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THE EFFECT OF CROP ROTATION SEQUENCE ON ORGANIC
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THE EFFECT OF CROP ROTATION SEQUENCE ON ORGANIC CARBON AND NITROGEN LEVELS OF A CHARITY CLAY

By

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ABSTRACT

THE EFFECT OF CROP ROTATION SEQUENCE ON ORGANIC CARBON AND NITROGEN LEVELS OF A CHARITY CLAY

By

Richard Cleveland Zielke

The objective of this work was to evaluate changes in soil organic C and N and their effect on N mineralization characteristics of six crop rotation sequences. The six rotation sequences consisted of combinations of corn (*Zea mays* L.), oats (*Avena sativa* L.), alfalfa (*Medicago sativa* L.), sugarbeets (*Beta vulgaris* L.) and navy beans (*Phaseolus vulgaris* L.). After 9 years of cropping there were significant differences between sequences in organic C and N losses, mineralizable N and N uptake in a greenhouse study. These differences were attributed to differences in organic matter return rates. Losses ranged from 0.180 % C (4030 kg C/ha) and 0.019 % N (417 kg N/ha) for the navy bean-sugarbeet rotation, to 0.053 % C (1200 kg C/ha) and 0.0066 % N (148 kg N/ha) for the corn-corn-corn-sugarbeet rotation. Nitrogen mineralization estimates were linearly correlated with soil C and N levels but were not linearly related to sugarbeet yield parameters measured in the field or uptake of N measured in the greenhouse.

DEDICATION

To Betty

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TABLE OF CONTENTS

LIST OF TABLES

Table

Table

INTRODUCTION

The contributions of soil organic matter to the productivity of mineral soils have long been documented and observed. The impetus for this research is based on two such observations. First and foremost, long term organic C and N changes may be culturally induced. Secondly, environmental and economic concerns arising from over-fertilization with N have resulted in a need to more accurately predict the amount of N that can be supplied through the decomposition of soil organic matter. For these reasons there has been a resurgence in research directed at developing management practices which conserve and restore soil organic matter. One such practice is the sequence in which crops are produced.

Numerous investigators have reported large losses of organic C and N as a result of long term cropping when compared to losses from virgin soils of the same type, location and properties. Salter and Green (1933) demonstrated that soil organic matter transformations follow first order kinetics (i.e. the rate of transformation is proportional to the initial concentration), and more importantly that organic matter losses can be minimized under different cropping systems. Determination of the decomposability of different crop residues via addition of isotopically labeled plant materials has helped to elucidate the nutrient dynamics of humus formation and degradation.

Unfortunately, certain problems are inherent in the determination of organic matter content of soils. Distinctions between humified and non-humified fractions in the soil are not obtained by ordinary analysis. On the reliability of the oxidation of organic matter by chromic acid, Walkley (1946) stated, "Evidently there are many who have rated convenience and speed more highly than accuracy". Fractionation itself does not produce absolute values, however the quantitative nature of these fractions may be ascertained if uniformity of procedure is maintained. Thus the term "organic matter" which appears in the literature is somewhat obscure and relative to the method of determination. For purposes of this discussion, the term "organic C" is used and is defined as the amount of organically bound C that is oxidized by the method of Walkley-Black. It is for the above reasons that many investigations (the present one included) have focused on the transformations of organic N, an organic matter constituent whose concentration in the soil can be more accurately determined.

Nitrogen, the nutrient found in highest concentration in most plants occurs in surface soils primarily in organically bound forms. Little if any organic N is assimilated by plants and uptake is predominantly in the NH_4^+ and NO_3^- forms. Excluding biological N fixation, non-fertilizer N is made available to plants only through the microbial oxidation of soil organic matter. Such a process is called N mineralization, but because of the biological and chemical dynamics of N in the soil system, the term "N mineralization" as used in this discussion will refer to the net amount of N mineralized over time.

With these definitions and framework in mind, the principles and assumptions behind this research may be stated. Few attempts have been made at evaluating the effect of crop rotation sequence on the N mineralization characteristics of soils where differences in added residue are observably small. This is such an attempt, and it is based upon the premise that crop rotation sequences which return a larger amount of crop residue to the soil will result in higher concentrations of organic C and N. The equilibrium concentration will be a function of the nature and amount of residue returned to the soil if all other factors affecting decomposition or loss are constant. Furthermore, since N mineralization and organic matter decomposition are assumed to follow first order kinetics, it is reasonable to expect that soils under different sequences for long periods of time will have variable potentials to mineralize N.

The specific objectives of this research are as follows: (1) determine the magnitude and direction of change of organic C and N levels of a Charity clay after 9 years of cropping in specific rotation sequences which are assumed to differ in the quantity and quality of crop residue returned; (2) evaluate the effect of crop rotation sequence on soil N availability as measured by selected N availability indices; (3) test the hypothesis that sugar yields may eventually be affected through correlation of N availability indices with sugarbeet yield and quality parameters observed in the field and uptake of N as observed in the greenhouse.

LITERATURE REVIEW

Effect of Cropping on Carbon and Nitrogen Levels

Early investigations of the loss of organic C and N due to cropping were primarily limited to observing the magnitude under different systems and attempting to describe the kinetics of such losses. Salter and Green (1933) recognized that the nature and amount of crop produced had an effect on the rate of N loss, and attempted to differentiate between the effects due to cultural practices and effects due to the amount of residue returned. They observed that organic C and N losses were greatest under continuous corn, less under continuous wheat and oats and least for 5 and 3 year rotations that contained legumes. Based upon the assumption that N loss was proportional to the total N content of the soil, they derived the following equation to describe N loss:

$$N = N_o K^t \qquad (1)$$

where K is the amount of N remaining after one year.

If K = (1-r), equation (1) can be rearranged to:

$$N = N_o(1-r)^t \qquad (2)$$

where N = N remaining after t years, N_o = initial N content and r = the annual rate of N loss (decomposition rate constant).

The differential and integral forms of equation (2) give rise to equations (3) and (4):

$$dN/dt = -rN \qquad (3)$$

$$N = N_o \exp -rt \tag{4}$$

Jenny (1933) investigated the loss of N from midwest, humid region soils and found N losses during the first, second and third 20 year periods of cultivation to be 25, 10 and 7 %, respectively. Myer et al. (1943) showed that losses from dry region soils also followed first order kinetics. Larson et al. (1972) and Rasmussen et al. (1980) confirmed the validity of Salter and Greens assumption for both gains and losses of C and N. Jenny (1941) further pointed out that equation (4) predicted total loss of soil N as t became very large. Based on his observations, he concluded that soil N levels would not decline to zero but to some equilibrium level, therefore he modified equation (4) with a factor (A) to account for additions of N. The resulting differential equation is:

$$dN/dt = A - rN \tag{5}$$

Integration of this equation yields:

$$N = N_o(\exp -rt) + A/r(1 - \exp -rt) \tag{6}$$

Woodruff (1949) expanded equation (6) to account for the presence of organic matter constituents which decomposed at different rates, therefore yielding:

$$N = N_1(\exp -r_1t) + A/r_1(1 - \exp -r_1t) + N_2\ldots \tag{7}$$

Using the integrated form of Jenny's equation and graphical methods, Bartholomew and Kirkham (1960) calculated the equilibrium concentration of N expected for the Morrow plots in Illinois and the Sanborn field in Missouri. They assumed (unlike Woodruff) that all fractions of organic matter were equally susceptable to decomposition and that annual additions from residues and other sources were constant. Furthermore, they calculated the equilibrium time to be 50 to 100 years and concluded that the rapidity of equilibrium was wholly dependent upon the rate constant (r). The equation accurately predicted the N levels expected despite the questionable validity of their assumptions.

Russell (1975) subjected data for the Sanborn and Morrow studies to computer based, mathematical procedures to demonstrate that increased yields can have a "feedback effect" on the equilibrium level of N due to the increased residue production, therefore demonstrating that (A) in equation (5) is not a constant. His results indicated that increasing crop yield of continuous corn on the Morrow plots had little effect on soil N although small increases were observed for yield increases in oats and alfalfa. At the Sanborn site, oats had the largest feedback while alfalfa had the least. Larson et al. (1972) and Bloom et al. (1980) conducted actual short term field experiments and found that the feedback effect significantly affected soil N levels of continuous corn plots.

With the kinetics of C and N loss well established, more recent research has been directed at conserving organic C and N through specific cultural practices. Many researchers reported that inclusion of a sod crop into a rotation functioned to raise soil N levels (Jackman, 1964; Clement and Williams, 1967; Giddens et al., 1971; and White et al., 1976). Greenland (1971) reported that the value of (A) in equation (5) exceeded (rN) under pasture conditions, thus total N increased. During cropping however, (A) was small and N levels decreased. The N levels tended to cluster about a mean value when several pasture-cultivated crop cycles were repeated.

Other investigators looked at manuring as a means of maintaining or increasing soil C and N levels. Hobbs and Brown (1965) estimated that addition of 56 to 67 metric T manure/ha every three years would prevent loss of organic matter from Kansas soils. McIntosh and Varney (1972) studied the effects of manuring on continuous corn and reported that N levels of plots receiving no manure declined by 8.7 % in five years. They concluded that an annual addition of 44 metric T manure/ha was required to maintain organic matter levels. Anderson and Peterson (1973) observed increases in soil C and N by applying 27 metric T manure/ha/yr. Jenkinson and Johnson (1977) observed that application of 35 metric T manure/ha/yr to continuous barley plots at the Rothamstead Experiment Station resulted in large increases in soil C and N over a period of 123 years. At this particular site, non-manured plots showed a gradual decrease in soil N, indicating that equilibrium was nearly established. Rasmussen et al. (1980) studied the long term effects of crop residue on C and N levels of a wheat-fallow system in Oregon. They concluded that 22.4 metric T manure/ha added to straw residue prevented C loss and predicted that 5 metric T mature crop residue/ha/yr was required to maintain soil C levels.

Other researchers recognized the problems associated with animal waste management and use in cash crop operations and looked at the effects of the more conventional practice of incorporating crop residue on C and N levels. Hedlin et al. (1957) observed that returning the residue of cereal crops increased C and N levels but reduced the yield of subsequent grain crops, apparently because of N immobilization. Sanford (1968) found that incorporating corn stover in the spring reduced yields while fall incorporation followed by spring fertilization with N not only increased yields but increased soil C and N levels as well. Morachan et al. (1972) also reported decreased corn yields when corn stover was applied, however this was attributed to a K-induced Ca deficiency. In an 11 year study, Larson et al. (1972) investigated the effects of increasing organic residues on continuous corn plots and predicted that 6 metric T corn stalks/ha/yr were required to prevent loss of soil C. Applications of corn stalks ranging from 0 to 16 metric T/ha/ yr raised organic C and N levels of the soil. Linear changes were observed only in the top 15 cm, although a nonsignificant trend toward increasing C and N was observed at deeper depths. Black (1973) studied the effect of increasing straw residue on wheat-fallow rotations on a sandy loam. After four crop-fallow cycles, addition of large amounts of wheat straw resulted in increased organic matter levels but again only in the top 15 cm. He concluded, "Changes in the chemical and soil properties measured were proportional to the quantity of crop residue added over time".

