO_2-C production rates for the control in both experiments were expected since the soils used were the same and no manure had been added.

After 32 days of incubation, the 112, 224 and 561 kg N ha^{-1} loading rates of Incubation I yielded considerably higher cumulative CO_2-C evolutions than the respective loading rates in Incubation II. Although Incubation I evolved more cumulative CO_2-C than Incubation II, the latter evolved more C when expressed as a percentage of the manure C evolved from the soil relative to the original manure C added (Figure 4.3). As discussed previously, the N content of the dry manure was low whereas the wet dairy manure had a high N content when expressed on a equivalent basis (Table 3.1.). Therefore, to achieve

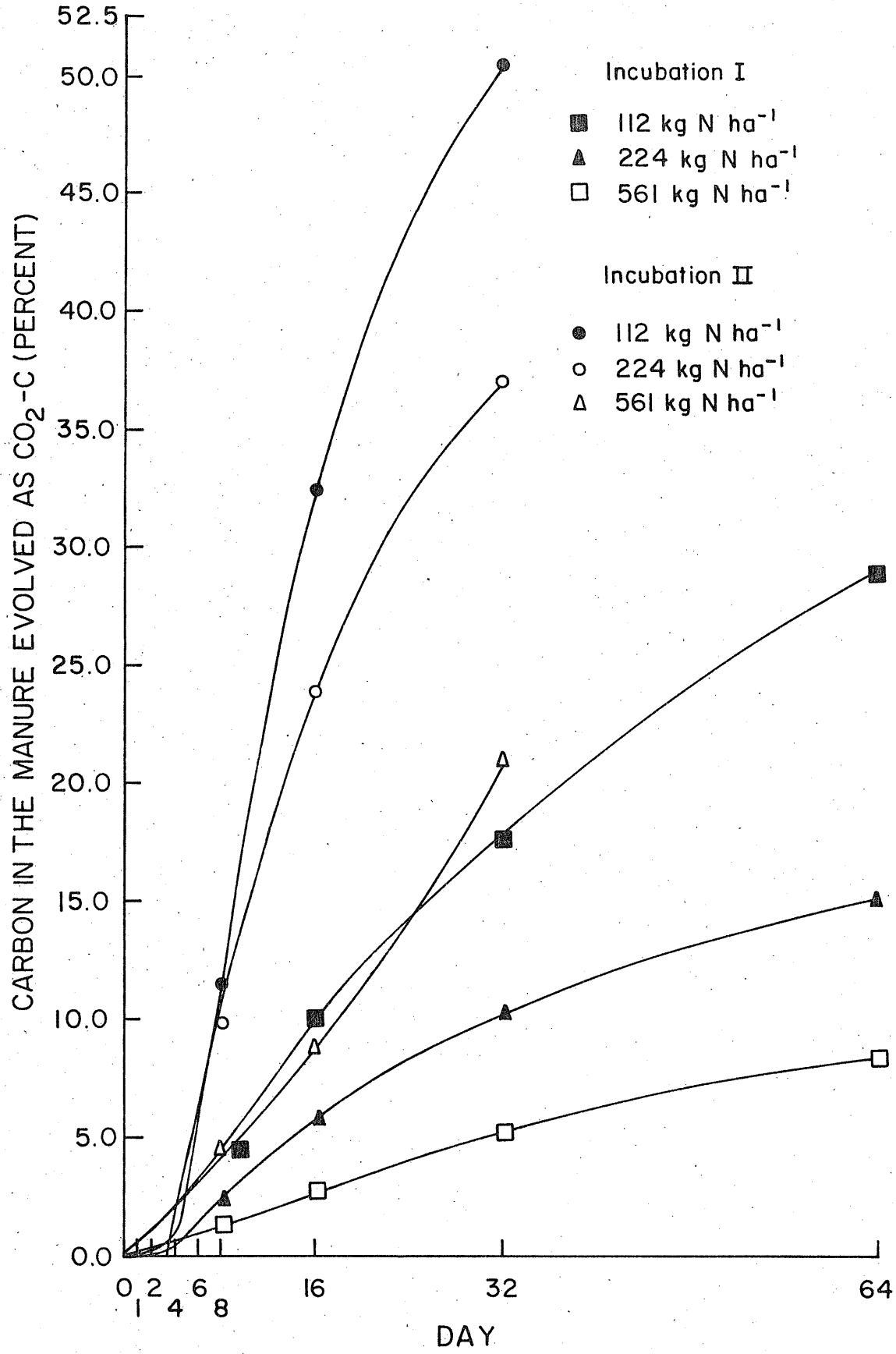

Fig. 4.3. Percent of Carbon in the Manure that is Evolved as CO_2-C

a desired N loading rate, more dry manure was required in Incubation I than in Incubation II. Consequently, more C was added in Incubation I. This indicates that wet dairy manure (containing a large quantity of N and no bedding material, i.e. a low C:N ratio) added to clay soil would release, in a shorter period of time, a greater percentage of the original manure C added due to the greater microbial activity in comparison to dry manure added to a similar soil.

The lowest manure loading rate (112 kg N ha^{-1}) for both incubations yielded the highest percent of C evolved for each respective incubation run. This result was contradictory to what Mathers and Stewart (1970) obtained. They stated that 49, 45, 45, 45 and 57 percent of the C added in the beef manure was evolved from the 1, 2.5, 5, 10 and 20 per cent (w.b.) manure treatments, respectively, after 90 days of incubation at a temperature of 27°C. They used a 10 g sample which probably permitted better aeration and a higher concentration of microorganisms to break down the organic matter.

Figures 4.4 and 4.5 were derived from Figure 4.3. These figures show plots of manure loading rate versus the percent of the original manure C remaining in the manure at various times. From these figures an estimate of percent C remaining in the manure (depending on whether dry or wet manure is chosen) for any loading rate can be obtained. For instance, a loading of 300 kg N ha^{-1} at day 32 would yield 92 percent and 68 percent C remaining in the manure for the dry and wet dairy manure, respectively.

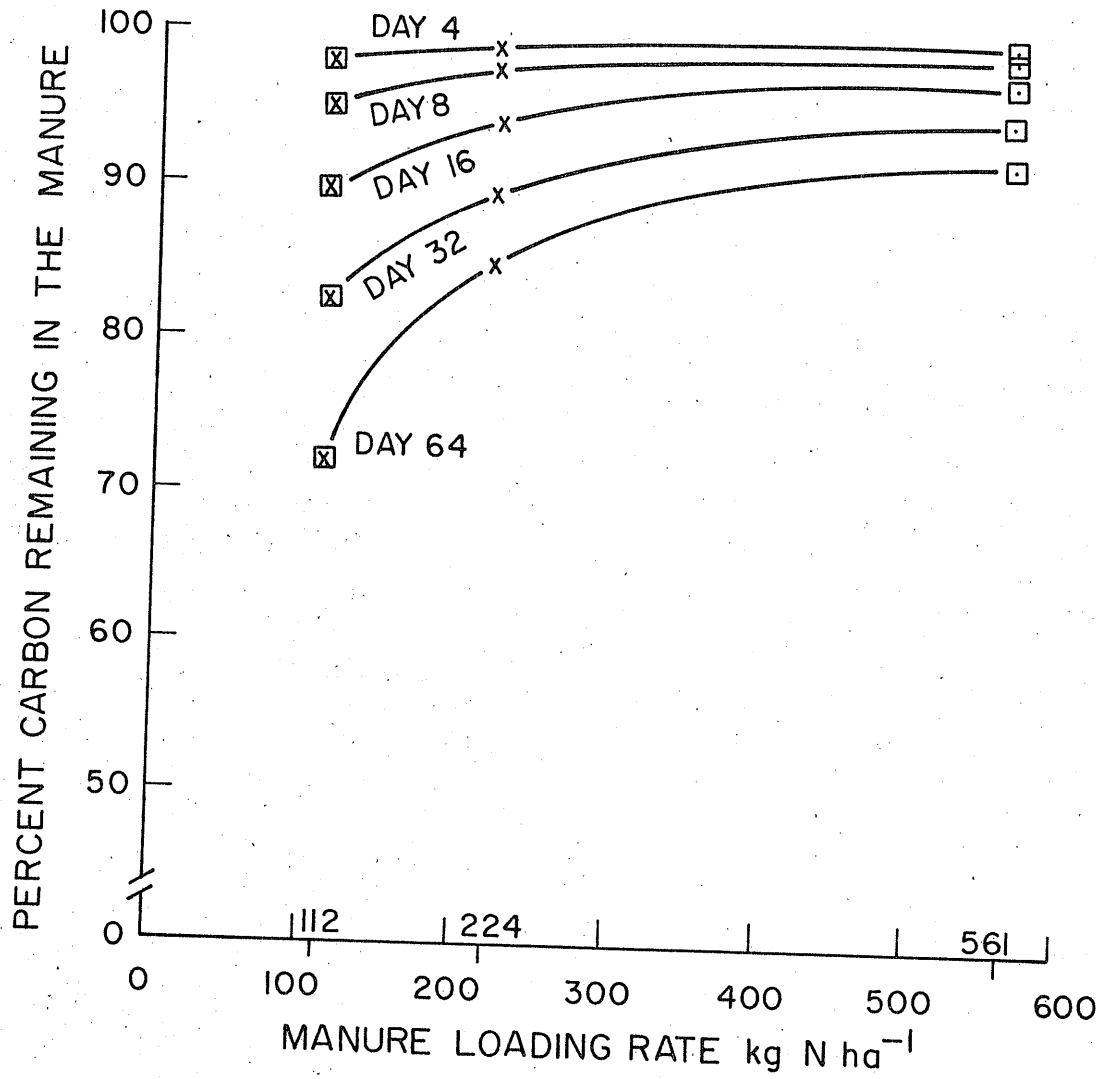

Fig. 4.4. Manure Loading Rate and Percent Carbon Remaining in the Manure at Various Time Intervals for Incubation I

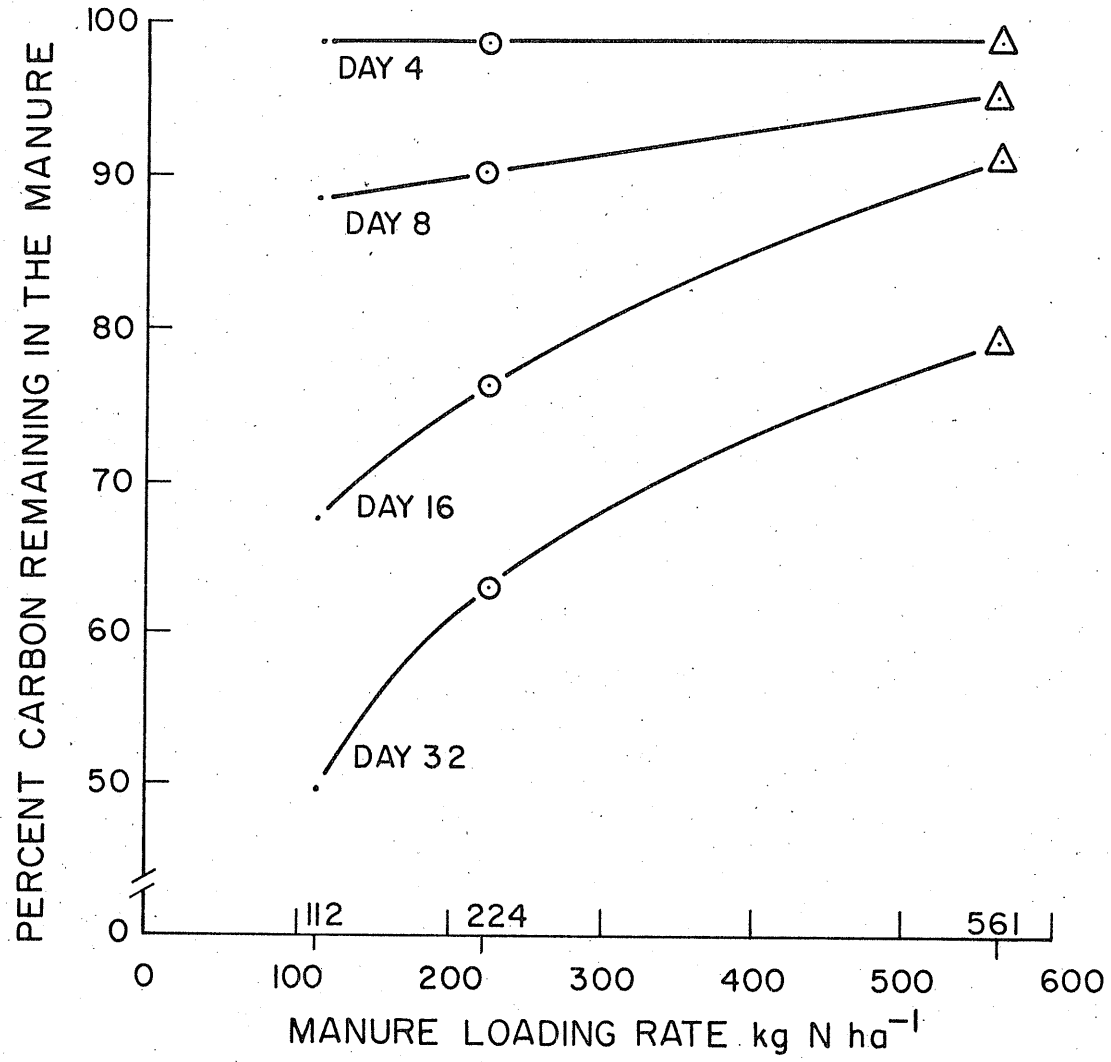

Fig. 4.5. Manure Loading Rate and Percent Carbon Remaining in the Manure at Various Time Intervals for Incubation II

4.4.3. Kinetics of C Loss

Sawyer and McCarty (1967) noted that the kinetics of biochemical oxygen demand reactions for most practical purposes is "first-order" in character. That is, the rate of the reaction is proportional to the amount of oxidizable organic matter remaining at any time, as modified by the population of active microorganisms. In this experiment, the population of active microorganisms would also have been modified by the remaining organic C. To obtain the necessary data, the percent CO_2-C evolved (Figure 4.3) was subtracted from 100 percent to yield percent C remaining in the manure (Figures 4.4 and 4.5). By making a semi-log plot of the data (first-order plot), it was possible to fit a straight line through the data points (Figure 4.6). The equations for the lines can be found in Table 4.4 with corresponding "r" values. The high "r" values indicate a strong relationship between the straight line and data points. Due to the sudden change in CO_2-C evolution at day 4 of Incubation II for the 112 and 224 kg N ha^{-1} loading rates as shown in Figure 4.3, the first terms (term A) of the respective equations in Table 4.4 were slightly higher than expected (100 should have been the value).