In a ten year study on a typic haplaudalf, Ketcheson and Beauchamp (1972) compared the effects of removing vs incorporating corn stover on yield and organic matter levels of continuous corn plots. Incorporating corn stover functioned to reduce grain production and dry matter yield, but increased soil organic matter when compared to removing the residue. Barber (1979) studied the effect of removing crop residue, returning the residue produced and returning double the amount of corn residue produced on Indiana soils over a ten year period. When the single crop residues were returned, the organic matter content was 0.3 % higher than plots receiving no residue, and 0.31 % lower than organic matter levels of plots receiving double residues.

Based on the above evidence, there can be little doubt that cultural and management practices affect the kinetics and equilibrium levels of soil organic matter. The assumption in the present study is that management practices have remained constant and that gains or losses are the result of crop residue factors. In recent reviews, Stevenson (1982), Campbell (1978), Parr and Papendick (1978) and Allison (1973) all support the idea that the rate of decomposition of an organic residue depends upon the nature of the residue and on factors affecting the soil environment. Soil factors are ones which affect microbial populations and are confined to water content, temperature, pH, aeration, and available nutrients (Alexander, 1977). Residue factors include chemical composition, C/N ratio, lignin content, age of plant, fineness of residue, method, rate and depth of incorporation, and associated microflora (Parr and Papendick, 1978).

Estimating Potentially Mineralizable Soil Nitrogen

Environmental and economic concerns resulting from over-fertilization with N are the primary reasons that a reliable method of estimating potentially mineralizable N is needed. Nitrogen fertilizer is no longer inexpensive, and it is now well known that sugarbeet quality is reduced as a result of excess fertilizer and/or residual N (Carter and Traveller, 1981; Hills and Ulrich, 1971). Furthermore, over-fertiliztion can result in contamination of ground water (Hills et al., 1978), and is suspect in the increased occurence of biological denitrification which is important in the catalytic destruction of statospheric ozone (Mosier and Hutchinson, 1981; Crutzen, 1970).

Many of the earlier methods for estimating mineralizable N in soil were reviewed by Brown (1916). Such tests were called nitrification tests up to 1955 when Harmsen and Van Schreven (1955) argued that these estimates were empirical in nature and therefore represent the "potential" or "relative" N supplying power of the soil. For purposes of this discussion, potentially mineralizable N is a measure of extractability of soil N under imposed experimental conditions.

Several investigations support the concept of a definable pool of potentially mineralizable N as opposed to the idea that all soil N is readily mineralized. The equilibrium concept of Jenny sparked a great deal of interest in the decomposability of different fractions of organic matter. Henin and Dupis (1945) suggested that the non-humified fraction of organic matter was the most active fraction, and Henin and Turk (1949) developed a method to separate low density, non-humified materials from soil by floating them on heavy liquids. Greenland and Ford (1964) and Ford et al. (1969) perfected a method to separate this light fraction by ultrasonic dispersion in halogenated hydrocarbons. Greenland (1971) reported that this light fraction comprised 7-23 % of the total N in the soil, yet accounted for 25-60 % of the N mineralized in laboratory incubations.

Concurrently, a great deal of work was being conducted on the rate of decomposition of isotopically labeled plant material in soils (Broadbent and Bartholomew, 1948; Jenkinson, 1964; Legg et al., 1971; and Broadbent and Kakashima, 1974). Jenkinson (1965) observed that a rapid flush of labeled C occurs when a labled substrate is added to soil, but that this flush falls off quickly. He further reported that "four years later, the labeled organic C still mineralizes several times faster than the soil organic C as a whole". The use of ^{14}C dating of humic and other organic fractions confirms the existence of increments which mineralize at markedly different rates (Jenkinson and Rayner, 1977). Results of such studies leave no question that different organic fractions can supply mineral N at different rates, and have helped to elucidate even the biochemical mechanisms of mineralization and immobilization of soil N (Ladd and Jackson, 1982), yet the dilemma of developing a reliable test to quantify the potentially mineralizable soil N still persists.

Bremner (1965a) discussed the advantages and disadvantages of many chemical and biological methods. Incubation (biological) methods appear to be the most useful since N mineralization is biologically regulated, however these methods are tedious, expensive, and subject to variation due to sample pretreatment. Chemical methods are highly empirical, yet relatively inexpensive and unaffected by sample pretreatment. The accuracy of any method is measured by the magnitude of the correlation coefficient between uptake of N in some vegetative test and laboratory results, or correlation with previously established methods.

Incubation techniques have received the most attention. Two methods which show the greatest potential for success are the aerobic method of Bremner (1965a), and the anaerobic method proposed by Waring and Bremner (1964). The aerobic method is used in the present investigation and is described in a later section. The anaerobic (waterlogged) method consists of measuring the amount of NH_4^+-N mineralized after incubation at 40 C for 7 days. Keeney and Bremner (1966) established that anaerobic incubations at 40 C were better correlated with uptake of N by ryegrass (*Lolium perenne* L.) than at 30 C. Robinson (1967) modified the anaerobic method to allow for steam distillation of a filtered extract so as to prevent the alkaline hydrolysis of soil organic matter. Several investigators have since compared the correlation coefficients of these methods with uptake of N in the greenhouse. (Hanway and Ozus, 1966; Kadirgamathoiyah and MacKenzie, 1970; Cornforth, 1968; Smith, 1966; Ryan et al., 1971; Gasser and Kalembasa, 1976; and Geist, 1977). See Table 1 for a summary of the magnitude of these correlations.

Smith and Stanford (1971) modified the method of Robinson (1967) by removing initial mineral N with 0.01 M $CaCl_2$ followed by incubation of the soil in a saturated "minus N" nutrient solution at 35 C for 14 and 28 days. The NH_4^+ released was recovered by a series of centrifugations and washings. Correlation between results obtained after 4 week incubations by the above method and the aerobic method was higher ($r = 0.93$) than the correlation obtained in 2 week incubations ($r = 0.86$). These results indicate that long term incubations are a better indication of potentially mineralizable N since results obtained the first two weeks may be affected by the presence of recent crop residues of varying C/N ratio. Chickester et al. (1975) confirmed that the presence of crop residue with varying C/N ratios affects the degree of mineralization observed in short term incubations.

Stanford and Smith (1972) devised a long term method based on 30 week incubations from which the term N mineralization potential has materialized. The method involves incubation of 1:1, sand:soil mixtures at 35 C followed by periodic leaching with 0.01 M $CaCl_2$ to remove the mineralized N. Nitrogen mineralization potential (N_{mp}) is defined as the amount of soil N that is susceptable to mineralization according to first order kinetics. Eventually N_{mp} is calculated from the first order equation:

Table 1. Simple correlation coefficients obtained in studies comparing aerobic[1] and anaerobic[2] incubation methods with uptake of N in the greenhouse.

Plant species (tops only)	Aerobic	Anaerobic	Reference
	Correlation coefficient (r)		
Corn (*Zea mays* L.)	0.862^{**}	0.818^{**}	Cornforth (1968)
Ryegrass (*Lolium perenne* L.)	0.93^{**}	0.93^{**}	Gasser and Kalembasa (1976)
Orchard grass (*Dactylis glomerata* L.)	----	0.907^{**}	Geist (1977)
Ryegrass	0.57^{*}	0.85^{**}	Hanway and Ozus (1966)
Sudan-sorghum hybrid	0.700^{**}	0.805^{*}	Kadirgamathayah and MacKenzie (1970)
Sorghum (*Sorghum bicolor* L.)	0.86^{**}	0.84^{**}	Ryan (1971)
Orchard grass	0.690^{**}	0.351^{**}	Smith (1966)

[1] Bremner (1965a)

[2] Waring and Bremner (1964)

*,**Significantly correlated at alpha = 0.05, 0.01 level respectively.

$\log(N_{mp}-N_t) = \log N_{mp} - kt/2.303$ (8)

where N_t = cumulative amount of N mineralized at time t, and k = the mineralization rate constant.

The first estimates of N_{mp} were obtained using the equation:

$1/N_t = 1/N_{mp} + b/t$ (9)

where b = slope.

Therefore N_{mp} could be obtained by finding the reciprocal of the intercept of the regression line obtained from a plot of $1/N_t$ vs 1/t for each individual soil.

Next, $(N_{mp}-N_t)$ was calculated using the value of N_{mp} obtained in equation (9) and the N_t observed. Plotting $(N_{mp}-N_t)$ vs t on semilog paper produced a concave up curve if N_{mp} was smaller than, concave down curve if N_{mp} was larger than, or a straight line if N_{mp} was equal to the N_{mp} denoting best linear fit. The N_{mp} denoting best linear fit was finally obtained using an iterative process involving successive evaluation of $(N_{mp}-N_t)$ vs t. The mineralization rate constant (k) was evaluated from equation (8) whereby the slope of a plot of $\log(N_{mp}-N_t)$ vs t is equal to k/2.303, and hence k = 2.303 x slope. Stanford concluded the pool of potentially mineralizable N was similar based on the observation that the rate constants observed for many different soils were statistically poolable. The average rate constant obtained for 39 diverse soils was 0.054 weeks^{-1}. As can be seen, the mathematical method for estimating N_{mp} is tedious. The chemical determinations and extractions are equally laborious, however recent modifications by MacKay and Carefoot (1981) allow for incubation of the sample in a 0.2 um Falcon disposable filter unit which greatly simplifies the extraction and moisture equilibration processes.