Table 4.4 also shows that the C turnover period for the wet dairy manure was considerably lower than the dry manure. Assuming that a constant soil temperature and field capacity could be maintained, it would take 0.77 and $^{3.44}$

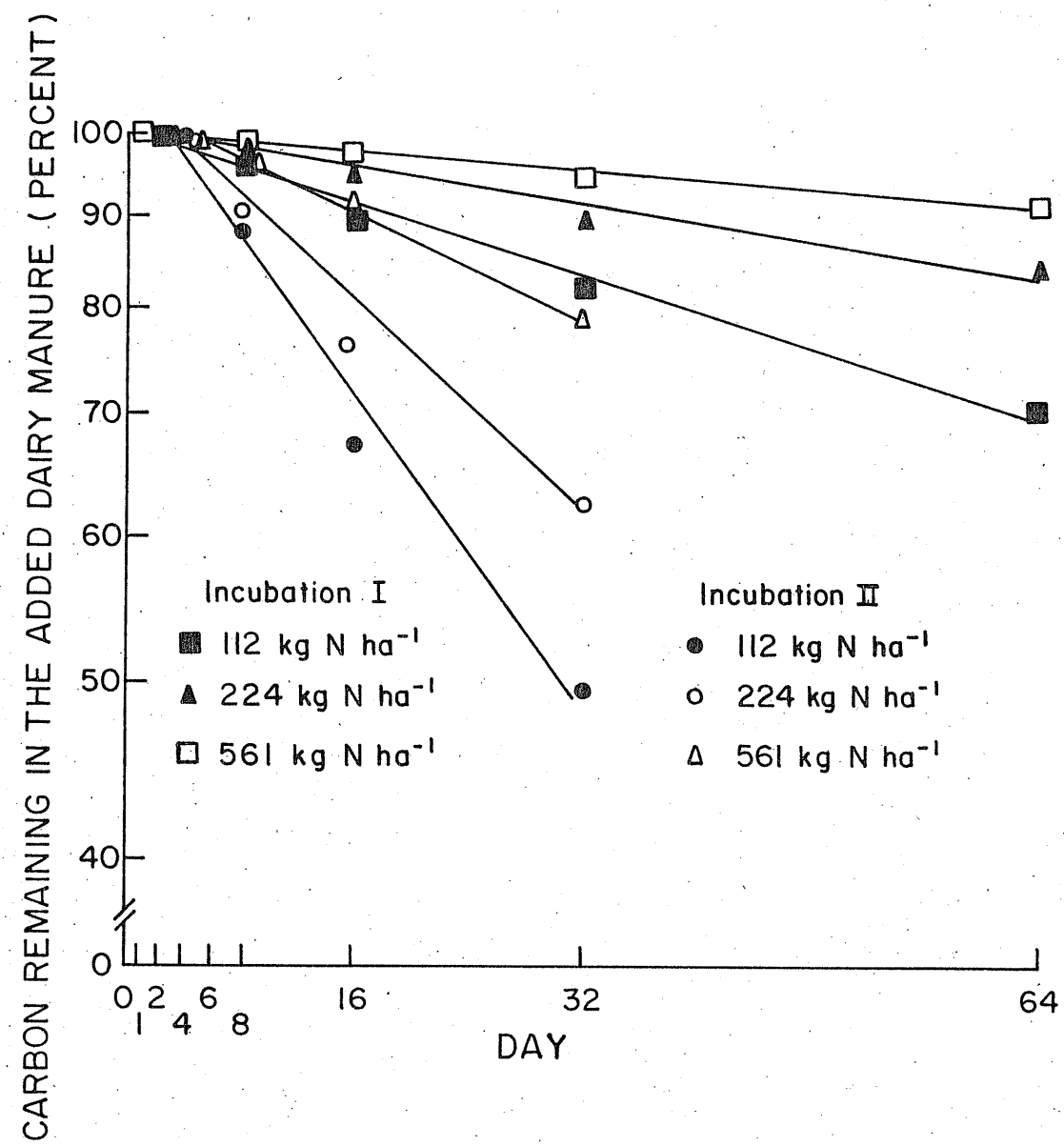

Fig. 4.6. Carbon Remaining in the Dairy Manure versus Time

TABLE 4.4 Equations Describing Carbon Remaining in the Added Dairy Manure Versus Incubation Time.

Incubation I

Manure Loading Rate, kg N ha^{-1}	Equation Form: $C=Ae^{-kt}$*	Interval, day	r	T**
112	$C=99.8e^{-0.0055t}$	1<t<64	-0.996	3.44
224	$C=99.6e^{-0.0027t}$	2<t<64	-0.983	7.00
561	$C=99.8e^{-0.0014t}$	2<t<64	-0.987	13.50

Incubation II

Manure Loading Rate, kg N ha^{-1}	Equation Form: $C=Ae^{-kt}$*	Interval, day	r	T**
112	$C=105.9e^{-0.0245t}$	2<t<32	-0.992	0.77
224	$C=103.6e^{-0.0159t}$	4<t<32	-0.987	1.12
561	$C=101.6e^{-0.0079t}$	1<t<32	-0.992	2.40

* where C = carbon remaining at time t, percent

A = initial carbon available, percent

k = rate constant, day^{-1}

t = time, day

** where T = turn over period to remove 99.9% of the added manure carbon, calendar years

calendar years to remove 99.9 percent of the added C in Incubation II and I respectively from manure applied at a rate equivalent to 112 kg N ha^{-1}. The higher manure loading rates increased the turnover period to 2.4 calendar years for the 561 kg N ha^{-1} loading rate in Incubation II and 13.5 calendar years for the same loading rate in Incubation I. The low "k" values in Incubation I relative to Incubation II in Table 4.4 imply long turnover periods.

Table 4.5 shows the rate of C removal at various incubation times. The higher the rate constant (k) shown in Table 4.5 the higher the rate of CO_2-C evolution and the higher the rate of removing C from the manure. As time progressed in the incubation, less CO_2-C was evolved (Figure 4.3) from the manure, and so the rate of C removal ($\frac{dC}{dt}$) also decreased (Table 4.5). The highest rate of C removal was in the 112 kg N ha^{-1} loading of Incubation II (Table 4.5) which was the loading rate with the shortest turnover period (Table 4.4).

4.5. Nitrogen Mineralization

4.5.1. Ammonia Evolution

Ammonia evolution from the controls as well as soil-manure mixtures of both incubations was measurable in either trace amounts or not at all. At these low concentrations (less than 0.4 µg g^{-1} of soil), it was difficult to determine whether there actually was NH_3 pre-

TABLE 4.5 Rate of Decrease of Carbon Remaining in the Manure

Manure Loading Rate, kg N ha^{-1}	Rate Equation Form: $\frac{dC}{dt} = -Se^{-kt}$*	Rate of C removal at incubation times of Day				
		4	8	16	32	64
		% Carbon per day				
Incubation I						
112	$\frac{dC}{dt} = -0.549e^{-0.0055t}$	-0.537	-0.525	-0.503	-0.460	-0.386
224	$\frac{dC}{dt} = -0.269e^{-0.0027t}$	-0.266	-0.263	-0.258	-0.247	-0.226
561	$\frac{dC}{dt} = -0.140e^{-0.0014t}$	-0.139	-0.138	-0.137	-0.134	-0.128
Incubation II						
112	$\frac{dC}{dt} = -2.59e^{-0.0245t}$	-2.35	-2.13	-1.75	-1.18	
224	$\frac{dC}{dt} = -1.65e^{-0.0159t}$	-1.55	-1.45	-1.28	-0.992	
561	$\frac{dC}{dt} = -0.803e^{-0.0079t}$	-0.778	-0.754	-0.708	-0.624	

*where C = carbon remaining at time t, percent

k = rate constant, day^{-1}

t = time, day

S = product of A times k from Table 4.4

sent or an error in titration had occurred. On day 8 of Incubation II the NH_3 scrubbing solution was changed from 0.5 N H_2SO_4 to 0.05 N H_2SO_4 because a low concentration of NH_3 would be more readily noticeable in a lower H_2SO_4 concentration. This attempt failed to produce better results. The NH_3 evolution results obtained appeared contradictory to those of Floate and Torrance (1970) who stated that if the pH of the decomposing substrate rose significantly above 7.0, NH_4-N would be volatilized as NH_3. They also referenced Doak (1952) as demonstrating the above occurrence when urea or urine was added to soil resulting in a rapid rise in pH from 6.0 to more than 8.0. In this experiment, the pH did reach 7.0 and, in some cases, the pH reached 8.3 (Table 4.2).

Lack of NH_3 evolution was probably due to the high cation exchange capacity (CEC) of the soil. In Appendix B, the analysis of cultivated Scanterbury clay shows the CEC for NH_4 to be 52.75 millequivalents (Meq) per 100 g of dry soil at a pH of 6.54. Cation exchange is the exchange of one cation for another at the exchange sites in the soil. Brady (1975) defines an equivalent as 1 gram atomic weight of hydrogen or the amount of any other ion that will combine with or displace this amount of hydrogen. For monovalent ions such as NH_4^+, the equivalent weight and atomic weight are the same since they can replace or react with one H^+ ion. A milliequivalent weight of a substance is one thousandth of the atomic weight and since the equivalent

weight of hydrogen is about 1 g, the term milliequivalent may be defined as 1 milligram of hydrogen or the amount of any other ion that will combine with or displace it (Brady, 1975). Thus, the Scanterbury clay soil has a CEC of 52.75 mg per 100 g of soil or 527.5 ppm. Brady (1975) also noted that as pH increases the cation exchange capacity of most soils increase. Therefore, the CEC of the Scanterbury clay used in this experiment could have increased during the experiment as the pH increased. Grinding the soil probably also increased CEC by increasing the number of exchange sites. Due to the high CEC of the Scanterbury clay, the NH_4-N produced was probably held by the soil itself.

4.5.2. Total Kjeldahl Nitrogen (TKN)

The TKN curves (Figures 4.7, 4.8, 4.9 and 4.10) of Incubation I fluctuated considerably and were difficult to interpret. The data did not produce the "smooth curves" preferred for determining kinetic information as discussed earlier. There was variation in TKN as much as 1320 ppm from one date to the next. At some point in time for each loading rate, the TKN was higher than that originally present at day 0. The above results should not have occurred since no organic or ammonium N was added after the onset of the incubation. There were also considerable differences in the TKN among the triplicate samples taken for each analysis. Variations were greater than 20 percent in some cases (Appendix E).

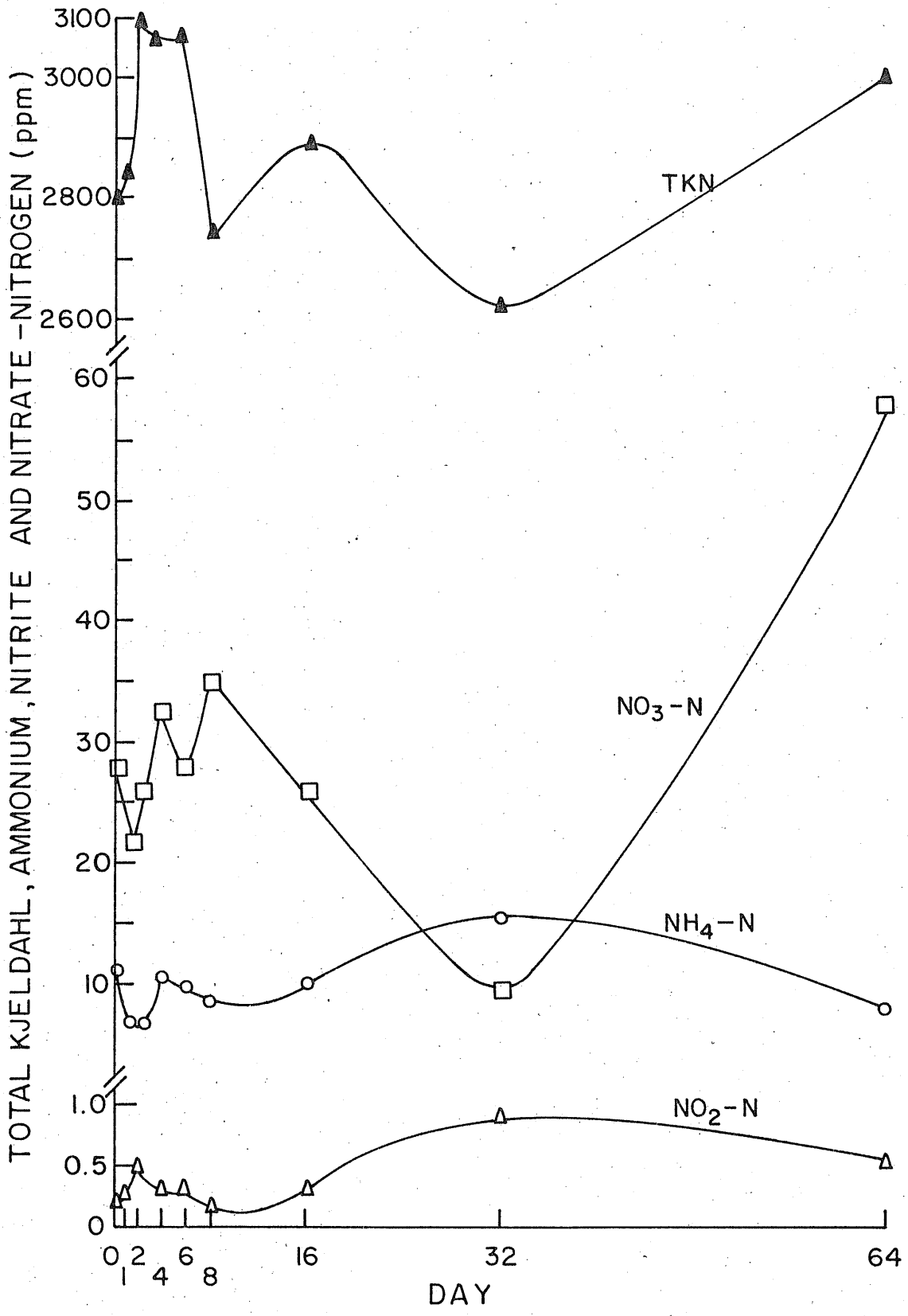

Fig. 4.7. Soil Nitrogen Curves for the Control in Incubation I.