Stanford et al. (1973b) conducted greenhouse experiments on soil N availability using labeled plant materials and concluded that N_{mp} may have use in predicting N mineralized under varying field and greenhouse conditions. These conclusions were also based on work that established moisture and temperature coefficients for optimum N mineralization (Stanford et al., 1973a; Stanford and Epstein, 1974). Stanford et al. (1974) attempted to develop a short term (8 week) incubation method using the same procedure described above. Thus N_{mp} could be calculated using the following regression equation and the pooled rate constant ($k = 0.054\ wk^{-1}$ @ 35 C):

$$N_{mp} = N_t/(1-10^{-kt/2.303}) \qquad (10)$$

Since their development, the short and long term methods have received a considerable amount of attention, primarily for purposes of establishing their validity under field and greenhouse conditions. Verstraete et al. (1976) investigated the use of N_{mp} in predicting N mineralized as a function of crop, organic amendment, and liming variables. His conclusion was that N_{mp} was not useful in detecting differences in N mineralized due to the above factors but also added "lack of sensitivity and/or accuracy to differentiate in a statistically significant way the impact of the experimental variants examined is not surprising in view of the relatively small differences in soil characteristics between the various variants".

Smith et al.(1977) reported that the correlation of N_{mp} with estimates of N mineralized under modified field conditions using a buried polyethylene bag technique was quite high ($r = 0.92$). Westerman and Crothers (1980) also used the buried polyethylene bag technique to determine N mineralized under field conditions. They reported that correlation between predicted N uptake and measured N uptake by corn was highly significant ($r = 0.98$) and further concluded that the buried bag technique was as useful as N_{mp} in predicting N mineralized in a single growing season or several growing seasons if cropping practices do not change significantly. Hsieh et al. (1981) used the method of Stanford (1972) to estimate the possible N pollution potential associated with sewage sludge decomposition in soil. Marion et al. (1981) used the N_{mp} method to predict N mineralized by Chaparall soils. He also reported that the method has potential in predicting N mineralization in terrestrial ecosystems. Keeney (1981) discussed the problems associated with estimating N_{mp} in forest ecosystems.

Recently, the assumption that N mineralization follows first order kinetics has come under criticism. One such criticism is that a significant amount of soluble organic N is removed during leaching and thus is ignored in estimating N_{mp} if only mineral N is determined (Smith et al., 1980; Legg et al., 1971; Broadbent and Nakashima, 1971). Other criticisms exist (Talpaz et al., 1981; Molina et al., 1980; Stanford et al., 1980), but they are difficult to verify because of the complex and obscure mathematical principles involved. Probably the most important criticism is that most researchers air dry the soil before incubation, a process which tends to retard the soil biological activity for some time. It would seem that these estimates would be more meaningful if the soil used were maintained more closely to "steady state" field conditions. The obvious problem with this concept however is that heterogeneity of subsamples (particularly in the fine textured soils) increases drastically with field moist soils. Still the method of Stanford and Smith (1972) has become quite popular primarily because of the potential for its use in kinetic and quantitative (as opposed to relative) measurement of N availability. Correlation studies of N_{mp} with uptake of N in greenhouse and field experiments are still neccesary to better establish the validity of N_{mp}.

There are an enormous number of proposed chemical indices of soil N availability. These methods have been reviewed by Bremner (1965a), Jenkinson (1968), Danke and Vasey (1973), Cambell (1978) and Stanford (1982). The disadvantage of chemical methods is that they can in no way simulate the microbial considerations that are involved in N mineralization. Their use lies primarily in demonstrating a high degree of correlation with previously established methods or with uptake of N in the field or greenhouse. One method which meets these criteria is the autoclavable NH_4^+ method of Stanford and Demar (1970). Thus the following dicussion will be limited to the development of the autoclavable NH_4^+ method as an index of N availability.

Stanford and Demar (1969) compared the autoclavable method with the anaerobic method of Waring and Bremner (1964) and obtained a correlation coefficient $r = 0.94$. Smith and Stanford (1971) reported that the correlation between NH_4^+ released upon autoclaving and N mineralized by the aerobic and anaerobic methods previously discussed were quite different ($r = 0.70$ and 0.92 respectively). Smith et al. (1977) reported significant correlation of field estimates of N_{mp} with autoclavable NH_4^+ ($r = 0.86$). Stanford and Smith (1976) were the first to attempt to estimate N_{mp} from a chemical index of N availability (autoclavable NH_4^+) for a large number of U.S. surface soils. Excepting a few calcareous western soils, N_{mp} could be estimated fairly accurately. The following equation is the pooled regression equation for 275 diverse soils:

$$N_{mp} = 4.1\ N_i + 6.6 \qquad (11)$$

where N_i = NH_4^+-N released upon autoclaving at 121 C for 16 hrs. The correlation between N_{mp} and N_i was high (r = 0.92) indicating that the method does indeed have potential for use in predicting N_{mp}. The reason for the lack of fit for the western calcareous soils is unknown.

More recently, Fox and Piekielek (1978) reported significant correlation coefficients (r = 0.92) resulting between autoclavable NH_4^+ and soil N supplying capacity ([total plant N - (.75 x amount of starter N added)]) under field conditions.

The autoclavable NH_4^+ method has been shown to be well correlated with all three biological methods previously mentioned. The significance of this correlation is that N_i is easier and cheaper to determine therefore lending itself to adoption by soil testing laboratories for routine use.

MATERIALS AND METHODS

General Overview

The objectives of these investigations have been mentioned. In the spring of 1981 a soil sample consisting of 20 probes per plot was collected from a long term rotation experiment and chemical analyses for organic C and N were conducted. These values were compared to values obtained from corresponding 1972 soil samples. The soils at the field site are very uniform and it was assumed that any changes in organic C and N level resulted from differences in factors such as the amount of crop residue produced and its associated decomposability, therefore no biomass estimates were obtained. If the foregoing assumption is valid, it is reasonable to expect that differences in N availability will eventually result. Therefore N availability estimates were obtained and correlated with sugarbeet parameters. Sugarbeets were chosen as an "indicator crop" because of their sensitivy to excess mineral N. Results obtained the first year suggested that some significant differences existed and therefore warranted a more in-depth study of the N status of these soils as affected by rotation sequence.

In the fall of 1981 a soil sample consisting of 80 probes per plot was collected from plots that would be planted in sugarbeets the following spring. Chemical determinations were repeated and compared to values from corresponding plots sampled in 1972. Since sugarbeet parameters would not be available the second year, a detailed investigation of the relationship between uptake of N in the greenhouse and estimates of potentially available soil N (including N_{mp}) was undertaken. The following sections describe specific procedures that were used in these investigations.

Field Experiment

A field study was conducted at the Saginaw Valley Bean and Beet Research Farm in 1981 to determine the magnitude and direction of change of organic C and N levels of the soil due to rotation sequences which differ in crop residue return rates and to determine if these changes had a significant effect on yield and quality of sugarbeets. The study was composed of rotation sequences which were selected from a larger, long term experiment that was initiated in 1972, and was arranged as a randomized complete block with six treatments and four replications. Each experimental unit measured 20.13 by 5.69 m, comprising a total area of 1.15×10^{-2} ha. Sequences selected include combinations of corn (*Zea mays* L.), oats (*Avena sativa* L.), alfalfa (*Medicago sativa* L.), sugarbeets (*Beta vulgaris* L.), and navy beans (*Phaseolus vulgaris* L.). The six treatments were as follows; (1) corn-sugarbeets (C-B); (2) navy beans-sugarbeets (Be-B); (3) corn-corn-corn-sugarbeets (C-C-C-B); (4) corn-corn-navy beans-sugarbeets (C-C-Be-B); (5) oats-navy beans-sugarbeets (O-Be-B); and (6) oats-alfalfa-navy beans-sugarbeets (O-A-Be-B).

Tillage practices remained constant since 1972, and consisted of fall plowing and harrowing before planting in the spring using a spring tooth-spike tooth harrow combination. Fertilizer applied in a band below and to the side of the seed in 1981 consisted of 336 kg 18-46-0 plus 1% B and 3% Mn/ha. The navy bean-sugarbeet sequence was sidedressed with an additional 28 kg N/ha in June. Nitrogen fertilizer applied to plots sampled in the spring of 1981 since the initiation of the experiment can be found in Table 2. Potassium fertilization was unnecessary since soil test K levels were sufficient (500 kg exchangeable K/ha). Sugarbeets (variety US H20) were planted at a 71 cm row spacing on April 22 and were thinned to 20 cm between plants approximately five weeks after planting. Weeds were controlled by a preemergence application of 6.72 kg trichloro-acetic acid (TCA) and 4.48 kg 5-amino-4-chloro-2-phenyl-3(2H)-pyridazmone (Pyrimin)/ha. On October 24, the sugarbeets were topped with a beater/topper and mechanically harvested. Yields were calculated by weighing the beets harvested from two 20.13 m rows. Ten average sized sugarbeets were selected from each plot, mechanically sliced, the pulp hand squeezed and the resulting juice was sent to Michigan Sugar Company where determinations of clear juice purity, recoverable sucrose and alpha-amino N were conducted according to the methods described by Dexter et al. (1967), Caruthers and Oldfield (1961), and Moore and Stein (1954), respectively.

Sample Collection and Preparation

All soils used in these investigations were obtained from the Saginaw Valley Bean and Beet Research Farm located in Swan Creek Township, Saginaw County, Michigan. There were three sampling dates; the summer of 1972 when the larger study was initiated; the spring of 1981 from plots that were planted in sugarbeets in 1981 (designated Sp 1981); and the fall of 1981 from plots that were planted in sugarbeets in 1982 (designated F 1981). All soils were sampled to a depth of 20 cm

Table 2. Crops grown and N applied to crop rotation sequences since 1972[1].

	C-B		Be-B		C-C-C-B		C-C-Be-B		O-Be-B		O-A-Be-B	
Year	Crop	N	Crop	N	Crop	N	Crop	N	Crop	N	Crop	N
		kg/ha		kg/ha		kg/ha		kg/ha		kg/ha		kg/ha
1972	C	150	Be	30	C	150	Be	30	B	60	Be	30
1973	B	50	B	84	B	50	B	50	O	56	B	50
1974	C	224	Be	28	C	224	C	224	Be	28	O	56
1975	B	50	B	84	C	168	C	140	B	50	A	0
1976	C	224	Be	28	C	106	Be	28	O	56	Be	28
1977	B	56	B	84	B	56	B	56	Be	28	B	56
1978	C	224	Be	28	C	168	C	168	B	56	O	56
1979	B	56	B	84	C	168	C	168	O	56	A	0
1980	C	224	Be	28	C	168	Be	28	Be	28	Be	28
1981	B	56	B	84	B	56	B	56	B	56	B	56

[1] Each crop in each sequence is grown each year. The above table corresponds to research plots that were sampled in the spring of 1981.

C = Corn, Be = Navy beans, B = Sugarbeets, O = Oats, A = Alfalfa and consisted of 20 probes per plot with the exception of the fall 1981 sampling which consisted of 80 probes per plot. Samples collected in the field were air dried for 72 hours, ground in a hammer mill until the entire sample passed a 20 mesh sieve and stored in airtight containers until chemical analysis and laboratory experiments could be conducted. Soils used for total N and dry combustion C determinations were subsampled and ground in a Spex Mixer/Mill until they passed a 100 mesh sieve.

General Chemical and Mineralogical Properties

The soil used in this study is tentatively classified as Aeric, Haplaquept, fine, illitic (calcareous) mesic (Charity clay)[1], with 15% sand, 39.3% silt and 45.7% clay. Textural analysis was determined by a modified hydrometer method after reducing the soluble salt content with acidified sodium acetate followed by removal of easily oxidizable organic matter with hydrogen peroxide (Day, 1965). Preparation and X-ray diffraction of an oriented aggregate was conducted according to methods described by Kunze (1965) and Whittig (1965) after each of three consecutive treatments; glyceration and Mg saturation; saturation with 1 N KCl followed by air drying; and heating the K saturated sample to 500 C. The clay fraction was found to contain a large amount of vermiculite and smaller amounts of montmorillonite, chlorite, illite, kaolinite and quartz. The relative abundance of each of the various minerals (particularly vermiculite), was determined from information on the diffractogram as well as from differences in estimates of the cation

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[1] Personal communication, Dr. E.P. Whiteside, Professor Emeritus, Crop and Soil Science Department, Michigan State University.

exchange capacity (CEC) of the purified clay sample obtained in Ca/Mg and K/NH_4 matrices.

The CEC of the soil was estimated to be 290 cmmol (p^+)/kg soil and was determined by saturating 2 g soil with neutral NH_4OAc followed by three washings and centrifugations to remove excess NH_4^+ in solution. Ammonium on the CEC was determined by alkaline steam distillation into H_3BO_3 and titration with standardized H_2SO_4.[2] Soil pH was 7.9 and was measured on a 1:1, soil:water suspension using a glass electrode.

Greenhouse Experiment

A greenhouse experiment using soils samples collected in the fall of 1981 was conducted to investigate the relationship between estimates of potentially available soil N and uptake of N at three levels of N fertility, and to determine if future sugarbeet yields would be affected by culturally induced soil N changes. The experimental design consisted of a 3 x 6 factorial (arranged as a ramdomized complete block) with three levels of N fertility (0, 37.5 and 75 ppm N), six rotation sequences, and four replications, giving a total of 72 separate experimental units. Three consecutive crops were grown; oats (var. Garry), corn (var. Pioneer 3780) and oats, respectively. Before each growth period, each block was rerandomized and placed under high intensity discharge lights in the greenhouse. Daytime temperatures ranged from 20 to 29 C over the entire experiment. Night temperatures were constant at 20 C and the photoperiod was 16 hours per day. Moisture was periodically adjusted to 20 % (w/w) by gravimetric means.

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[2] Unpublished mimeo, D.D. Warncke, Crop and Soil Science Department, Michigan State University.

Initially, the potting and fertilization procedure was as follows. Twelve hundred fifty g air dried soil (20 mesh) was deposited in tared pots with plastic liners. In order to ensure that N was the only growth limiting nutrient, each pot received applications of 63 and 50 ppm K and P respectively by the addition of an aqueous solution of KH_2PO_4. The moisture content was adjusted and the pots were allowed to stand unplanted for three days. At the end of this period the moist soil was screened through a 4 mesh sieve, carefully mixed and returned to the pot for planting. One week after planting of the first crop, each pot received a predetermined amount of N fertilizer as an aqueous solution of $Ca(NO_3)_2 \cdot 4\ H_2O$. No additional N, P, or K fertilizer was applied in the second or third cropping periods.

When oats were grown, thirty seeds were planted to a depth of 1 cm and thinned to 25 plants per pot upon emergence. Five corn seeds were planted and thinned to 3 plants per pot upon emergence. At the end of five weeks the above ground portion was harvested by clipping at the soil surface. The moist soil was then sieved through a 4 mesh screen and 50 g were removed for chemical analysis.

All soils sampled in the greenhouse experiment were rapidly air dried, ground in a mortar and pestle to pass a 20 mesh sieve, carefully mixed, extracted in a 10:1 v/w ratio of 2 $\underline{N}$ KCl and analyzed for NO_3^- and NH_4^+-N by the automated methods discussed in the section on laboratory analyses. Results obtained were reported as ppm NO_3^- and NH_4^+-N in air dry soil.

All plant samples were dried in an oven at 60 C for 48 hours, after which time dry weights were obtained and the samples were ground to pass a 40 mesh sieve in a Wiley Mill. Total N in the plant tissue

was determined by the micro-Kjeldahl method.

Laboratory Analyses and Experiments

Organic carbon determinations

Organic C content of the soil was estimated by two methods; Walkley-Black wet combustion with chromic acid (Walkley and Black, 1934; Walkley, 1935, 1947), and dry combustion using a Leco 70-second C analyzer (Tabatabai and Bremner, 1970). The dry combustion method was used only on the 1981 spring and corresponding 1972 samples.

In the Walkley-Black method, 10 ml 1 N $K_2Cr_2O_7$ was added to 1.5 g soil (20 mesh) in a 500 ml Erlenmeyer flask followed by the addition of 20 ml 36 N H_2SO_4. The sample was allowed to digest and cool for 30 minutes. To this mixture, 170 ml distilled water and 3 drops of Ferroin (1,10-phenantroline ferrous sulfate) were added, and the sample was titrated to a maroon endpoint using 0.5 N $Fe(NH_4)_2(SO_4)_2$. Results of duplicate determinations are reported as % organic C in air dry soil (% moisture = 4.60 w/w).

Estimates of organic C by the dry combustion method were obtained by weighing 100 mg soil (100 mesh) into a combustion crucible and adding three 1 ml aliquots of 5% H_2SO_3 to destroy carbonate C (Allison, 1965). The sample was allowed to stand overnight after which time the crucible was dried in an evacuated desiccator containing NaOH pellets. The sample was then analyzed for C by combusting in a Leco 70-second C analyzer. The mechanism for this process is described in detail by Belo (1970) and Tabatabai and Bremner (1970), but basically consists of two steps: first the sample is combusted in a high temperature, high frequency induction furnace, and secondly, the amount of CO_2 evolved is detected by a thermocouple which allows the C content of the sample to be read directly from the instrument. Results obtained for duplicate determinations of each sample are reported as % organic C in air dry soil.

Total nitrogen determinations

Total N content (not including NO_3^-) of the soil was determined by the micro-Kjeldahl method described by Bremner (1965b). For soils, two ml distilled water was added to 400 mg soil (100 mesh) in a 100 ml Kjeldahl flask and allowed to stand for 30 minutes. Each flask received 1.1 g catalyst mixture (100:10:1 ratio of K_2SO_4 : $CuSO_4$: Se, respectively), 3 ml 36 N H_2SO_4 and was digested for 3 hours. After cooling, each sample was diluted with 20 ml distilled water. Ammonium liberated by alkaline steam distillation was collected in H_3BO_3 and total N was determined by titration with standardized H_2SO_4. Results obtained for duplicate determinations of each sample are reported as % N in air dry soil. For plant samples, 100 mg plant tissue (40 mesh) were digested using 2 ml distilled water, 2 ml 36 N H_2SO_4 and 1.1 g catalyst mixture. Results are reported as % N in oven dry plant tissue.

Nitrate and ammonium determinations

Nitrate and NH_4^+-N in the soil were determined by a modified automated colorimetric Cd reduction method and automated alkaline phenate method, reqpectively (Technicon industrial methods 1973a and 1973b). Duplicate 5 g soil samples were extracted with 50 ml 2 N KCl on a reciprocating shaker for 1 hour. The samples were then filtered and analyzed immediately or stored at -4 C until the analysis could be conducted. Simultaneous determination of NO_3^- and NO_2^--N and NH_4^+-N was accomplished using a Technicon Autoanalyzer System II. Values obtained are reported as ppm NO_3^- and NH_4^+-N in air dry soil (NO_2^- was considered to be negligible and therefore was contained in the NO_3^- fraction).

Potentially available soil nitrogen

Three methods of estimating potentially available N were investigated. These involved; (1) aerobic incubation (Bremner, 1965); (2) N released by autoclaving in 0.01 M $CaCl_2$ (Stanford and Demar, 1969); and (3) N mineralized in short term incubations at 35 C (Stanford, Carter and Smith, 1974). Due to the effect of long term storage on the mineralization of N by soil, estimates were obtained only for samples collected in 1981.

In the aerobic method, 10 g air dry soil (20 mesh) was mixed with 30 g acid washed quartz sand (30-60 mesh), moistened with 6 ml distilled water and allowed to incubate in 8 ounce bottles at 30 C for 14 days. Water loss was prevented by covering each vessel with polyethylene plastic and aerobic conditions were maintained by puncturing three small pin holes in each top. Mineralized nitrogen (NO_3^- and NH_4^+-N) was extracted by shaking for 1 hour with 100 ml 2 N KCl. Mineral N in the filtered extracts was determined by the automated Cd reduction and alkaline phenate methods previously mentioned. Estimates of mineralizable N were obtained by subtracting the amount of NO_3^- and NH_4^+-N contained in an unincubated 3:1, sand:soil mixture from mineral N contained in the extracts of incubated samples. Values obtained for triplicate determinations are reported as ppm NO_3^- and NH_4^+-N mineralized.

Nitrogen released by autoclaving in 0.01 M $CaCl_2$ was determined in the following manner. Twenty-five ml 0.01 M $CaCl_2$ was added to 10 g air dry soil (20 mesh) in a 50 ml plastic centrifuge tube. Each tube was covered with Al foil and placed in an autoclave for 16 hours at 121 C. Each sample was centrifuged and resuspended in two more 25 ml aliquots of $CaCl_2$ and the extracts were combined and adjusted to 100 ml in a volumetric flask. Distillable N in the filtrate was determined by micro-Kjeldahl alkaline distillation whereby 10 ml 0.1 N NaOH was added to 30 ml extract in a 100 ml Kjeldahl flask and NH_3-N was distilled into H_3BO_3 and titrated with standardized H_2SO_4. Estimates of N released by autoclaving were obtained by subtracting NH_4^+-N contained in a non-autoclaved sample. Results obtained for duplicate determinations of each sample are reported as ppm NH_4^+-N released by autoclaving at 121 C for 16 hours.