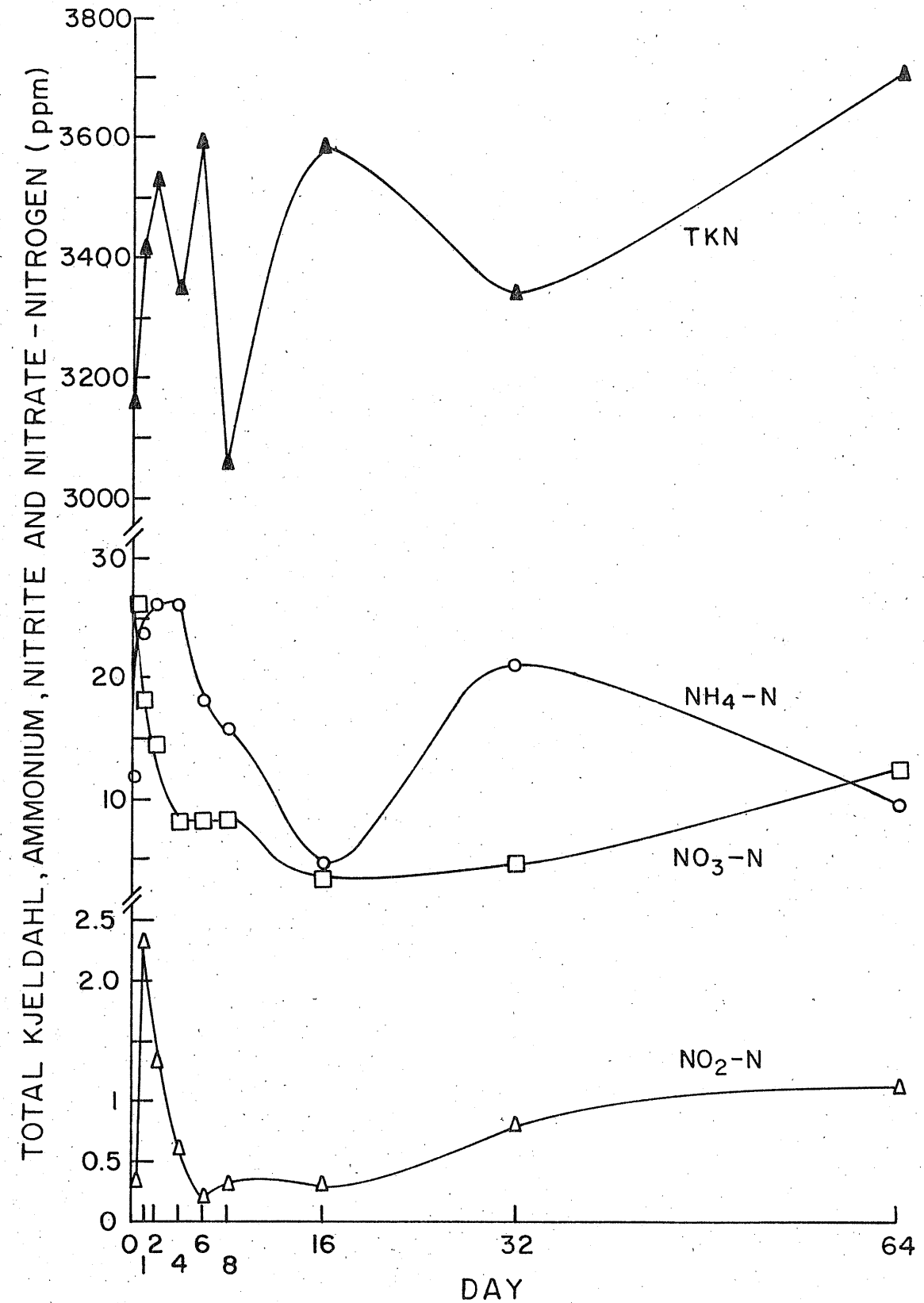

Fig. 4.8. Soil Nitrogen Curves for the 112 kg N ha^{-1} Loading Rate in Incubation I

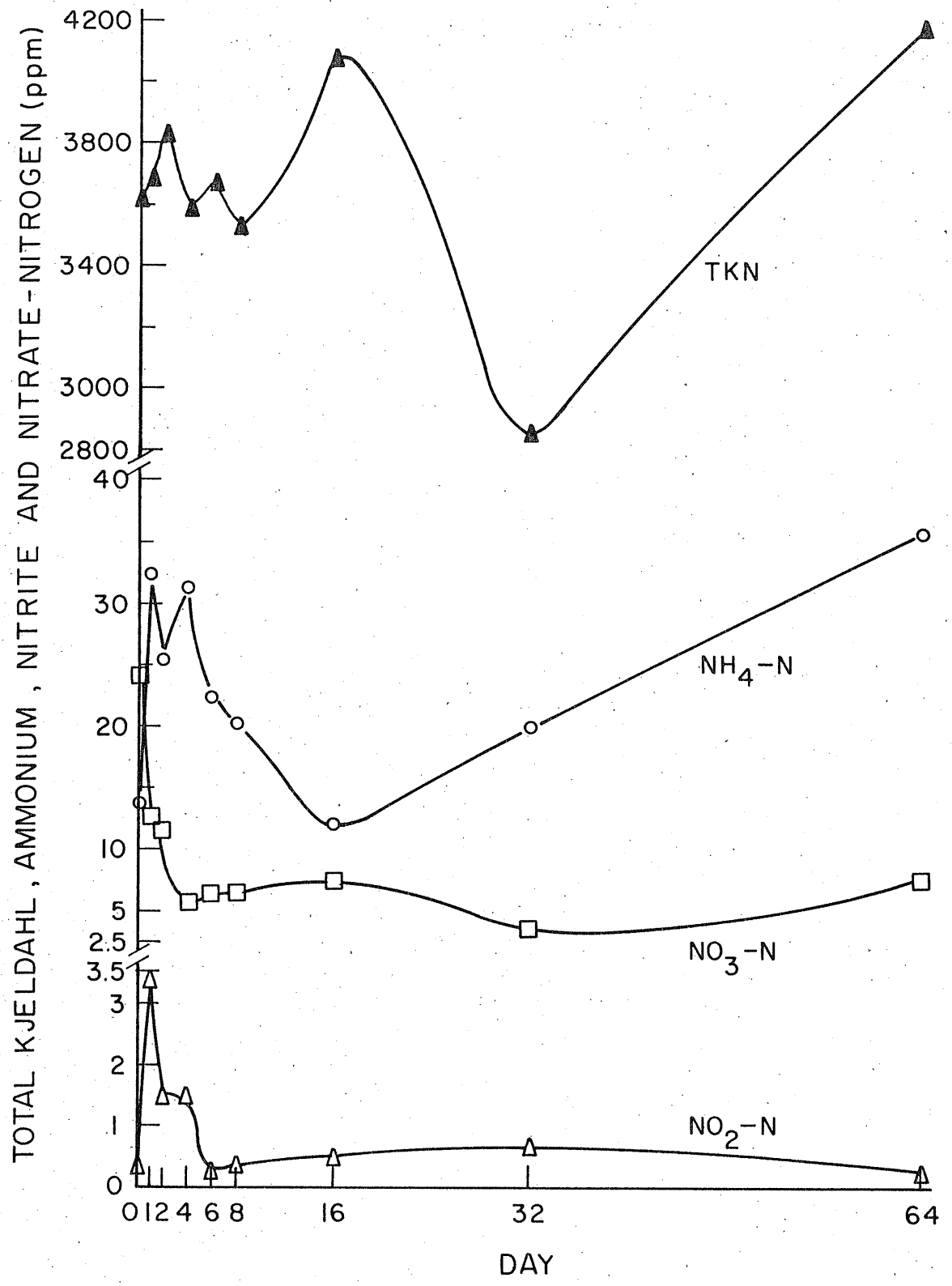

Fig. 4.9. Soil Nitogen Curves for the 224 kg N ha^{-1} Loading Rate in Incubation I

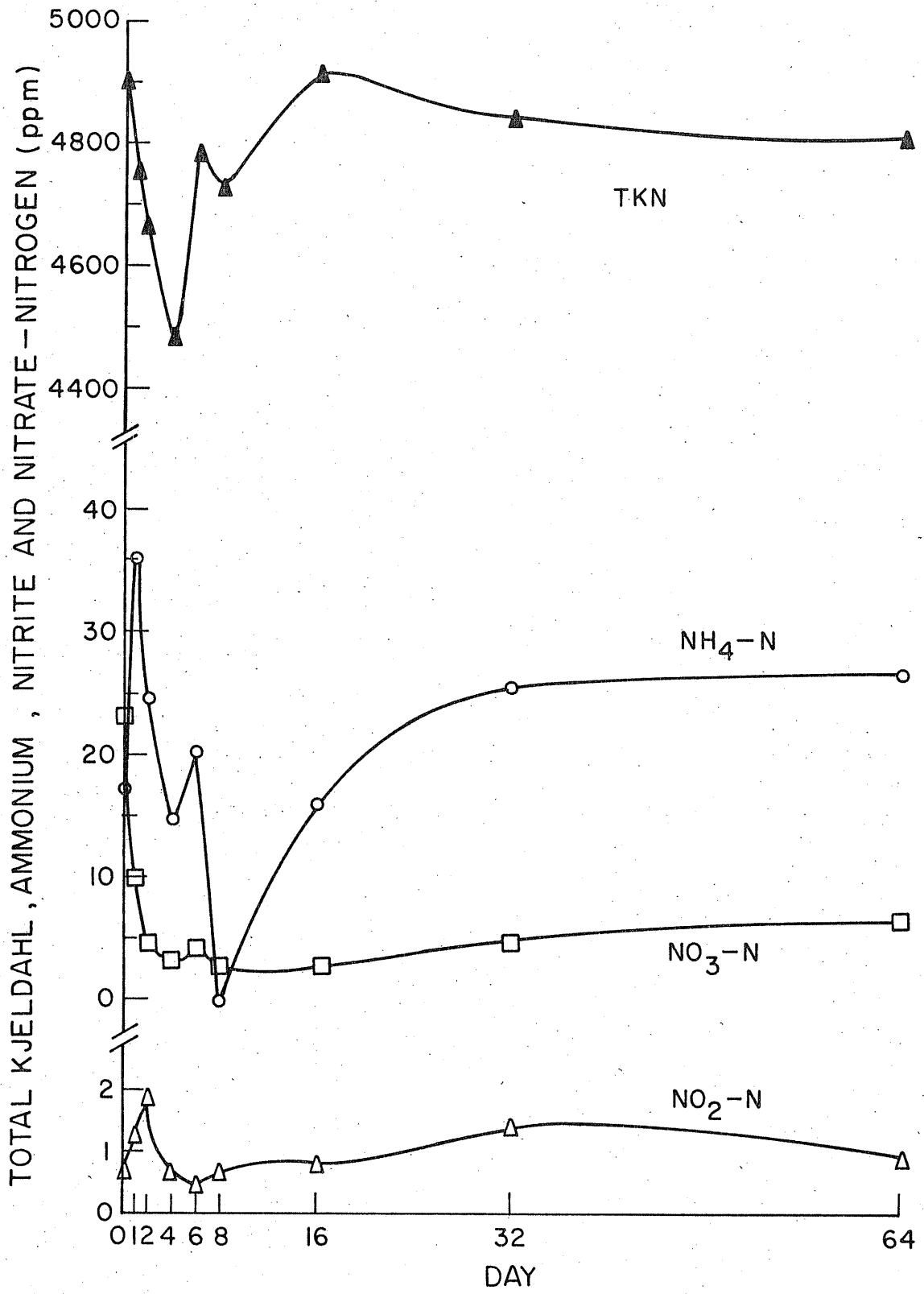

Fig. 4.10. Soil Nitrogen Curves for the 561 kg N ha^{-1} Loading Rate in Incubation I

The use of boric acid and flame heat in the analysis was considered the primary reasons for the variations. Boric acid has a gradual color change making it difficult to obtain a consistent end point from day to day and, in fact, from sample to sample. Also, flame heat did not produce uniform heat throughout the digestion flask which could result in only a portion of the organic matter being converted to NH_3. Bartholomew (1965) stated that the determination of total organic N before and after incubation generally is not feasible because the total quantity of organic N usually is large in comparison to the expected net change. This condition makes it difficult to obtain precise results from the analysis.

Noting the above results and Bartholomew's statement, the TKN analysis of Incubation II was performed only for day 0 (Figures 4.11, 4.12, 4.13 and 4.14). Comparing both incubations at day 0, the control of Incubation II had a lower TKN than the control of Incubation I probably due to the longer storage period. As noted previously under section 4.3, storage time probably caused a decrease in pH.