Nitrogen released in short term incubations was determined by a method similar to the aerobic incubation method. Triplicate 20 g soil samples (20 mesh) were mixed with 20 g acid washed quartz sand (30-60 mesh) and placed in Nalgene disposable filter membrane units (model # 120-0020) each containing a 0.20 um Millipore filter. A piece of filter paper was placed on the surface of each vessel to prevent particle separation during leaching. Samples were leached with 4-10 ml aliquots of 0.01 M $CaCl_2$, and the filtrate saved for NO_3^- and NH_4^+-N analysis. Twenty-five ml of a minus N nutrient solution (0.002 M $CaSO_4 \cdot 2H_2O$; 0.002 M $MgSO_4$; 0.005 M $Ca(H_2PO_4)_2 \cdot H_2O$; and 0.0025 M K_2SO_4) was then added to each sample. The samples were placed on a vacumm manifold and allowed to equilibrate under 60 cm suction for 12 hours. The resulting filtrate was combined with the previously collected filtrate and equilibration weights were obtained for each tared vessel to ensure uniform moisture content. Each vessel was covered with polyethylene plastic and placed in the incubation chamber at 35 C. The system remained aerobic because of diffusion of O_2 through the bottom of the filter unit. The leaching and equilibration processes were repeated for each successive incubation period. Successive incubation periods consisted of 1, 1, 1, 2 and 3 weeks respectively, corresponding to cumulative incubation periods of 1, 2, 3, 5 and 8 weeks respectively. Nitrate and NH_4^+-N contained in the filtrate were determined by the automated Cd reduction and alkaline phenate methods previously cited. Values obtained are reported as ppm NO_3^- and NH_4^+-N mineralized in the soil.

Statistical analyses

Statistical methods described by Snedecor (1978) and Steel and Torrie (1980) were used for pertinent statistical analyses.

RESULTS AND DISCUSSION

Comparison of Organic Carbon Levels by Two Methods

Before a major investigation of the magnitude and direction of change of organic C and N levels of soils could be conducted it was necessary to decide upon an appropriate analytical method. The criteria for selection was reproducibility of results (i.e. duplicate determinations were required to have a relative error of less than 5%). Organic N in soil is readily determined by the micro-Kjeldahl method. However, organic C is more difficult to measure. This problem is compounded in these investigations because of the presence of free carbonates in the soil.

Table 3 contains a comparison of organic C levels of the Sp 1981 and corresponding 1972 soil samples obtained by two methods. Both methods indicate that organic C levels have declined over the cropping period. Furthermore, organic C levels between rotation sequences upon initiation of the experiment were not significantly different, whereas after nine years of cropping, significant differences have resulted as measured by both methods. In general, the dry combustion results are slightly lower than the Walkley-Black estimates. This observation suggests that the correction factor used in calculating % C via the Walkley-Black method may be too large or that pretreatment with dilute H_2SO_3 to destroy carbonate-C in the dry combustion method may oxidize organic C as well. The latter explanation is more feasible owing to the

Table 3. Comparison of organic carbon levels of soils sampled in the spring of 1981 and their corresponding 1972 levels as measured by two different methods.[1]

	Walkley-Black			Dry Combustion		
Sequence[2]	1972	1981	72-81	1972	1981	72-81
	% C					
C-B	1.67a[3]	1.55a	0.128ab	1.46a	1.38ab	0.078a
Be-B	1.65a	1.47b	0.175b	1.49a	1.34b	0.143a
C-C-C-B	1.68a	1.63c	0.053a	1.56a	1.50c	0.060a
C-C-Be-B	1.66a	1.58ac	0.083ab	1.62a	1.55c	0.068a
O-Be-B	1.63a	1.46b	0.173b	1.59a	1.46ac	0.128a
O-A-Be-B	1.66a	1.48b	0.180b	1.62a	1.50c	0.118a
CV	4.96%	2.27%	46.04%	6.61%	4.47%	79.67%

[1] Means of 4 replications, duplicate determinations.

[2] C = Corn, Be = Navy bean, B = Sugarbeet, O = Oat, A = Alfalfa

[3] Means followed by same letter within a column are not significantly different, alpha = 0.05 (Duncans new multiple range test).

large number of samples that had to be rerun in order to obtain a relative error of less than 5%. Conversely, the Walkley-Black method had a very low relative error test failure rate. A closer look at the organic C levels in 1972 as estimated by both methods will show that results obtained by the dry combustion method are more highly variable than the Walkley-Black estimates. Consequently, the Walkley-Black method was employed in these investigations. The dry combustion method would be extremely useful for determining organic C in acid soils where free carbonates are not present and hence do not result in positive errors or variation due to sample pretreatment.

Trends in Culturally Induced Soil Carbon and Nitrogen Changes

Gains and losses of organic C and N in soil are considered to be first order processes. The major objective in this study was to first identify the magnitude and direction of change of these organic matter constituents. Verification that these changes proceed via first order kinetics using equations derived by Salter and Green (1933), Jenny (1941), Bartholomew and Kirkham (1960), and Russell (1976) is not possible since the data base is only nine years old and is comprised of two measurements. Thus the discussion of C and N changes is relative to initial organic C and N levels without regard to quantitative kinetics.

Each crop in each sequence is grown every year. Table 2 shows the crops grown and N rates from 1972 to 1981 for the experimental units sampled Sp. 1981. A similar table could be constructed for the plots sampled F 1981. Distinction between date of sampling, size of sample obtained and experimental unit represented is critical, since these samples appear to represent significantly different populations (their variances are not homogeneous for all soil parameters estimated). Results of these investigations are contained in Table 4 (Sp 1981 sampling) and Table 5 (F 1981 sampling). In both cases, C and N levels observed are compared to levels observed for corresponding 1972 samples.

Table 4 (Sp 1981) shows that organic C levels in 1972 ranged from 1.63 to 1.67 % C (36500 and 37400 kg C/ha, respectively). Total N levels ranged from 0.192 to 0.197 % N (4300 and 4410 kg N/ha, respectively). The lack of significance in both cases verifies the assumption of homogeneity of experimental units upon initiation of the experiment. Organic C and N levels observed in Sp 1981 are significantly different. A useful quantity in assessing the effect of rotation sequence upon these levels is obtained by difference (1972 minus 1981 levels). In all cases, C and N levels of Sp 1981 samples have declined after nine years of cropping.

Specific organic C changes range from 0.053 % for the C-C-C-B rotation to 0.180 % for the O-A-Be-B rotation, corresponding to losses of 1180 and 4030 kg C/ha, repectively. The change in C for the C-C-C-B rotation was significantly lower than the O-Be-B, Be-B and O-A-Be-B rotations, but not significantly different from the C-C-Be-B and C-B rotations. Organic N losses ranged from 0.0066 % for the C-C-C-B rotation to 0.0186 % for the Be-B rotation (148 and 417 kg N/ha, respectively). The change in N for the C-C-C-B rotation was significantly lower than the O-Be-B, Be-B and O-A-Be-B rotations, but again not different from the other rotations containing corn. The change in N for the C-C-Be-B rotation was significantly less than the change for the Be-B rotation, but not different from the O-Be-B rotation.

Table 4. The effect of rotation sequence on organic C and N levels of soils sampled in the spring of 1981 after 9 years of cropping.[1]

	Walkley-Black C			Total Nitrogen			C/N Ratio	
Sequence[2]	1972	1981	72-81	1972	1981	72-81	1972	1981
	% C			% N				
C-B	1.67a[3]	1.55a	0.128ab	0.197a	0.187a	0.0104abc	8.59a	8.27a
Be-B	1.65a	1.47b	0.175b	0.196a	0.177c	0.0186a	8.47a	8.31a
C-C-C-B	1.68a	1.63c	0.053a	0.196a	0.190a	0.0066c	8.53a	8.59b
C-C-Be-B	1.66a	1.58ac	0.083ab	0.194a	0.186ab	0.0077bc	8.47a	8.48ab
O-Be-B	1.63a	1.46b	0.173b	0.192a	0.176c	0.0164ab	8.40a	8.28a
O-A-Be-B	1.66a	1.48b	0.180b	0.197a	0.179bc	0.0180a	8.57a	8.28a
CV	4.96%	2.76%	46.06%	4.25%	2.75%	45.25%	2.46%	1.69%

[1] Means of 4 replications, duplicate determinations.

[2] C = Corn, Be = Navy bean, B = Sugarbeet, O = Oat, A = Alfalfa

[3] Means followed by the same letter within a column are not significantly different, alpha = 0.05 (Duncans new multiple range test).

Table 5. The effect of rotation sequence on organic C and N levels of soils sampled in the fall of 1981 after 9 years of cropping.[1]

	Walkley-Black C			Total Nitrogen			C/N Ratio	
Sequence[2]	1972	1981	72-81	1972	1981	72-81	1972	1981
		% C			% N			
C-B	1.61a[3]	1.52a	0.090a	0.175a	0.178a	-0.0030a	9.23a	8.62a
Be-B	1.76a	1.56a	0.200b	0.188a	0.168a	0.0198c	9.40a	9.31a
C-C-C-B	1.66a	1.60a	0.068a	0.182a	0.175a	0.0076ab	9.12a	9.12a
C-C-Be-B	1.74a	1.64a	0.095a	0.192a	0.178a	0.0134bc	9.06a	9.22a
O-Be-B	1.68a	1.52a	0.158b	0.183a	0.167a	0.0156bc	9.19a	9.11a
O-A-Be-B	1.64a	1.58a	0.055a	0.179a	0.175a	0.0036ab	9.18a	9.05a
CV	4.98%	4.50%	56.70%	5.04%	5.28%	79.05%	1.58%	3.99%

[1] Means of 4 replications, duplicate determinations

[2] C = Corn, Be = Navy Bean, B = Sugarbeet, O = Oat, A = Alfalfa

[3] Means followed by the same letter within a column are not significantly different, alpha = 0.05 (Duncans new multiple range test).

In general, where losses of C were high, N losses were high. For Sp 1981 samples, it was observed that rotations containing corn tended to result in higher levels of soil C and N, while rotations containing combinations of a legume and oats resulted in lower C and N levels. Salter and Green (1933) observed the opposite although this anomally may be due to the fact that substantial use of N fertilizer did not occur until 1950 and alfalfa is grown only one season. Furthermore, alfalfa has been grown twice since the initiation of the experiment and apparently the beneficial effects on soil organic C and N are not observable under these management practices in the time interval investigated. For the system studied, it is reasonable to assume that rotations containing 50 % corn return a larger amount of crop residue than the Be-B and O-Be-B rotations, and therefore result in higher C and N levels.

Table 5 (F 1981) shows that organic C and N levels in 1972 were not significantly different between sequences. The range of these values is slightly wider and CV's are slightly larger than those observed in Table 4. Furthermore, organic C and N levels observed in F 1981 were not significantly different, however the differences between the C and N levels in 1972 and F 1981 were significant. Losses of C ranged from 0.055 % for the O-A-Be-B rotation to 0.200 % for the Be-B rotation (1230 and 4000 kg C/ha, respectively), while losses of N ranged from -0.0030 % for the C-B rotation to 0.0198 % for the Be-B rotation (-67 and 444 kg N/ha respectively). Changes in C for the C-C-C-B, C-C-Be-B, C-B and O-A-Be-B rotations were all significantly lower than changes for the O-Be-B and Be-B rotations. With respect to N changes, the C-B rotation was significantly lower than the C-C-Be-B, O-Be-B and

Be-B rotations, while the N changes for the O-A-Be-B and C-C-C-B rotations were significantly lower than the Be-B rotation.

The increase in N observed for the C-B rotation is inconsistent with results obtained on Sp 1981 samples, as is the higher level of C and N of the O-A-Be-B plots, although this observation for a rotation containing alfalfa is more in line with results obtained in similar long term cropping experiments. These inconsistencies may be due to the fact that a larger sample (80 probes per plot) was collected in the fall, and that these measurements were made on a different set of experimental units. Regardless, the trend toward higher C and N levels of sequences containing 50% corn is evident in both the spring and fall sampling. The lower values of C and N obtained for the Be-B and O-Be-B rotations parallel observations made on Sp 1981 samples, again suggesting that these rotations return a smaller amount of crop residue to the soil. The C and N status of the O-A-Be-B and C-B rotations is less definitive since these rotations behaved differently the second year.

Also noteworthy is the fact that C/N ratios calculated for Sp 1981 samples and corresponding 1972 samples are lower than those calculated for F 1981 and corresponding 1972 samples despite the fact that all the 1972 samples were collected at the same time. The lack of significance of the 1972 C/N ratios of both sampling dates supports the hypothesis that experimental units were homogenous. The significant difference between the Sp 1981 C/N ratio for the C-C-C-B rotation and the other rotations is not suprising due to the larger differences in its components.

Estimates of Potentially Mineralizable Nitrogen

Table 6 contains estimates of potentially mineralizable N for both sampling dates. The Sp 1981 autoclavable mineralizable N estimates (AMN) were not significantly different, however they follow a pattern similar to the Bremner mineralizable N estimates (BMN) for both sampling dates. The Sp 1981 BMN estimates were significantly different. The C-C-Be-B rotation provided the largest estimate and was significantly different from the O-A-Be-B, C-B, O-Be-B and Be-B estimates, but not different than the C-C-C-B value. The C-C-C-B, O-A-Be-B, C-B and O-Be-B rotations all behaved similarly, however C-C-C-B and O-A-Be-B estimates were significantly higher than the Be-B rotation. The C-B, O-Be-B and Be-B estimates were not significantly different from each other.

The F 1981 AMN estimates indicate that the C-B rotation is significantly lower in potentially mineralizable N than all the other rotations. This is surprising Table 5 indicates that the level of soil N increased over the nine year cropping period whereas all the other levels decreased. The F 1981 BMN estimates paralleled Sp 1981 estimates, although the N levels and change in N levels did not parallel Sp 1981 observations. The C-C-C-B rotation is significantly higher in potentially mineralizable N than the O-Be-B and Be-B rotations, but not different from the other rotations. Furthermore, the C-C-Be-B rotation is significantly higher than the O-Be-B rotation but not different from the other rotations. The O-A-Be-B, C-B, Be-B and O-Be-B estimates were not significantly different from each other.

Table 6. The effect of rotation sequence on estimates of potentially mineralizable soil N for both sampling dates.[1]

	Sp 1981		F 1981			
	Total N (NO_3^- + NO_2^- + NH_4^+) mineralized					
Sequence[2]	BMN[3]	AMN[4]	BMN	AMN	$N_{mp}A$[5]	$N_{mp}U$[6]
	ppm					
C-B	30.5ab[7]	29.9a	27.1abc	21.3a	2.07a	6.30a
Be-B	29.0b	29.1a	26.4bc	25.3b	1.38b	5.14a
C-C-C-B	33.4ac	32.3a	30.6a	24.8b	1.82a	5.98a
C-C-Be-B	35.3c	31.4a	29.9ab	27.3b	2.08a	6.83a
O-Be-B	30.2ab	27.2a	25.9c	26.3b	1.81a	4.96a
O-A-Be-B	31.6ab	30.2a	28.8abc	26.6b	1.93a	6.20a
CV	6.89%	7.00%	8.02%	8.79%	10.65%	16.71%

[1] Means of 4 replications, BMN-triplicate determinations, AMN-duplicate determinations.

[2] C = Corn, Be = Navy bean, B = Sugarbeet, O = Oat, A = Alfalfa

[3] BMN = Bremner (1965) aerobic incubation method.

[4] AMN = Stanford and DeMar (1969) autoclavable method.

[5] $N_{mp}A$ = Stanford et al. (1974) short term incubation method, adjusted.

[6] $N_{mp}U$ = Stanford et al. (1974) short term incubation method, unadjusted.

[7] Means followed by the same letter within a column are not significantly different, alpha = 0.05 (Duncans new multiple range test).

There are several observations from Table 6 which are noteworthy when these values are compared to each other and to corresponding C and N levels of the soil for the different sampling dates. For both sampling dates BMN estimates are slightly higher than AMN estimates. This difference is not surprising since N mineralization in the BMN method proceeds via biochemical mechanisms. Secondly, the AMN and BMN estimates for the F 1981 samples are lower than the Sp 1981 estimates. This is thought to result from a higher concentration of mineralizable substrate in the spring samples because crop residues were fall incorporated. Fall samples were obtained before residue incorporation. Biological activity is higher during the growing season, and hence one would expect available and potentially available N to be depleted at the end of the season. The mineral N content of the soil is also lower for the fall samples (Table 7). Little statistical inference is implied by the significant differences between sequences for these quantities since mineral N is a highly variable soil parameter, however the higher value of NO_3^- for the F 1981 C-B sample may be attributed to a higher N fertilizer rate for that sequence (Table 2).

If one excludes the F 1981 AMN and N_{mp} values in Table 6, ranking of the remaining means suggests that the chemical method may be as useful as the biological method, and more importantly, N mineralization as measured by the BMN method is affected by rotation sequence to the same degree on the separate experimental units. This ranking operation results in the following ordering: C-C-C-B = C-C-Be-B > O-A-Be-B = C-B > Be-B = O-Be-B. The reason F 1981 AMN estimates deviate from this pattern is unexplained, however the AMN estimates of mineralizable N at the end of the growing season for rotations containing legumes appear to

Table 7. Mineral N concentrations observed in the spring and fall soil samples in relation to rotation sequence.[1]

	Sp 1981		F 1981	
Sequence[2]	NO_3^-	NH_4^+	NO_3^-	NH_4^+
	ppm N			
C-B	5.03a[3]	3.30a	5.43a	2.60a
Be-B	5.63a	2.90a	2.91cd	2.63a
C-C-C-B	5.22a	3.37a	3.73b	2.76a
C-C-Be-B	6.54a	2.76a	3.22bc	2.88a
O-Be-B	5.57a	3.05a	2.36d	2.51a
O-A-Be-B	5.41a	2.47a	3.21bc	2.29a
CV	11.83%	14.50%	13.80%	21.02%

[1] Means of 4 replications, duplicate determinations.

[2] C = Corn, Be = Navy bean, B = Sugarbeet, O = Oats, A = Alfalfa

[3] Means followed by the same letter within a column are not significantly different, alpha = 0.05 (Duncans new multiple range test).

be higher than rotations containing only corn and sugarbeets. This anomally may arise from differences in the biochemical as opposed to chemical stability of the mineralizable substrate, and hence may be a reflection of the chemical nature of organic matter present.

Another characteristic of the data in Table 6 is the large difference between estimates of N mineralized by the short term incubation method of Stanford et. al. (1974), and those obtained by other methods. Owing to the fact that these incubations were carried out for 8 weeks at 35 C, and that the BMN method was carried out for only two weeks at 30 C, the results obtained are indeed unusual. Because of this the N mineralization potential as defined by Stanford and Smith (1972) could not be calculated. During the experiment, it was thought that N mineralized during the short term incubation was being lost through ammonia volatilization or biological denitrification. A monitoring of N lost by these processes for a few randomly selected samples indicated that little or no gaseous evolution occured. Nitrogen immobilization was possibly occurring due to the presence of carbonaceous filter paper which was placed in each vessel to prevent erosion and particle separation during the leaching process. The presence of visible microbial colonies residing on the filter paper in each vessel may be further evidence of immobilization due to an artificially high C/N ratio. Regardless of the cause of immobilization, the inhibitory effect seems to be quantitative since a similar ranking of the means reveals that although the values are much lower, they follow a pattern similar to the other estimates of N availability (again excepting the F 1981 AMN estimates).

Previous crop residue which is easily decomposed may influence the degree of N mineralization, hence adjusted and unadjusted values of N_{mp} are contained in Table 6. Adjusted means that N mineralized during the first week of incubation was subtracted from the cumulative N mineralized at the end of 9 weeks. Unadjusted values follow the trends observed for adjusted values although there are no significant treatment differences. It is important to note that the adjusted estimate for the Be-B rotation is significantly lower than the other rotations, a fact which is consistent with estimates obtained by the other methods.

The effect of crop rotation sequence on estimates of potentially mineralizable N is best illustrated using simple correlation. Tables 8 and 9 contain simple correlation coefficients obtained by correlation between organic C and N, AMN, BMN, and estimates of short term mineralizable N for the separate sampling dates. The Sp 1981 correlations (Table 8) will be considered first.

Highly significant linear relationships are shown to exist between Walkley-Black C (WBC) and estimates of mineralizable N by the AMN and BMN methods ($r = 0.89$ and 0.79, respectively). Similar relationships exist between total N (TN) and AMN and BMN ($r = 0.87$ and 0.80 respectively). This significant linear relationship suggests that the degree of mineralization is proportional to the amount of mineralizable substrate present in the soil, and substantiates the assumption that N mineralization (under controlled conditions) proceeds via first order kinetics. Further evidence exists in the significant relationships observed between the change in C and N (D%C and D%N, respectively) and AMN and BMN (Table 8). The correlation coefficients in this case are negative and can be interpreted to mean that large losses of organic C and N will result in smaller estimates of mineralizable N. One must be

Table 8. Simple correltion matrix of sugarbeet yield parameters observed in the field with selected soil properties of the spring 1981 soil samples.