When comparing the TKN for each manure loading rate between Incubation I and II (Figures 4.7 to 4.14), differences in TKN were also evident, especially at the 561 kg N ha^{-1} rate. Incubation I at day 0 for the 561 kg N ha^{-1} rate had approximately 4900 ppm while Incubation II had approximately 3470 ppm for the same day and loading rate. The results should have been fairly close together since the same soil and loading rate were used. Since the soil

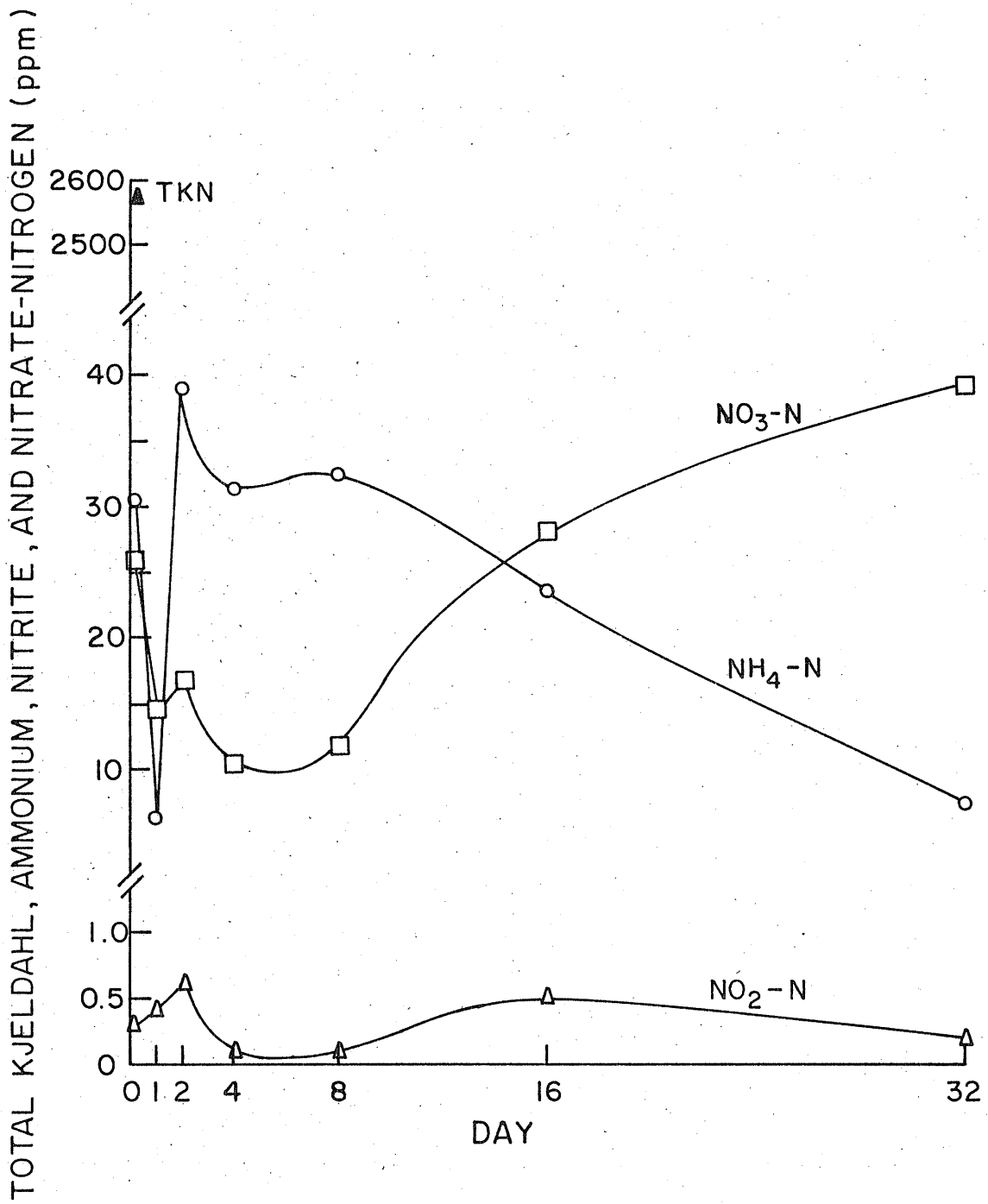

Fig. 4.11. Soil Nitrogen Curves for the Control in Incubation II.

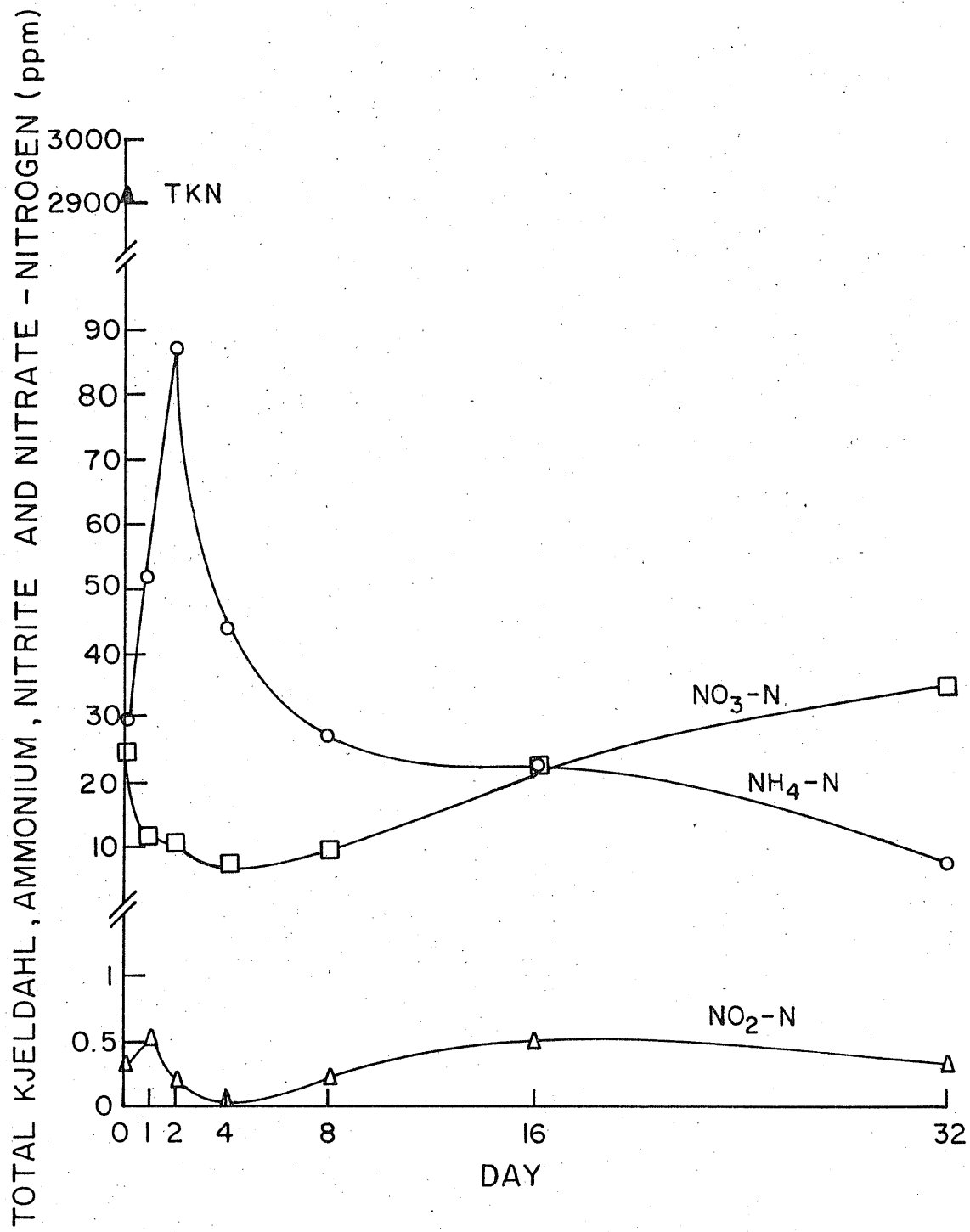

Fig. 4.12. Soil Nitrogen Curves for the 112 kg N ha^{-1} Loading Rate in Incubation II

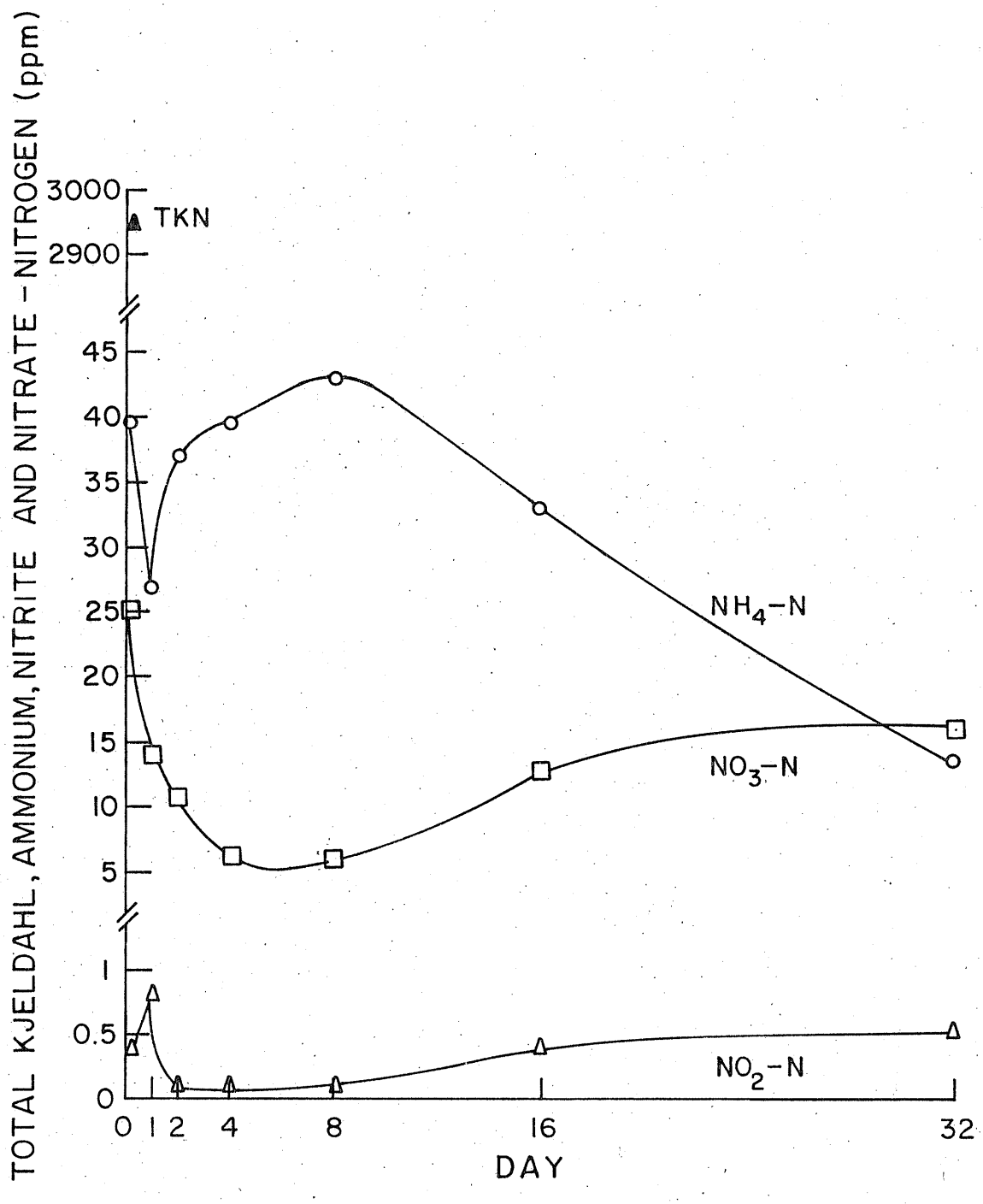

Fig. 4.13. Soil Nitrogen Curves of the 224 kg N ha^{-1} Loading Rate in Incubation II.

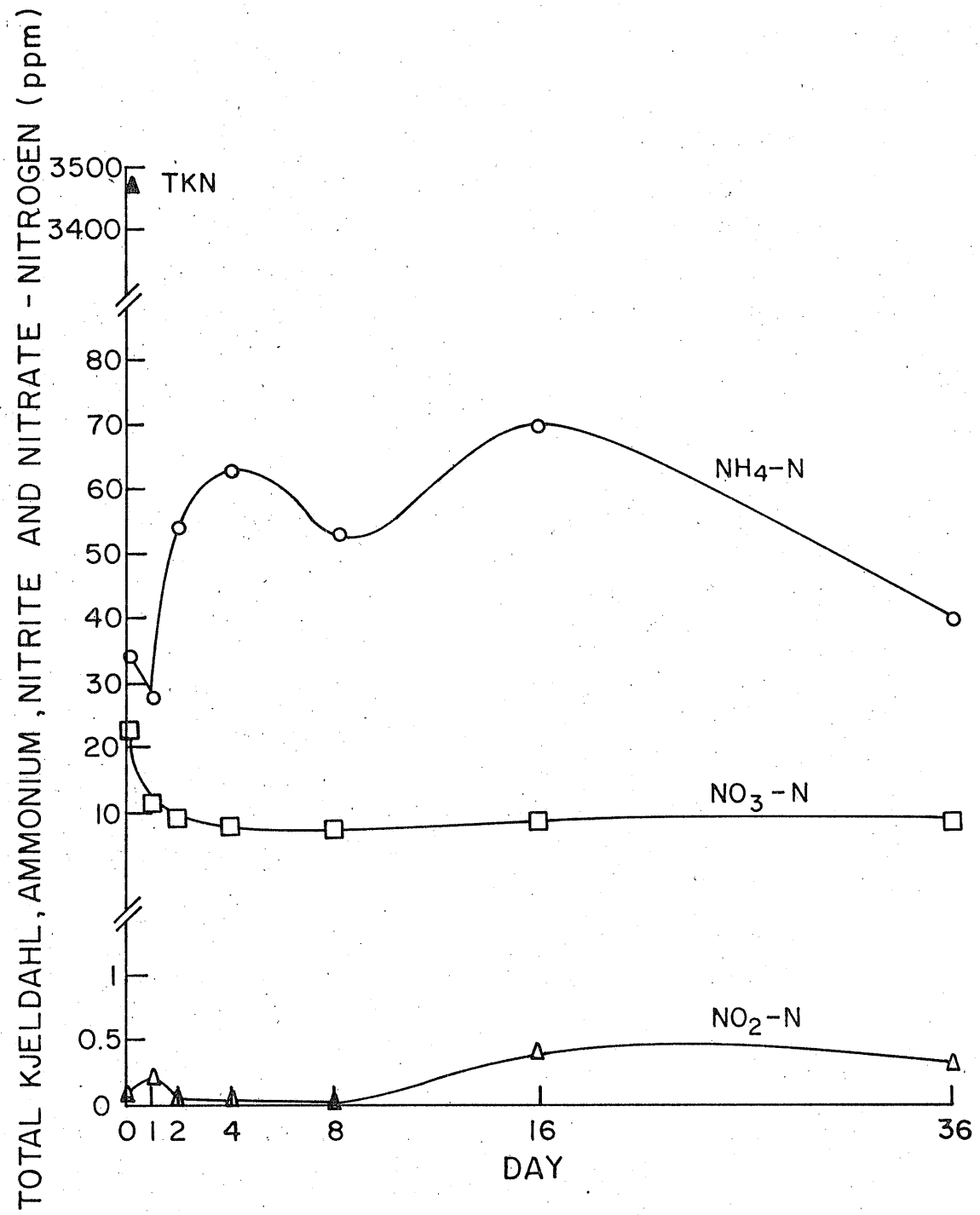

Fig. 4.14. Soil Nitrogen Curves for the 561 kg N ha^{-1} Loading Rate in Incubation II

in Incubation II had a slightly lower TKN, as noted previously, a slight decrease in overall TKN was expected. However, the only other difference between incubations was that wet manure was added in Incubation II whereas Incubation I utilized dry manure. Such a large difference in TKN at the same loading rates could not be explained satisfactorly.