Parameter	WBC	TN	AMN	BMN	YLD	%S	- CJP	AAN	D%C	D%N	RST	RSH
Walkley-Black % C (WBC)	1.00											
Total % N (TN)	0.96**	1.00										
Autoclavable N (AMN)	0.89**	0.87**	1.00									
Bremner mineralizable N (BMN)	0.79**	0.80**	0.61**	1.00								
Sugarbeet yield (YLD)	0.15	0.26	0.26	0.03	1.00							
% sucrose (% S)	-0.27	-0.21	-0.23	-0.36	0.11	1.00						
Clear juice purity (CJP)	-0.42*	-0.37	-0.40	-0.30	0.20	0.67**	1.00					
Alpha-amino N (AAN)	0.22	0.20	0.26	0.06	-0.02	-0.55**	-0.83**	1.00				
Delta % C (D%C)	-0.60**	-0.50*	-0.43*	-0.58**	0.36	0.19	0.24	0.03	1.00			
Delta % N (D%N)	-0.54**	-0.51*	-0.42*	-0.57**	0.29	0.27	0.19	0.04	0.84**	1.00		
Recov. sugar per ton (RST)	-0.32	-0.26	-0.28	-0.37	0.13	0.99**	0.79**	-0.65*	0.21	0.27	1.00	
Recov. sugar per ha (RSH)	0.04	0.15	0.15	-0.10	0.93**	0.46*	0.43*	-0.24	0.39	0.36	0.48*	1.00

*,**Significantly correlated at alpha = 0.05, 0.01 level repectively.

Table 9. Simple correlation matrix of uptake of N at the zero N level as observed in the greenhouse with selected soil properties of the fall 1981 soil samples.

Parameter	WBC	TN	AMN	BMN	UNC1	UNC2	UNC3	CUUP	A	U
Walkley-Black % C (WBC)	1.00									
Total % N (TN)	0.95**	1.00								
Autoclavable N (AMN)	0.75**	0.79**	1.00							
Bremner min. N (BMN)	0.23	0.26	0.03	1.00						
Uptake of N, crop 1 (UNC1)	0.47*	0.47*	-0.03	0.27	1.00					
Uptake of N, crop 2 (UNC2)	0.17	0.18	0.31	0,08	0.04	1.00				
Uptake of N, crop 3 (UNC3)	0.11	0.11	-0.12	0.07	0.28	-0.46*	1.00			
Cumulative uptake (CUUP)	0.48*	0.48*	0.02	0.25	0.94	0.17	0.47*	1.00		
N_{mp} adjusted (A)	-0.38	-0.36	-0.41*	0.03	-0.26	-0.36	0.08	-0.26	1.00	
N_{mp} unadjusted (U)	0.07	0.12	-0.15	0.30	0.29	-0.41*	0.14	0.16	0.48*	1.00

*,**Significantly correlated at alpha = 0.05, 0.01 level resectively.

careful with this interpretation since the magnitude of these correlation coefficients are not as large as those previously discussed. A significant correlation between the AMN and BMN methods ($r = 0.61$) was also obtained.

Table 9 contains similar correlation coefficients for the F 1981 sampling, however D%C and D%N coefficients are not included since they were not significant. Here, AMN is significantly correlated with WBC and TN ($r = 0.75$ and 0.79, respectively) whereas BMN is not significantly correlated ($r = 0.23$ and 0.26, respectively). As expected from the previous observation, the relationship between BMN and AMN is poor ($r = 0.03$). Similarly, the adjusted and unadjusted N_{mp} estimates are poorly correlated with WBC, TN and BMN. The negative correlation between the adjusted estimates and AMN although small, is unusual.

In conclusion, the relative magnitude and uniformity of results obtained for the Sp 1981 estimates of mineralizable N suggest that it is best to collect soil samples for this purpose in the spring instead of the fall. This is supported by a similar observation for organic C and N levels of the soil. Furthermore, a first approximation of N mineralization appears to follow first order kinetics. Therefore a rotation which returns a larger amount of crop residue can supply a larger amount of mineralizable N to a growing crop, and is consistent with the "feedback effect" (increasing yields increase crop residue production and soil C and N levels) described by Russell (1976) and results obtained by Larson et al. (1972). For the system studied, rotations containing 50 % corn have the potential to mineralize more N than the Be-B and O-Be-B rotations. The C-B and O-A-Be-B rotations are intermediate in their N supplying capacity. This pattern is similar to that observed for changes in organic C and N levels of the soil.

Effect of Crop Rotation Sequence on Sugarbeet Yield Parameters

The relationship between culturally induced soil N changes and estimates of mineralizable N has been established in this and other similar studies. In general, N mineralization proceeds via first order kinetics and the rate of mineralization is therefore proportional to the amount of mineralizable substrate. Similarly, the detrimental effects of excess mineral N on the yield and quality of sugarbeets are well documented. Table 10 contains the results of a field study which was conducted in 1981 to determine if sugarbeet yield parameters were significantly affected by culturally induced soil N changes. With respect to % sucrose (% S) and recoverable sugar per metric ton of beets (RST), only the C-C-Be-B rotation produced significant differences. Yield, clear juice purity (CJP), recoverable sugar per ha (RSH) and alpha amino N (AAN) were not significantly different.

Table 8 contains simple correlation coefficients obtained from correlations between sugarbeet yield parameters and selected soil properties. Most importantly, there is no significant correlation between estimates of mineralizable N and yield parameters. There are some significant linear relationships between the yield parameters themselves. For example, yield is highly related to RSH ($r = 0.93$). Percent sucrose in the beet juice was positively correlated with CJP, RST, and RSH ($r = 0.67$, 0.99, and 0.46, respectively), and negatively correlated with AAN ($r = -0.55$). Clear juice purity was negatively correlated with AAN ($r = -0.83$) and positively correlated with RST and RSH ($r = 0.79$ and 0.43, respectively). Alpha amino N was negatively

Table 10. The effect of crop rotation sequence on sugarbeet yield, % sucrose (S), clear juice purity (CJP), recoverable sugar, and alpha-amino N (AAN) concentrations in sugarbeet juice obtained from sugarbeets produced on soils sampled in the spring of 1981.[1]

Sequence[2]	Yield	S	CJP	Recoverable S		AAN
	Mg/ha	%	%	kg/Mg	Mg/ha	meq/100g
C-B	61.58a[3]	16.7a	96.2a	145.6a	10.28a	7.68a
Be-B	60.91a	16.1a	95.7a	138.6a	9.77a	9.93a
C-C-C-B	58.62a	16.2a	95.6a	139.4a	9.48a	8.05a
C-C-Be-B	57.57a	15.2b	95.0a	129.0b	8.76a	11.3a
O-Be-B	59.31a	16.3a	95.7a	141.0a	9.67a	8.43a
O-A-Be-B	64.58a	16.3a	95.8a	141.0a	10.55a	8.45a
CV	8.62%	2.49%	0.62%	3.20%	9.68%	22.14%

[1] Means of 4 replications, duplicate determinations.

[2] C = Corn, Be = Navy bean, B = Sugarbeet, O = Oat, A = Alfalfa

[3] Means followed by the same letter within a column are not significantly different, alpha = 0.05 (Duncans new multiple range test).

correlated with RST (r = -0.48) while the relationship between RST and RSH was positively correlated (r = 0.48). These relationships are in agreement with the findings of Hills and Ulrich (1971), Hills et al. (1978), Carter and Traveler (1981) and numerous others.

One must conclude that culturally induced soil N changes are not substantial enough to significantly influence sugarbeet yield parameters in the field. The significant differences arising in the C-C-Be-B rotation are not likely due to differences in mineralizable N since correlation between yield parameters and mineralizable N indexes were not significant. It is important to note that CJP which is a moderate indicator of sucrose recoverability, is negatively correlated with WBC and TN (r = -0.42 and -0.37, respectively). Although the first correlation is small and the latter is not significant, it establishes a trend and provides evidence indicating that in time sugar yields may decline. This trend will be further considered in the next section.

Effect of Crop Rotation Sequence on Uptake of Nitrogen in the Greenhouse

Results of the field study clearly established that sugarbeet yield parameters were little affected by culturally induced soil N changes, yet some significant differences in potentially mineralizable N appear to exist. A greenhouse experiment was conducted to determine if uptake of N was linearly related to the N availability indices. Three consecutive crops were produced (oats, corn and oats, respectively), and yield, % N in tops, uptake and mineral N remaining in soil after cropping were determined. Results obtained are reported in Table 11. A complete statistical interpretation of Table 11 is not presented because uptake is the best indictor of N availability due to the inherent

Table 11. The effect of crop rotation sequence and N fertility level on yield, N content, N uptake and mineral N remaining in the fall 1981 soil after three consecutive cropping periods in the greenhouse.[1]

Sequence[2]	N level	Yield			N in tops			Uptake of N				Mineral N in soil		
		Crop1	Crop2	Crop3	Crop1	Crop2	Crop3	Crop1	Crop2	Crop3	Cum.	Crop1	Crop2	Crop3
	ppm	g/culture			%			mg/culture				ppm		
C-B	0	1.153	0.708	0.845	2.28	1.00	1.19	26.3	6.98	9.98	43.2	1.97	2.42	0.34
	37.5	1.565	1.185	0.911	4.01	1.68	1.11	62.6	19.8	10.1	92.5	32.0	2.83	0.16
	75.0	1.668	0.830	1.109	3.88	3.50	3.16	64.7	29.1	35.0	128	90.7	42.2	2.35
Be-B	0	1.043	0.868	0.716	2.13	0.90	1.20	22.1	7.74	8.60	38.5	1.59	2.08	0.23
	37.5	1.665	1.533	0.752	4.15	1.11	1.09	68.7	16.9	8.21	93.8	27.2	1.82	0.16
	75.0	1.633	1.788	1.129	4.26	3.38	2.16	69.4	43.5	24.1	137	80.2	28.0	1.24
C-C-C-B	0	1.093	0.788	0.855	2.32	1.01	1.17	25.3	7.94	9.97	43.2	1.89	2.30	0.18
	37.5	1.628	1.383	0.943	4.29	1.41	1.11	70.0	19.3	10.4	99.7	29.9	2.21	0.11
	75.0	1.580	1.130	1.171	4.34	3.25	2.71	68.7	36.6	31.6	137	82.7	39.4	2.34
C-C-Be-B	0	1.130	0.773	0.785	2.29	0.95	1.11	25.8	7.22	8.74	41.8	2.42	2.42	0.26
	37.5	1.588	1.533	0.852	4.33	1.34	1.06	68.4	20.8	9.07	98.3	31.5	2.29	0.18
	75.0	1.655	1.165	1.755	4.28	3.19	2.41	70.8	37.2	30.1	138	92.1	36.4	1.96
O-Be-B	0	1.013	0.865	0.744	2.06	0.92	1.18	20.7	7.84	8.72	37.3	2.38	1.94	0.20
	37.5	1.545	1.560	0.713	4.16	1.23	1.14	64.1	18.5	8.01	90.6	31.6	1.77	0.33
	75.0	1.495	1.218	1.088	4.35	3.26	2.70	65.0	39.9	29.3	134	88.2	36.7	2.58
O-A-Be-B	0	1.053	0.898	0.764	2.27	1.03	1.11	23.9	9.00	8.52	41.4	2.31	2.11	0.20
	37.5	1.508	1.730	0.806	4.28	1.32	1.07	65.5	22.5	8.62	95.6	36.2	1.61	0.28
	75.0	1.560	1.415	1.125	4.21	3.17	2.49	65.6	44.7	27.6	138	83.8	35.3	2.20

[1] Means of four replications, duplicate determinations.