4.5.3. Ammonification

The extractable NH_4-N curves of Incubation I (Figures 4.7, 4.8, 4.9 and 4.10) fluctuated with no consistent trends evident. Again boric acid was considered the primary reason for the fluctuations since it was difficult to obtain a consistent end-point with a small sample. This made it difficult to state whether ammonification, immobilization or nitrification was occurring. The extractable NH_4-N results of Incubation I showed low NH_4-N levels with the maximum level obtained being 38 ppm (Figure 4.9). Such low levels of NH_4-N and a high CEC combined to prevent NH_3 evolution.

To obtain a consistent end-point, a Fisher model 35 automatic titrimeter was utilized in Incubation II. The end-point obtained by the titrimeter was considerably higher than the manual titration procedure which was based on the color change of the indicator. The extractable NH_4-N of Incubation II (Figures 4.11, 4.12, 4.13 and 4.14) fluc-

tuated for the first four days. The control as well as the 224 and 561 kg N ha^{-1} loading dropped from the initial day indicating that immobilization was occurring, that is, the microorganisms required an inorganic N substrate (NH_4-N) for growth. For the same loadings, an increase in NH_4-N occurred after the initial decrease indicating that ammonification then exceeded immobilization. The extractable NH_4-N of the 112 kg N ha^{-1} loading initially increased to 87 ppm indicating that at the outset ammonification must have exceeded immobilization and that at least initially, nitrification was not occurring fast enough to lower the NH_4-N level.

At day 32, the NH_4-N content of the soil and soil-manure mixtures were all decreasing. The control, 112 and 224 kg N ha^{-1} rates had less than 15 ppm NH_4-N. This suggests that either immobilization or nitrification was occurring. The NH_4-N content of the 561 kg N ha^{-1} rate was 40 ppm on day 32, considerably higher than that of the other loadings. The NH_4-N level in the 561 kg N ha^{-1} loading did not fall below the initial 35 ppm NH_4-N present at day 0.

4.5.4. Extractable NO_2-N

Extractable nitrite levels for both incubations (Figures 4.7 to 4.14) were always low being less than 3.5 ppm NO_2-N. The 112, 224 and 561 kg N ha^{-1} loadings of

Incubation I showed a slight increase in NO_2-N levels at day 1 or 2 but these increases were only temporary. Such increases also occurred in Incubation II but were not as great as Incubation I. These increases in NO_2-N could be due to ammonium oxidation or denitrification.

4.5.5. Extractable NO_3-N

The greatest NO_3-N levels of both incubations occurred on day 0 due to the fact that the soil was obtained in the fall from a fallow field in which mineralization of N had taken place. Normally, at the end of a growing season, low levels (less than 5 ppm) NO_3-N are obtained from a stubble field.

4.5.5.1. Incubation I

The extractable NO_3-N levels of Incubation I showed a definite decline from the initial day in the 112, 224 and 561 kg N ha^{-1} loadings and never rose higher than the initial NO_3-N level for each respective loading (Figures 4.8 to 4.10). The control, with no manure addition, did not follow the same NO_3-N trend as in the soil-manure mixtures. The NO_3-N levels in the control were always higher than the soils amended with manure. The above result was not expected since the addition of manure generally causes an increase in NO_3-N.

In the control of Incubation I, the NO_3-N levels remained fairly constant initially, dropped slightly and then rose to a NO_3-N level of 57.9 ppm at day 64 which was higher than that on the initial day (28.2 ppm). The NH_4-N level of the control (Figure 4.7) increased as the NO_3-N decreased indicating that nitrification initially was inhibited. However, the NO_3-N level increased near the end of the experiment while the NH_4-N decreased indicating that nitrification was occurring.

The low levels of NO_3-N in the soil-manure mixtures could be the result of one of the following possible pathways:

(1) nitrification-denitrification

(2) net immobilization

(3) no nitrification combined with denitrification and/or net immobilization.

Each of the above pathways will be discussed as to the conditions that could cause the low NO_3-N levels obtained in the manure amended soil (Figure 4.8, 4.9 and 4.10).

The first pathway is nitrification-denitrification. Nitrification occurs under aerobic conditions and this incubation experiment was designed to aerate the soil to create conditions favourable for nitrification. This process by itself, could not account for the slight decrease in NO_3-N. Denitrification could account for the decrease in NO_3-N. The soil was quite wet at field capacity and the high moisture content could have aided the denitrification

process. Although the control operated at field capacity and some NO_3-N accumulated, the moisture content at field capacity increased as the manure loading rate increased (Table 3.1). A combination of high moisture levels and increased microbial activity may have caused the micro-environment of the bacteria to become oxygen deficient, stimulating denitrification. Furthermore, the addition of manure, a carbon source, could also have caused nitrate reduction as noted in the literature review and may have also stimulated denitrification. Since N_2 and N_2O gases were not monitored, the amount of denitrification, that occurred cannot be stated with certainty.

The second pathway, net immobilization (the formation of organic N from NH_4-N or NO_3-N), was considered the prime factor for lack of NO_3-N accumulation from the initial day in the soils amended with manure in Incubation I. When a low-N manure, containing much straw, is added to soil, immobilization exceeds ammonification[1]. The strawy manure of Incubation I had a C:N ratio of 124:1 (Table 4.3) which is certainly high enough to cause net immobilization. The manure increased the C:N ratio of the 112, 224 and 561 kg N ha^{-1} loading rate in comparison to the C:N ratio of the control (Table 4.3). Although the resulting C:N ratios were less than 20:1, the added manure probably disrupted the steady-state conditions of the microbial environment

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[1] G. Racz, Department of Soil Science, University of Manitoba, personal communication, December, 1975.

and allowed immobilization to exceed ammonification.

In the third pathway, if nitrification was inhibited, the decrease in NO_3-N could be a result of either net immobilization, denitrification or both. However, near the end of the incubation period (day 64) the NO_3-N levels rose slightly indicating that nitrification was occurring to some degree.

4.5.5.2. Incubation II

To contrast the effect of the high C:N ratio of the manure used in Incubation I, wet dairy manure containing no straw was utilized in Incubation II. The C:N ratio of the wet manure was 17.5 to 1 (Table 4.3).

The NO_3-N levels for all manure loading rates in Incubation II decreased from the initial day (Figures 4.12 to 4.14). Water added to reach the field capacity of the soil and the increased microbial activity due to the presence of the manure could have caused the micro-environment to become anaerobic, thus causing NO_3-N to be denitrified. A more likely loss would be net immobilization, that is, the microorganisms used NO_3-N for growth and metabolism as the microorganism population increased. The addition of manure to soil has been known to cause increased microbial activity that require inorganic NH_4-N or NO_3-N for growth which can thus lower NO_3 levels. As the NO_3-N levels decreased from the initial day, the NH_4-N increased implying that denitrification exceeded NH_4 oxidation. After 8 days of

incubation the NO_3-N started to increase in the control, and in the 112 and 224 kg N ha^{-1} loading. As the NO_3-N increased, the NH_4-N level decreased showing that nitrification was occurring. After 32 days of incubation, the control (as in Incubation I) produced the most NO_3-N (39.5 ppm), followed by the 112 kg N ha^{-1} (35.0 ppm), the 224 kg N ha^{-1} (16.1 ppm) and the 561 kg N ha^{-1} (8.7 ppm).

With a large drop in NH_4-N and only a small increase in NO_3-N, especially for the 112 and 224 kg N ha^{-1} loading rates, it would appear that the moisture content was too high which probably lead to partial anaerobic conditions and some loss of NO_3-N. The increase in microbial activity could also aid in producing anaerobic conditions. For optimum microbial activity, 10 percent air space should be available for O_2 to diffuse easily[1]. However, it is difficult to estimate the amount of water necessary to attain this air space in a clay soil[1].

In the 561 kg N ha^{-1} loading rate, the NH_4-N level remained fairly constant between 40 and 60 ppm and the NO_3-N level also remained fairly constant (Figure 4.14) implying that nitrification was not occurring. Harmsen and Kolenbrander (1965) noted that most investigators agree that reduced aeration can curb or even entirely suppress nitrification but ammonification is less affected. Inorganic N levels, they noted, as high as 100 ppm may be reached but

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[1] C.F. Shaykewich, Department of Soil Science, The University of Manitoba, personal communication, August, 1976.

mainly as NH_4 and not as NO_3. However, if Harmsen and Kolenbrander's statement were applied to this experiment, increased NH_4-N levels would have been expected as time progressed but instead the NH_4-N levels remained fairly constant. If an equilibrium between nitrification and denitrification occurred, then the NH_4-N levels would remain fairly constant.

The controls in both incubations nitrified whereas none of the manure-amended soils in Incubation I clearly exhibited nitrification. In Incubation II, the lower C:N ratio of the dairy manure enabled nitrification in the lower rates of manure addition to the soil.

4.5.6. Kinetics of N Mineralization

A kinetic interpretation of the N data of these experiments was unsuccessful due to the immobilization and denitrification that apparently occurred in the clay soil. As mentioned previously, "smooth" curves are required to obtain kinetic constants. The experiments were not a failure but helped explain what could happen in the field. For instance, the results from the incubation conducted in the laboratory can help explain why Bergson (1975) noted no accumulation of NO_3-N after heavy applications of dairy manure at Glenlea, Manitoba. Thus, the lack of N mineralization reinforces the observation that high manure loading rates may not be a serious problem in terms of NO_3-N accumulation in a Scanterbury clay soil.

4.6. Experimental Design

4.6.1. Manure Loading Rates

Racz[1] noted that manure can be safely applied on land at rates of 89.8 to 112.3 kg N ha^{-1} yr^{-1} (80 to 100 lb N $acre^{-1}$ yr^{-1}) where cereal crops are grown. Furthermore as noted in the previous section, heavy application rates of dairy manure (201 kg N ha^{-1}) showed no accumulation of NO_3-N in the soil. Thus, this experiment utilized loading rates of 0 (control), 112, 224 and 561 kg N ha^{-1} loadings in which the manure was thoroughly mixed with the soil.

The loading recommended by Racz was based on wet manure (urine and faeces) but Incubation I used dried dairy manure containing straw bedding whereas Incubation II used wet manure but did not include urine or bedding. Without urine, which contains a high percentage of N, more of the dry and wet manure was required for each incubation in order to reach the desired N loading rate.

4.6.2. Drying Dairy Manure

It was noted in Chapter 3 that the dairy manure for Incubation I was oven-dried. Oven-drying probably caused NH_3 evolution resulting in a loss of N prior to incubation and an abnormal increase in the C:N ratio. Incubation II was improved by incorporating wet dairy manure into the

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[1] G. Racz, Department of Soil Science, The University of Manitoba, classroom lecture notes of course 65.302, Fall, 1974.

soil. The latter procedure made the manure loading rate more realistic. The kind of manure management system - for example; solid, liquid or dried manure - has a very large influence on actual nutrient content at time of application and should be considered when determining the amount of N applied to the land (Committee of the Manitoba Institute of Agrologists, 1973).

The results of using dried and wet manure (faeces only) in the two incubations (Table 3.1) certainly verifies the above statement.

4.6.3. Effect of Manure Addition to Soil

Brady (1974) noted that the addition of organic matter not only binds but also lightens and expands the soil. He also noted that the organic matter is of much importance of modifying the effects of clay and that the humus has a high absorptive capacity for water which helps to disrupt the effects of temperature changes and moisture fluctuation. This increase in moisture content as the manure loading rate increased can be seen in Table 3.1.

Fresh manure, as noted by MacLean and Hore (1974), is better suited to clay and loam soils than to sandy soils because its coarseness improves their physical condition by opening them to air and making them more friable. The addition of manure in this experiment, either dry or wet, caused the soil to be more friable after drying and the ease

of fracture increased as the manure loading rate increased. The ease of fracture is of importance when preparing - for example, ploughing or cultivating - a field. Schulte and Tokarz (1976) pointed out that manure helps build and maintain soil fertility, improves tilth, increases the water-holding capacity of the soil, lessens wind and water erosion, improves soil aeration and promotes the growth of beneficial soil microorganisms. Manure has thus many good effects besides nutrient addition and so should not be treated as a "waste" but as a valuable product.

4.6.4. Soil Particle Size

The importance of the fact that the manure and soil were ground and dried prior to incubation, which differ from actual field conditions, cannot be over-emphasized. Grinding the soil or manure increases the surface area on which microorganisms can attack organic and inorganic substances. It also decreases the pore space which aids in holding more water and prevents good aeration. A 2-mm mesh was probably too small for grinding the soil; a 4-mm mesh would have been better since it would have increased the pore space[1]. Probably the best method is not to grind the soil at all since the incubation would be more realistic of what is happening in the field.

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[1] C.F. Shaykewich, Department of Soil Science, The University of Manitoba, personal communication, August, 1976.

4.6.5. Soil Drying

The soil and soil-manure mixtures were air-dried after each incubation period, and changes in the N levels could have occurred during this drying period. In this experiment, the above drying was assumed to produce negligible N changes.

4.6.6. Bulk Density of the Scanterbury Clay

The bulk density of the Scanterbury clay was 0.8 g cm^{-3} which was considered quite low[1,2]. The normal bulk density for Scanterbury clay ranges between 1.12 and 1.24 g cm^{-3}. However, since the soil was sampled from the dry soil surface, was rather loose and contained some straw, a low bulk density was possible[2]. Furthermore, the bulk density varies with depth and time of year (lack of water may cause the soil to crack and a bulk density of 1.7 to 1.8 may be reached). The low bulk density obtained for this soil meant that the soil weight per hectare for a 15.2 cm depth was somewhat low (1 222 000 kg ha^{-1}). The equivalent N requirements per hectare based on the manure to soil weight would demand a higher percentage of manure by weight if the bulk density was higher.

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1 F. Penkava, Department of Agricultural Engineering, The University of Manitoba, personal communication, August, 1976.

2 W. Michalyna, Department of Soil Science, Soil Survey, The University of Manitoba, personal communication, August, 1976.

4.6.7. Sample Size

Float and Torrance (1970) noted that by using small sample sizes (2 g), better agreement of duplicate samples occurred owing to more uniform aeration within the samples. The incubation experiments, using considerably larger sample size (120 g), showed some variation from tube to tube in the chemical analysis (Appendix E) probably due to the lack of uniform aeration within the sample. Larger samples, however, represent field conditions better than small samples.

4.6.8. Cation Exchange Capacity

The CEC measures the available exchange sites for positive ions, such as NH_4^+, in a soil (clay for this experiment). The available water affects the CEC since the NH_4^+ can hydrolize to form ammonium hydroxide which may or may not attach to the exchange sites[1]. Lack of NH_3 evolution in the incubations was attributed to the high CEC (527.5 ppm).

4.6.9. Organic Carbon

When determining the organic C from the organic matter of the soil, a 0.58 factor was recommended[2]. Brady

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1 W. Michalyna, Department of Soil Science, The University of Manitoba, telephone communication, August, 1976.

2 G. Racz, Department of Soil Science, The University of Manitoba, personal communication, December, 1975.

(1974) pointed out that the C:N ratio of mineral soils is rather constant and the organic C is 0.58 times the organic matter. For the Scanterbury clay soil which had manure added, the 0.58 factor may not be the correct factor since the addition of manure upset the "constant" C:N ratio of the soil. The factor, however, is the best available estimate. If the factor was different, it would change the C content and, in turn, alter the C:N ratio.

This same factor was also assumed to apply to animal manures. Brady (1970) stated that manures are, to a considerable extent, partially degraded plant materials with hemicellulose, lignin and ligno-protein complexes similar to those found in soil humus. The 0.58 may not be correct for manure, it again is the best estimate available. Although the C measured and the C calculated in Table 4.3 were similar in value, the "true" C content may be different as mentioned above due to the use of the 0.58 factor.

4.6.10. CO_2 Production and N Mineralization

Daknke and Vasey (1973) stated that the principle of the CO_2 estimation procedure for estimating N mineralized is that when a soil sample is incubated with an excess of easily decomposable organic material, the amount of CO_2 produced will be proportional to the amount of mineral N initially present in the soil plus the amount made available during incubation. In these experiments, the high C:N ratio of

incubation I and the high moisture content of Incubation I and II prevented a NO_3-N build-up and, in turn, the CO_2 production could not be correlated to N mineralized. Thus, CO_2 production may not always imply that net N mineralization will occur.

CHAPTER 5

CONCLUSIONS

(1) A first-order kinetic equation successfully described the amount of carbon remaining in the dairy manure undergoing decomposition in soil.

(2) The carbon evolved from oven-dried, strawy dairy manure as CO_2-C was 17.6, 10.3 and 5.3 percent of the original carbon added in the 112, 224 and 561 kg N ha^{-1} loading rates, respectively, after 32 days of incubation at 15°C. For the same loading rates, but after 64 days of incubation, CO_2-C evolved was 29.1, 15.2 and 8.9 percent, respectively, of the original carbon added.

(3) The carbon evolved from fresh wet dairy manure was 50.5, 37.1 and 20.9 percent of the original carbon added in the 112, 224 and 561 kg N ha^{-1} loading rates, respectively, after 32 days of incubation at 15°C.

(4) The turnover period required to decompose 99.9 percent of the manure carbon ranged from 0.77 calendar years for the 112 kg N ha^{-1} loading rate of fresh wet dairy manure to 13.5 calendar years for the 561 kg N ha^{-1} of oven-dried strawy dairy manure when incubated at a temperatore of 15°C.

(5) No evolution data of NH_3 was obtained because the high cation exchange capacity of the Scanterbury clay soil prevented NH_3 evolution.

(6) Nitrate accumulation occurred only in the control of Incubation I reaching a NO_3-N level of 58 ppm after 64 days of incubation. In Incubation II, after the eighth day of incubation NO_3-N began to accumulate in the control and in the 112 and 224 kg N ha^{-1} loading rates with the control, at the end of 32 days of incubation, producing the most NO_3-N (39.5 ppm) followed by the 112 kg N ha^{-1} rate (35.0 ppm) and the 224 kg N ha^{-1} rate (16.1 ppm), respectively.

(7) A kinetic explanation of the nitrogen data failed due to the fact that the N transformations did not produce "smooth" N curves.

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APPENDIX A

APPENDIX A

CALCULATION OF AIR FLOW RATE

Finstein (1972) obtained the following O_2 uptake rates:

(a) manure - 10 ml O_2 per g of poultry manure for a 24 hour period;

(b) soil - 0.05 ml O_2 per 10 g of soil for a 24 hour period.

Using the highest loading rate (561 kg N ha^{-1}) of Incubation I (Table 3.1) the O_2 required by the manure would be

$$\frac{10 \text{ ml } O_2}{\frac{\text{g manure}}{\text{day}}} \times \frac{17.1 \text{ g manure}}{\text{tube}} \times \frac{24 \text{ tubes}}{\text{incubation chamber}} = \frac{4100 \text{ ml } O_2}{\frac{\text{chamber}}{\text{day}}}$$

Using the highest loading rate of Incubation I (Table 3.1) the O_2 required by the soil is

$$\frac{0.05 \text{ ml } O_2}{\frac{\text{g soil}}{\text{day}}} \times \frac{102.9 \text{ g soil}}{\text{tube}} \times \frac{24 \text{ tubes}}{\text{incubation chamber}} = \frac{124 \text{ ml } O_2}{\frac{\text{chamber}}{\text{day}}}$$

Total O_2 required is $4224 \text{ ml } O_2 \Big/ \frac{\text{chamber}}{\text{day}}$

The O_2 content of air is approximately 20% by volume. Therefore, air flow rate is $4230 \div 0.2 = 21{,}120 \text{ ml air} \Big/ \frac{\text{incubation chamber}}{\text{day}}$

or approximately $15 \text{ ml air} \Big/ \frac{\text{incubation chamber}}{\text{minute}}$

An air flow rate of 40 ml min^{-1} was chosen in order to be reasonably in excess of the air flow rate calculated. Air flow rates lower than 40 ml air per minute produced considerably fewer bubbles and higher rates produced short contact time of the air bubbles with the scrubbing solution.

APPENDIX B

APPENDIX B

ANALYSIS OF SCANTERBURY CLAY (CULTIVATED)*

Depth	Sand	Silt	Clay	Organic C	Total N	C:N Ratio	CEC(NH_4)
cm	%	%	%	%	%		meq/100 g
0-17.8	6.2	23.28	70.52	2.59	0.305	8.5:1	52.75

* Unpublished report of the Detailed Soil Survey of Glenlea Research Station, Glenlea, Manitoba by W. Michalyna, Department of Soil Science, University of Manitoba.

APPENDIX C

APPENDIX C

C.1 DEVELOPMENT OF EQUATIONS TO DETERMINE THE WET AND DRY MANURE LOADING RATES

1. Wet Manure

Definitions

M_s - moisture content of the soil required for incubation, % weight basis

Mww - wet weight of a manure sample, g

Mdw - dry weight of a manure sample, g

MwwmI - wet weight of manure used per sample tube (MSm) for incubation, g

MdwmI - dry weight of manure used per sample tube (MSm) for incubation, g

WwwmI - weight of water in wet manure used for incubation, g

Nm - nitrogen content of manure, w.b., expressed as a fraction

Nwwnd - nitrogen contained in wet weight of a manure sample used in the N determination, g

Mwwnd - wet weight of a manure sample, g, used for N determination

MSm - total dry weight of manure and soil mixture required per sample tube, g

Sd - dry weight of soil required per sample, g

NLR - nitrogen loading rate, kg N ha^{-1} (lb N $acre^{-1}$)

Wt - total weight of water required to prepare a sample tube (MSm) for incubation, g (field capacity was used)

WwR - weight of distilled water required to bring the moisture content of a sample tube (MSm) to the desired level, g

m.c. - moisture content of manure, w.b., expressed as a fraction

Lwms - loading rate of wet manure to soil in the field expressed as a fraction

W_s - weight of soil surface 15 cm, (6 in) deep per hectare (acre), kg (ha x 15.2 cm)$^{-1}$ (lb (acre-6 in)$^{-1}$ in the Imperial System). The units used for NLR must be the same for Ws.

The equations are developed to determine the wet weight of manure required per sample tube.

Moisture Content

$$m.c. = \frac{Mww-Mdw}{Mww} \qquad (1)$$

also

$$m.c. = \frac{MwwmI-MdwmI}{MwwmI} = \frac{WwwmI}{MwwmI} \qquad (2)$$

Nitrogen Content of wet manure

$$Nm = \frac{Nwwnd}{Mwwnd} \quad (3)$$

Total weight of soil and dry manure per sample tube

$$MSm = Sd + MdwmI \quad (4)$$

Wet weight of manure for incubation

$$MwwmI = WwwmI + MdwmI \quad (5)$$

The moisture content of the soil, Ms, used for this experiment was determined for field capacity (Table 3.1). To obtain the amount of water, Wt, required in grams per sample tube, then

$$Wt = Ms \times MSm \quad (6)$$

Also, the total weight of water per sample tube equals

$$Wt = Wwr + MwwmI \quad (7)$$

Rearrange (4)

$$Sd = MSm - MdwmI \quad (8)$$

Rearrange (5)

$$MdwmI = MwwmI - WwwmI \quad (9)$$

Combine (8) and (9)

$$Sd = MSm - MwwmI + WwwmI \quad (10)$$

Rearrange (2)

$$WwwmI = m.c. \times MwwmI \quad (11)$$

Combine (10) and (11)

$$Sd = MSm - MwwmI + (m.c. \times MwwmI) \quad (12)$$

$$\text{or } Sd = MSm - MwwmI\ (1-m.c.) \quad (13)$$

The loading rate of wet manure to soil in the field

$$Lwms = \frac{NLR}{Nm\ Ws} \quad (14)$$

Note: the manure is assumed to mix with the top 15.2 cm (6 in) of soil.

Also, the above ratio must be equivalent to the wet manure added to the soil sample in an incubation run per sample tube.

$$Lwms = \frac{MwwmI}{Sd} \quad (15)$$

The dry weight of wet manure used for incubation

$$MdwmI = (1\text{-}m.c.)\ MwwmI \quad (16)$$

Combine (12) and (14)

$$Lwms = \frac{MwwmI}{MSm-(1\text{-}m.c.)\ MwwmI} \quad (17)$$

or

$$Lwms\ MSm = MwwmI + Lwms\ (1\text{-}m.c.)\ MwwmI \quad (18)$$

Rearrange (18)

$$MwwmI = \frac{Lwms\ MSm}{1 + Lwms(1\text{-}m.c.)} \quad (18)$$

Rearrange (18)

$$MwwmI = \frac{Lwms\ MSm}{1 + Lwms(1\text{-}m.c.)} \quad (19)$$

Now, rearrange (4)

$$MdwmI = MSm - Sd \quad (20)$$

Rearrange (5)

$$WwwmI = MwwmI - MdwmI \quad (21)$$

Rearrange (7)

$$Wwr = Wt - MwwmI \quad (22)$$

Combine, (20), (21) and (22)

$$Wwr = Wt - MwwmI + MSm - Sd \quad (23)$$

The key equations are (13), (19) and (23) for determining the wet manure loading rate.

2. Dry Manure

The equations used for wet manure are similar for dry manure but some redefinition is necessary.

m.c. - zero moisture

Nm - is changed to Nmd; nitrogen content of dry manure, weight basis, expressed as a fraction

Nwwnd - is changed to Ndwnd; the nitrogen contained in a dry manure sample used in the N determination, g

Mwwnd - is changed to Mdwmd; dry weight of manure sample, g, used for the N determination

MwwmI - is changed to MdwmI; the dry weight of manure used for incubation per sample tube

Lwms - is changed to Ldms; the loading rate of dry manure to soil in the field expressed as a fraction

MSm, Sd, NLR - defined previously

The following equations are developed to determine the dry weight of manure and water required per sample tube.

Nitrogen content of dry manure

$$Nmd = \frac{Ndwnd}{Mdwnd} \qquad (24)$$

The amount of water, Wt, in grams required per sample tube is calculated as shown in equation (6). The loading rate of dry manure to soil in the field is calculated the same manner as equation (14) but Nm is based on the N content of

dry manure as shown in equation (24). Thus,

$$Ldms = \frac{NLR}{Nmd \times Ws} \qquad (25)$$

The above ratio Ldms, must be equivalent to the dry manure added to the soil sample per sample tube in the incubation run

$$Ldms = \frac{MdwmI}{Sd} \qquad (26)$$

Combining (8) and (26)

$$Ldms = \frac{MdwmI}{MSm - MdwmI} \qquad (27)$$

or

$$MdwmI = \frac{Ldms \times MSm}{(1 + Ldms)} \qquad (28)$$

The key equations are (6), (8) and (28) for determining the dry manure loading rate.

APPENDIX C

C.2 EXAMPLE CALCULATION OF THE DRY MANURE LOADING RATE IN INCUBATION I

From Table 3.1, the TKN of the dry manure in Incubation I was 0.277%. This is equivalent to .00277 g N per g of dry manure and is equal to Nmd in equation (24) in Appendix C1. The total dry weight of manure and soil per sample tube (MSm) was 120 g (Table 3.1).

From Table 3.1, and using the highest loading rate (561 kg N ha^{-1}) in Incubation I, the moisture at field capacity was 82%. Therefore, using equation (6), 0.82 x 120 g = 98 g of distilled water (Wt) was added per sample tube.

The weight of the Scanterbury surface soil was 1 222 300 kg $(15.2\ cm \times ha)^{-1}$. The loading rate of dry manure to soil in the field (Ldms) expressed as a fraction (metric units) was obtained using equation (25) in Appendix C1:

$$Ldms = \frac{561\ kg\ N\ ha^{-1}}{\frac{.00277\ g/g}{1\ 222\ 300\ kg\ (\ 15.2\ cm \times ha)^{-1}}} = 0.1657$$

The manure dry weight used per sample tube was calculated using equation (28) in Appendix C1:

$$MdwmI = \frac{(0.1657)(120)}{1.1657} = 17.1\ g$$

Soil dry weight added per sample tube (Sd) was obtained using equation (8) in Appendix C1:

$$Sd = 120 - 17.1 = 102.9 \text{ g}$$

APPENDIX C

C.3 EXAMPLE CALCULATION OF THE WET MANURE LOADING RATE IN INCUBATION II

From Table 3.1, the TKN of the wet manure in Incubation II was 0.335%. This is equivalent to 0.00335 g N per g of wet manure and is equal to Nm in equation (3) in Appendix C1.

The total dry weight of manure and soil per sample tube (MSm) was 120 g (Table 3.1).

From Table 3.1, and using the highest loading rate (561 kg N ha^{-1}) in Incubation II, the moisture content at field capacity and of the wet dairy manure were 79.2% and 82.4%, respectively. The amount of water, Wt, required per sample tube to reach field capacity using equation (6) was .792 x 120 g = 95 g.

The loading rate of wet manure to soil in the field (Lwms) expressed as a fraction (metric units) was obtained using equation (14) in Appendix C1:

$$Lwms = \frac{561 \text{ kg N ha}^{-1}}{\frac{.00335 \text{ g/g}}{1\ 222\ 300 \text{ kg}}\ (15.2 \text{ cm x ha})^{-1}} = 0.137$$

The manure wet weight added per sample tube was calculated using equation (19) in Appendix C1:

$$MwwmI = \frac{(0.137)(120)}{1 + (0.137)(1 - 0.824)}$$

$$MwwmI = 16.1 \text{ g}$$

The soil dry weight added per sample tube as calculated using equation (13), Appendix C1:

$$Sd = 120 \text{ g} - 16.1 \text{ g } (1-.824)$$

$$= 117.2 \text{ g}$$

Check: $Lwms = \frac{MwwmI}{Sd} = \frac{16.1}{117.2} = 0.137$

The amount of water required to bring the moisture content to field capacity was obtained using equation (23) in Appendix C1:

$$Wwr = 95 \text{ g} - 16.1 \text{ g} + 120 - 117.2 = 81.7 \text{ g}$$

APPENDIX C

C.4 CALCULATED C:N RATIOS

The calculated values for the TKN, organic C and the C:N ratio shown in Table 4.3 were based on the amount of soil and manure used for each loading rate (Table 3.1) as well as the TKN and organic C of the control (0 kg N ha^{-1}) and the dairy manure. The 112 kg N ha^{-1} loading rate of Incubation I is used to show the procedure employed to obtain the calculated TKN, organic C, and C:N ratio.

TKN

The amount of soil used in 112 kg N ha^{-1} loading (Table 3.1) = 116.1 g.

The amount of manure added in the 112 kg N ha^{-1} loading (Table 3.1) = 3.9 g.

TKN of manure (Table 4.3) = 0.28%

TKN of soil (Table 4.3) = 0.28%

Total amount of soil per sample tube = 120 g

Calculated % TKN is calculated:

$$\left(\text{TKN of manure} \times \frac{\text{manure weight}}{\text{sample tube weight}} + \text{TKN of soil} \times \frac{\text{soil weight}}{\text{sample tube weight}}\right) \times 100$$

Using values yields

$$\left(.0028 \times \frac{3.9}{120} + .0028 \times \frac{116.1}{120}\right) \times 100 = 0.28\%$$

Organic C

The soil-manure weights for the TKN calculation are the same for the organic C calculations.

Organic C of manure (Table 4.3) - 34.70%

Organic C of soil (Table 4.3) - 3.93%

Total amount of soil per sample tube - 120 g

Organic C is calculated using the same equation as TKN but organic C is substituted for TKN. Therefore, using the above values yields

$$(0.3470 \times \frac{3.9}{120} + 0.0393 \times \frac{116.1}{120}) \times 100 = 4.93\%$$

C:N Ratio

Using the calculated % TKN and % organic C values the C:N ratio can be calculated:

$$\frac{4.93}{0.28} = 17.6:1$$

APPENDIX D

APPENDIX D

D.1 AMMONIA SCRUBBING

The NH_3 scrubbing system used 250 ml of 0.5 N H_2SO_4 to remove the NH_3 produced by the microorganisms in the incubation chamber. A reagent blank was also used. A 50 ml aliquot was titrated using 0.5 N KOH and methyl red indicator. The color change was from red to yellow.

Calculation:

$$\text{mg/l } NH_3 = (\text{Blank} - \text{Sample}) \times \frac{0.5\text{ N}}{\frac{1}{14}} \times 1.0 \frac{\text{mg}}{\text{ml}} \times \text{dilution factor}$$

CARBON DIOXIDE SCRUBBING

To remove the CO_2 produced by the microorganisms in the incubation chamber, 250 ml of 1N KOH was used. A 50 ml aliquot was titrated with 1 N HCl using excess $BaCl_2$ (3N $BaCl_2$ was prepared by dissolving 312 g $BaCl_2$ to 1 liter) and phenothalein indicator. The color change was from pink to clear. A reagent blank was also included.

Calculation:

$$\text{mg/l } CO_2 \text{ as } CO_2\text{-C} = (\text{Blank-sample}) \times 6 \times \text{normality of scrubbing solution} \times \text{aliquot } \frac{(50\text{ml})}{250\text{ml}}$$

To convert CO_2-C to CO_2 multiply by 3.67.

APPENDIX D

D.2 PERCENTAGE OF CARBON IN THE MANURE THAT IS EVOLVED AS CO_2-C

1. Dry Dairy Manure

The 561 kg N ha^{-1} loading rate at day 64 of Incubation I was used as an example.

From Table 3.1, 17.1 g of manure were added to each sample tube. From Table 4.3, the organic C content of the manure was 34.7%. The amount of organic C in the manure per g of soil-manure mixture for each sample tube is

$$\frac{17.1\ g}{120\ g} \times .347 = .0494\ \frac{g\ organic\ C}{g\ soil\text{-}manure\ mixture}$$

From Figure 4.1, the cumulative CO_2-C evolved at day 64 was 4.72 $\frac{mg}{g\ soil\text{-}manure\ mixture}$ or .00472 $\frac{g}{g\ soil\text{-}manure\ mixture}$. From the same figure, the cumulative CO_2-C evolved from the control at day 64 is 0.849 $\frac{mg}{g\ soil}$. The control is based on 120 g of soil per sample tube and the 561 kg N ha^{-1} loading rate contains 102.9 g of soil (Table 3.1) per sample tube. Therefore, in 1 g of soil-manure mixture there is $\frac{102.9\ g}{120\ g} \times 1\ g = 0.858$ g of soil. The amount of CO_2-C evolved from the 561 kg N ha^{-1} loading rate due to the soil is

$$0.858 \frac{\text{g soil}}{\text{g soil-manure mixture}} \times 0.849 \frac{\text{mg}}{\text{g soil}} = 0.728 \frac{\text{mg}}{\text{g soil-manure mixture}} = 0.000728 \frac{\text{g of } CO_2\text{-C}}{\text{g soil-manure mixture}}$$

The % of added organic C in the manure that is evolved as CO_2-C is

$$\frac{(.00472 - .000728)}{.0494} \times 100 = 8.1\%$$

2. Wet Dairy Manure

The calculations using wet dairy manure are basically the same as for the dry dairy manure. The dry matter in the wet manure is used for determining organic C in the manure. For instance, using the 561 kg N ha^{-1} loading rate at day 32 in Incubation II, the dry matter in the wet manure from Table 3.1 is 2.83 g. From Table 4.3, the organic C of the dry matter of the manure is 33.23% based on dry manure. The amount of organic C in the manure per g of soil-manure mixture for each sample tube is

$$\frac{2.83 \text{ g}}{120 \text{ g}} \times 0.3323 = 0.00784 \frac{\text{g organic C}}{\text{g soil-manure mixture}}$$

From Figure 4.2, the cumulative CO_2-C evolved at day 32 was 2.23 $\frac{\text{mg}}{\text{g soil-manure mixture}}$ or 0.00223 $\frac{\text{g } CO_2\text{-C}}{\text{g soil-manure mixture}}$. From the same figure, the cumulative CO_2-C evolved from the control at day 32 is 0.605 $\frac{\text{mg}}{\text{g soil}}$ or 0.000605 $\frac{\text{g}}{\text{g soil}}$. The control is based on 120 g of soil per sample tube and the 561 kg N ha^{-1} loading rate contains 117.2 g of soil (Table

3.1) per sample tube. Therefore, in one g of soil-manure mixture there is $\frac{117.2\ g}{120\ g} \times 1\ g = 0.977\ \frac{g\ of\ soil}{g\ soil\text{-}manure\ mixture}$.

The amount of CO_2-C evolved from the 561 kg N ha^{-1} loading rate due to the soil is $0.977\ \frac{g\ of\ soil}{g\ soil\text{-}manure\ mixture} \times$

$.000605\ \frac{g\ CO_2\text{-}C}{g\ soil} = 0.00059\ \frac{g\ CO_2\text{-}C}{g\ soil\text{-}manure\ mixture}$.

The % of added organic C in the manure that is evolved as CO_2-C is $\frac{(0.00223\ g - 0.00059)}{0.00784} \times 100 = 20.9\%$.

APPENDIX D

D.3 TOTAL KJELDAHL NITROGEN (KJELDAHL-GUNNING METHOD)*

Procedure:

(a) weigh 1, 2, or 5 g of soil and place into a 500 ml digestion flask;

(b) add 1 Kelpak #2, Fisher Scientific brand;

(c) add 25 ml of 36 N H_2SO_4 (rotate flask to wash down soil);

(d) digest for 30 minutes (after approximately 15 minutes of digestion, the liquid turns green. Digest for 15 minutes after this). In Incubation I a flame heat was used for digesting and distilling. Incubation II utilized a Precision Scientific Model # 10-AF-11 Kjeldahl apparatus using a coiled nickel-chromium heating element;

(e) cool, and then add 200 ml of distilled water;

(f) add 25 ml sodium thiosulfate (dissolve 80 g of $Na_2S_2O_3.5H_2O$ into 1 liter);

(g) add slowly 60 ml of 1-1 NaOH (weight of NaOH to weight of distilled water);

(h) add pumice, place in rack and twirl flask;

(i) distill 150 ml into 50 ml boric acid (Prepare boric acid monthly. Place 20 g pure boric acid into a 1 liter flask. Add 900 ml of distilled water and 10 ml of mixed indicator solution and then fill to the 1 liter mark. Mixed indicator solution is

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* Procedure used at the Manitoba Provincial Soils Testing Laboratory.

prepared by dissolving 0.2 g methyl red into 100 ml 95% ethanol and dissolving 0.1 g methyl blue into 50 ml ethanol The two solutions are then combined);

(j) lower receiver and wash out the tube;

(k) titrate the distilled fraction with 0.02 N H_2SO_4 (color change is from green to purple). In Incubation II, a Fisher model 35 automatic titrimeter was utilized for titrating the distilled fraction;

(l) run a blank;

(m) calculation.

$$\% \text{ N} = (\text{sample} - \text{blank}) \times \text{Normality} \times \frac{1.4}{\text{sample weight, g}}$$

$$\text{ppm} = \% \text{ N} \times 1{,}000{,}000$$

APPENDIX D

D.4 AMMONIUM BY STEAM DISTILLATION

The procedure used was similar to the one described by Bremner (1965).

Procedure:

(a) place 5 ml of boric acid (see Kjeldahl procedure for preparation) into a 50 ml Erlenmeyer flask marked to indicate 30 ml;

(b) place under steam distillation apparatus 4 cm above the surface of the boric acid;

(c) pipette on aliquot (10 ml to 20 ml) of soil extract. The soil extract was prepared by adding 30 ml of 2 N potassium chloride, KCl to 3 g of soil and shaken for one hour. The sample was then filtered using Whatman #30 filter paper;

(d) add 0.2 g magnesium oxide (heat in a muffle furnace at 600-700^{o}C for 2 hours and store in a tightly stoppered bottle in a dessicator);

(e) when distillate reaches 30 ml mark, stop distilling and rinse condenser;

(f) titrate using 0.001 N H_2SO_4. Normally, a microburette containing 0.005 N H_2SO_4 is used. But, when the analysis was performed in Incubation I, the microburette did not function properly, and a 25 ml burette graduated in 0.1 ml

intervals was used to titrate a 0.001 N H_2SO_4 solution. In Incubation II, a Fisher model 35 automatic titrimeter was used to titrate a 0.001 N H_2SO_4 solution using a 0.1 ml graduated burette;

(g) Calculation

$$\% NH_4 - N = (\text{ml of sample titrated} - \text{ml of blank titrated}) \times \frac{1.4}{\text{sample weight}} \times N \times \text{aliquot fraction used}$$

$$\text{ppm} = \% NH_4^+ - N \times 1{,}000{,}000$$

APPENDIX D

D.5 NITRITE AND NITRATE EXTRACTION*

Procedures:

(a) extraction of NO_2 and NO_3 is accomplished by shaking 2.5 g of dry soil (ground to pass a 2-mm sieve) in 50 ml sodium bicarbonate, $NaHCO_3$ pH 8.5. Shake for 30 minutes at slow speed;

(b) add 1.0 g activated carbon prior to shaking;

(c) filter the solution using Whatman #30 filter paper into 50 ml beakers. The filtrate was transferred to test tubes and sent to the Manitoba Provincial Soils Testing Laboratory. The extracts were analyzed on a Technicon Auto Analyzer;

(d) a reagent blank was included with each set of 24 samples analyzed.

$$NO_3 - N \text{ ppm} = 20 \text{ (Sample - Reagent Blank)}$$

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* Procedure used at the Manitoba Provincial Soils Testing Laboratory.

APPENDIX D

D.6 ORGANIC CARBON*

Procedure:

(a) 0.5 g of less than 2-mm soil was weighed into a 500 ml Erlenmeyer flask;

(b) 10 ml of 1.0 N potassium dichromate (dissolve 49.04 g in water and dilute to 1 liter) was added;

(c) 20 ml of 36 N H_2SO_4 was added rapidly, directing the stream into the solution. The solution immediately was swirled vigorously for 1 minute and then allowed to stand on a sheet of asbestos for 30 minutes;

(d) the solution was diluted with 200 ml of distilled water;

(e) a Radiometer Copenhagen automatic titrator II was used to titrate the ferrous sulphate solution (0.5 N ferrous sulphate solution was prepared by dissolving 140 g reagent grade $FeSO_4.7H_2O$ in 900 ml distilled water. To this solution was added 40 ml 36 N H_2SO_4 and diluted to 1 liter). The titrator was adjusted such that the endpoint occurred at 375 mv.

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* Procedure used at the Manitoba Provincial Soils Testing Laboratory.

(f) calculations

$$\text{ml 1.0 N } K_2Cr_2O_7 \text{ reduced} = \frac{\text{blank} - \text{ml } Fe_2SO_4 \text{ titrated}}{\text{blank}} \times 10$$

$$\% \text{ organic matter} = \frac{(\text{ml 1.0 N } K_2Cr_2O_7 \text{ reduced})}{\text{weight of sample, g}} \times 0.67$$

$$\% \text{ organic carbon} = \% \text{ organic matter} \times .58$$

APPENDIX D

D.7 pH

A 1:1 water to soil ratio was used as noted by Jackson (1958).

Procedure:

Place 20 g of soil sample and 20 g distilled water into a 50 ml beaker. Stir for 1 hour. The pH was measured using a Fisher Model 230 pH meter. Stir prior to immersing the glass electrode.

APPENDIX E

APPENDIX E

NITROGEN ANALYSIS OF SOIL AND SOIL-MANURE MIXTURES

Nitrogen Analysis of the Control for Incubation I

Day	TKN	NH_4-N	NO_2-N	NO_3-N
	ppm			
0	2930	11.8	0.2	28.0
	2800	11.2	0.2	28.0
	2710	10.5	0.2	28.6
Average	2810	11.2	0.2	28.1
1	2460	6.2	0.4	19.8
	2990	7.6	0.4	22.2
	3070	7.5	0.2	23.4
Average	2840	7.1	0.3	21.8
2	2970	6.3	0.4	24.0
	3160	7.6	0.8	26.2
	3150	6.3	0.4	28.2
Average	3093	6.7	0.5	26.1
4	2960	8.4	0.2	29.8
	3180	13.3	0.2	25.2
	3050	10.3	0.4	43.2
Average	3063	10.7	0.3	32.7
6	2910	9.8	0.4	43.6
	3120	11.2	0.2	17.2
	3180	8.3	0.2	23.2
Average	3070	9.8	0.3	28.0
8	2770	8.3	0.2	28.8
	2610	10.4	0.2	35.8
	2830	7.0	0.2	41.2
Average	2737	8.6	0.2	35.3
16	2670	9.0	0.2	29.8
	3180	9.8	0.4	24.2
	2830	11.7	0.2	14.8
Average	2893	10.2	0.3	26.3
32	2360	16.0	1.0	17.2
	3220	12.5	1.0	1.2
	2300	18.9	0.8	10.0
Average	2627	15.8	0.9	9.5
64	3210	15.3	0.6	87.0
	2980	4.9	0.4	16.6
	2810	4.2	0.6	70.0
Average	3000	8.1	0.5	57.9

Soil Nitrogen Analysis of Control for Incubation II

Day	TKN	NH_4-N	NO_2-N	NO_3-N
	ppm			
0	3200	21.0	0.2	25.6
	2040	53.3	0.4	25.2
	2460	17.5	0.2	26.0
Average	2570	30.6	0.3	25.6
1		3.5	0.4	17.2
		1.4	0.4	14.8
		14.0	0.4	12.4
Average		6.3	0.4	14.8
2		27.8	0.4	19.6
		44.3	0.8	15.0
		44.8	0.6	16.2
Average		39.0	0.6	16.9
4		30.8	0.2	8.0
		28.0	0.0	8.2
		36.0	0.0	15.6
Average		31.6	0.1	10.6
8		39.0	0.0	16.2
		18.0	0.0	12.4
		40.5	0.2	7.0
Average		32.5	0.1	11.9
16		17.3	0.4	30.8
		25.5	0.4	22.6
		28.5	0.6	32.8
Average		23.8	0.5	28.7
32		4.5	0.2	44.8
		10.5	0.2	40.8
		7.5	0.2	33.0
Average		7.5	0.2	39.5

Nitrogen Analysis of the 112 $kgNha^{-1}$ Loading Rate for Incubation I

Day	TKN	NH_4-N	NO_2-N	NO_3-N
	ppm			
0	3250	11.9	0.2	27.0
	3160	11.9	0.2	26.4
	3050	12.2	0.4	26.4
Average	3153	12.0	0.3	26.6
1	3430	24.4	2.8	15.8
	3300	21.9	2.0	24.4
	3520	25.1	2.2	14.2
Average	3417	23.8	2.3	18.1
2	3340	25.2	0.8	12.8
	3780	31.5	1.2	15.8
	3490	22.4	2.0	15.0
Average	3537	26.4	1.3	14.5
4	3450	24.8	0.6	7.0
	3690	29.3	0.6	9.8
	2900	24.9	0.6	7.4
Average	3347	26.3	0.6	8.1
6	3780	16.0	0.2	9.8
	3820	15.3	0.2	8.8
	3190	23.5	0.2	6.2
Average	3597	18.3	0.2	8.3
8	3150	9.8	0.4	8.6
	2910	11.9	0.2	7.6
	3110	26.3	0.2	10.2
Average	3057	16.0	0.3	8.8
16	3560	7.7	0.6	3.8
	3710	1.4	0.2	2.8
	3480	5.6	0.2	3.6
Average	3583	4.9	0.3	3.4
32	3060	18.2	0.8	4.4
	3620	33.5	1.0	7.4
	3350	12.5	0.6	2.6
Average	3343	21.4	0.8	4.8
64	3760	5.6	0.4	17.0
	3800	7.0	2.6	16.4
	3560	16.7	0.4	4.0
Average	3707	9.8	1.1	12.5

Soil Nitrogen Analysis of the 112 $kgNha^{-1}$ Loading Rate for Incubation II

Day	TKN	NH_4-N	NO_2-N	NO_3-N
	ppm			
0	2430	18.0	0.2	25.8
	3170	43.5	0.4	26.0
	3140	26.3	0.4	24.8
Average	2910	29.3	0.3	25.5
1		44.8	0.4	11.0
		68.3	0.6	11.2
		42.8	0.6	12.8
Average		52.0	0.5	11.7
2		80.3	0.2	12.4
		149.3	0.2	10.0
		33.0	0.2	10.2
Average		87.5	0.2	10.9
4		39.0	0.0	7.2
		47.3	0.0	8.2
		45.8	0.0	7.0
Average		44.0	0.0	7.5
8		39.0	0.2	5.8
		19.5	0.2	15.2
		24.8	0.2	8.4
Average		27.8	0.2	9.8
16		15.8	0.4	29.4
		39.0	0.4	15.2
		13.5	0.6	23.6
Average		22.8	0.5	22.7
32		3.0	0.4	37.2
		3.0	0.4	40.8
		16.1	0.2	27.0
Average		7.4	0.3	35.0

Nitrogen Analysis of the 224 $kgNha^{-1}$ Loading Rate for Incubation I

Day	TKN	NH_4-N	NO_2-N	NO_3-N
	ppm			
0	3540	13.9	0.4	25.2
	3480	14.0	0.4	24.8
	3870	13.3	0.4	24.6
Average	3630	13.7	0.4	24.9
1	3500	33.1	5.2	13.8
	3760	30.6	1.8	13.4
	3830	40.4	3.2	10.6
Average	3697	34.7	3.4	12.6
2	3760	28.7	1.2	10.6
	3800	22.6	1.4	10.2
	3980	25.1	1.8	13.8
Average	3847	25.5	1.5	11.5
4	3320	34.7	0.6	5.8
	3720	32.0	2.6	5.4
	3750	30.6	1.2	6.2
Average	3597	32.4	1.5	5.8
6	3500	13.3	0.4	6.6
	3700	30.4	0.2	6.0
	3810	24.5	0.4	7.6
Average	3670	22.7	0.3	6.7
8	3830	23.6	0.2	5.8
	3360	11.8	0.6	7.8
	3400	25.9	0.4	6.2
Average	3530	20.4	0.4	6.6
16	4110	19.9	0.6	8.8
	4140	16.7	0.4	6.8
	4000	0.0	0.6	7.0
Average	4083	12.2	0.5	7.5
32	2900	60.1	0.8	4.4
	2920	0.0	0.6	2.8
	2810	0.0	0.8	3.6
Average	2877	20.0	0.7	3.6
64	4160	45.8	0.2	2.4
	4110	6.9	0.4	10.2
	4330	55.1	0.2	10.0
Average	4200	35.9	0.3	7.5

Soil Nitrogen Analysis of the 224 $kgNha^{-1}$ Loading Rate for Incubation II

Day	TKN	NH_4-N	NO_2-N	NO_3-N
	ppm			
0	3100	35.3	0.4	24.8
	2990	48.8	0.4	25.2
	2720	34.5	0.4	25.2
Average	2940	39.5	0.4	25.1
1		22.4	0.8	14.6
		32.3	0.8	15.0
		25.5	0.6	12.2
Average		26.7	0.7	13.9
2		36.8	0.2	10.6
		29.3	0.2	11.6
		45.8	0.0	10.2
Average		37.3	0.1	10.8
4		33.8	0.2	6.2
		47.3	0.0	7.0
		36.8	0.0	5.0
Average		39.3	0.1	6.1
8		46.2	0.2	5.4
		36.8	0.0	5.4
		47.3	0.0	6.8
Average		43.4	0.1	5.9
16		32.3	0.4	13.2
		35.3	0.4	14.4
		32.3	0.4	11.2
Average		33.3	0.4	12.9
32		15.8	0.4	7.6
		10.5	0.6	19.0
		14.3	0.4	21.8
Average		13.5	0.5	16.1

Nitrogen Analysis of the 561 kgNha^{-1} Loading Rate for Incubation I

Day	TKN	NH_4-N	NO_2-N	NO_3-N
	ppm			
0	4810	17.5	0.8	22.4
	5200	16.0	0.6	24.2
	4700	18.2	0.8	23.0
Average	4903	17.2	0.7	23.2
1	4770	47.5	1.2	9.8
	5010	18.6	0.8	10.0
	4490	42.3	1.8	10.0
Average	4757	36.1	1.3	9.9
2	4190	27.9	1.8	5.4
	4990	13.3	1.6	4.6
	4820	32.6	2.2	3.2
Average	4667	24.6	1.9	4.4
4	4410	17.4	0.6	3.0
	4470	26.7	0.8	2.8
	4580	0.0	0.8	3.8
Average	4487	14.7	0.7	3.2
6	4550	25.1	0.6	5.4
	4870	31.0	0.4	3.6
	4950	4.2	0.4	4.0
Average	4790	20.1	0.5	4.3
8	4700	0.0	0.8	2.8
	4710	0.0	0.6	2.4
	4770	0.0	0.6	2.6
Average	4727	0.0	0.7	2.6
16	4920	16.0	1.0	2.6
	4980	32.8	0.8	3.2
	4850	0.0	0.6	2.4
Average	4917	16.3	0.6	2.7
32	4550	52.4	1.2	4.8
	5060	0.0	1.6	5.2
	4930	25.2	1.4	4.6
Average	4847	25.9	1.4	4.9
64	4850	38.2	1.6	3.0
	4720	2.8	0.6	12.4
	4880	39.7	0.4	4.2
Average	4817	26.9	0.9	6.5

Soil Nitrogen Analysis of the 561 kgNha^{-1} Loading Rate for Incubation II

Day	TKN	NH_4-N	NO_2-N	NO_3-N
	ppm			
0	3400	30.0	0.0	23.2
	3580	28.5	0.2	22.6
	3440	45.0	0.2	24.4
Average	3470	34.5	0.1	23.4
1		26.3	0.2	11.8
		26.3	0.2	12.8
		31.5	0.2	10.4
Average		28.0	0.2	11.7
2		57.8	0.0	8.4
		57.0	0.0	9.0
		47.3	0.0	10.2
Average		54.0	0.0	9.2
4		60.2	0.0	8.4
		69.8	0.0	9.0
		58.5	0.0	8.4
Average		62.8	0.0	8.6
8		51.0	0.0	8.2
		56.3	0.0	7.8
		52.3	0.0	7.4
Average		53.2	0.0	7.8
16		78.0	0.4	9.0
		58.5	0.4	8.6
		72.8	0.4	9.0
Average		69.8	0.4	8.9
32		47.3	0.2	10.2
		47.3	0.4	9.0
		24.0	0.2	6.8
Average		39.5	0.3	8.7