[2] C = Corn, Be = Navy bean, B = Sugarbeet, O = Oat, A = Alfalfa

variability in yield, % N and mineral N estimates obtained for the different cropping periods, however some general observations are noteworthy.

During the first cropping period, N deficiency symptoms (stunting and yellowing of the older leaves) appeared in all pots at the 0 N level. Comparison of yield and % N in tops indicates no differential response to applied N occurred in cropping period 1 for the 37.5 and 75.0 ppm N levels. Near the end of the second cropping period, N deficiency was apparent at all N levels, though to a much lesser extent at the higher N level. All pots showed obvious signs of N deficiency during the final cropping period. As expected, yield, % N in tops and uptake of N increased in response to applied N and decreased in succesive cropping periods due to N depletion. The decreasing trend in mineral N remaining in the soil after cropping is further evidence of N depletion.

The quantitative discussion is limited to uptake of N (yield x % N) and the ratio of uptake of N (uptake at the 75 ppm level divided by uptake at the 0 N level), which is a measure of response to applied N. Table 12 contains the approximate level of significance of the F test for uptake of N by the three consecutive crops. There were significant differences between sequences and between N levels in all three cropping periods, and there was a significant sequence x N interaction for crop 2 and cumulative uptake values. Significant differences in response to applied N were observed only for the cumulative uptake values (Table 13). Thus the discussion is limited to cumulative uptake.

As expected, there was a significant difference in cumulative uptake due to N level (Table 14). There are also significant

Table 12. Approximate significance of uptake of N by three succesive crops in the greenhouse at three levels of N fertility.

	approximate level of significance			
Source	crop1	crop2	crop3	cum.
Replication	0.001	0.070NS	0.072NS	0.020
Sequence	0.015	0.001	0.028	0.001
Nitrogen	0.001	0.001	0.001	0.001
Sequence x Nitrogen	0.486NS	0.001	0.455NS	0.018

NS = F test was not statistically different at a probability level of 5%.

Table 13. Approximate significance of the F test for the response to applied N by three consecutive crops grown at three N fertility levels in the greenhouse on the fall 1981 soil samples.[1]

	Approximate level of significance			
Source	crop 1	crop 2	crop 3	cum.
Replication	.293NS	.122NS	.133NS	.024
Sequence	.145NS	.267NS	.725NS	.025

[1] Response = uptake at 75 ppm level divided by uptake at 0 ppm level.

NS = F test was not statistically different at a probability level of 5%.

Table 14. The effect of rotation sequence on the cumulative response to applied N and cumulative uptake of N by three consecutive crops grown at three N fertility levels in the greenhouse on the fall 1981 soil samples.[1]

Sequence[3]	Cumulative N uptake			Response[2]
	0 ppm N	37.5 ppm N	75 ppm N	
	mg/culture			
C-B	43.2Aa[4,5]	92.5Ba	128Ca	2.98a
Be-B	38.5Aab	93.8Bab	137Cb	3.61bc
C-C-C-B	43.2Aa	99.7Bc	137Cb	3.18ab
C-C-Be-B	41.8Aab	98.3Bbc	138Cb	3.32abc
O-Be-B	37.3Ab	90.6Ba	134Cb	3.64c
O-A-Be-B	41.4Aab	95.6Babc	138Cb	3.34abc

[1] Means of four replications, duplicate determinations.

[2] Response = uptake at 75 ppm N level divided by uptake at 0 ppm N level.

[3] C = Corn, Be = Navy bean, B = Sugarbeet, O = Oat, A = Alfalfa

[4] Means followed by the same letter (e.g. A) within a row (excluding the last column) are not significantly different, alpha = 0.05, (Duncans new multiple range test).

[5] Means followed by the same letter (e.g. a) within a column are not significantly different, alpha = 0.05, (Duncans new multiple range test).

differences between sequences at the individual N levels. Uptake at the 0 N level for the C-B and C-C-C-B rotations was significantly higher than the O-Be-B rotation but not different than the other rotations. The C-C-Be-B, O-A-Be-B, Be-B and O-Be-B rotations behaved similarly. Since uptake at the 0 N level is a reflection of the N supplying capacity of the soil, the greater uptake by the C-B rotation is not surprising owing to the apparent increase in soil N over the nine year cropping period (Table 5). Soil N and mineralizable N estimates were lower for the Be-B and O-Be-B rotations for both years, and hence lower values of uptake at the 0 N level were observed.

Uptake at the 37.5 ppm level for the C-C-C-B rotation was significantly higher than the Be-B, C-B, and O-Be-B rotations, while uptake for the C-C-Be-B rotation was significantly higher than the C-B and O-Be-B rotations. Uptake by the remaining rotations was not significantly different.

At the 75 ppm N level, C-C-Be-B, O-A-Be-B, C-C-C-B, Be-B, and O-Be-B rotations were all significantly higher in uptake than the C-B rotation. Uptake by the C-B rotation was highest at the 0 N level and lowest at the 75 ppm N level. A similar trend was observed for the C-C-C-B rotation. The significant sequence x N interaction appears to result from the presence of corn and the absence of legumes in a rotation, and from a differential response of the corn containing rotations when corn was grown as a second crop instead of oats. Addition of N to the corn containing rotations has apparently inhibited N mineralization or conversely, stimulted the mineralization for the legume containing rotations to a greater extent. This suggests that there are qualitative differences in the degradability and type of humus formed under these rotations.

The ratio of uptake inversely parallels uptake of N at the 0 N level. The response to applied N was significantly lower for the C-B and C-C-C-B rotations than for the O-Be-B rotation. This is most likely due to the sequence x N interaction. The C-B response was also significantly lower than the Be-B response. The response of the remaining rotations was not significantly different.

Table 9 contains simple correlation coefficients obtained from correlations between greenhouse results and selected soil properties. There were no significant linear correlations between uptake at the 0 N level (cumulative or otherwise) and N availability indexes, and hence these results are not consistent with results reported in Table 1. Similarly, it will be noted that Sp 1981 correlations with the same soil parameters are higher. For the system under study, one must therefore question the use and accuracy of N availability indexes in estimating mineralizable N in samples collected at the end of the growing season. Cumulative uptake and uptake during crop 1 at the zero N level were significantly correlated with WBC and TN (r = 0.47, 0.47, 0.48, and 0.48, repectively). The above observation and the significant linear relationship between AMN and BMN and their corresponding C and N levels (previously discussed) suggest that a nonlinear relationship may exist.

The highly significant linear relationship between uptake by crop 1 and cumulative uptake (r = 0.94) at the 0 N level is further evidence that a discussion of cumulative uptake is more useful than individual discussions of the results. It also tends to suggest that one extended cropping period is as useful as several consecutive cropping periods. A significant correlation between cumulative uptake and uptake by crop 3 at the 0 N level was also observed (r = 0.47). Uptake by crop three was negatively correlated with uptake by crop 2 (r = -0.46). This relationship is believed to result from the fact that corn was grown during the second cropping period, and corn containing rotations (C-B and C-C-C-B) behaved adversely. Larson (1972), Ketchenson and Beauchamp (1972) and Barber (1979) have all verified that corn and stover yields are reduced when corn follows corn (hence uptake of N may decline).

SUMMARY

Two methods of determining organic C in the soil were investigated. The dry combustion results were highly variable owing to problems associated with the removal of carbonate C before combustion, therefore the WBC method was employed. Because complete oxidation is accomplished, the dry combustion method would be extremely useful for soils which do not contain carbonates.

The same field experiment was conducted twice on separate experimental units. With respect to soil C and N levels, experimental units were shown to be statistically homogeneous upon initiation of the long term experiment. After 9 years of cropping to different rotation sequences, significant differences have resulted. Although there is a similarity of results, the variances of all soil parameters estimated were not homogeneous and hence results were not poolable between sampling dates. For the Sp 1981 samples, organic C losses ranged from 0.053% for the C-C-C-B rotation to 0.180% for the O-A-Be-B rotation (1180 and 4030 kg C/ha, respectively). Organic N losses ranged from 0.0066% for the C-C-C-B rotation to 0.186% for the Be-B rotation (148 and 417 kg N/ha, respectively). For the F 1981 samples, losses of C ranged from 0.055% for the O-A-Be-B rotation to 0.200% for the Be-B rotation (1230 and 4000 kg C/ha, respectively), while losses of N ranged from -0.0030% for the C-B rotation to 0.0195% for the Be-B rotation (-67 and 444 kg N/ha, respectively).

In general, losses of C paralleled losses of N. The trend toward higher C and N levels of sequences containing 50% corn is evident in both sampling dates, as is the trend toward lower C and N levels of the O-Be-B and Be-B rotations. One can conclude that these significant differences have resulted from differences in the amount of organic matter returned to the soil.

The next question which was asked is do these differences result in differences between estimates of potentially mineralizable N? Three methods were investigated, Bremner mineralizable N, autoclavable mineralizable N and N mineralization potential (BMN, AMN and N_{mp},repectively). The Sp 1981 AMN and BMN and F 1981 BMN estimates closely paralleled one another, indicating that N mineralization under controlled conditions was affected by rotation sequence to the same degree. The F 1981 estimates of AMN did not follow this pattern, possibly because of qualitative differences in organic matter. The N_{mp} method provided anomalously low results, and thus was not very useful since the N mineralization potential as defined by Stanford (1972) could not be calculated.

For both sampling dates the amount of N mineralized in the AMN and BMN methods was significantly and linearly correlated with Walkley-Black C and total N (WBC and TN). Thus one can conclude that the rate and amount of N mineralized in these processes was proportional to the amount of mineralizable substrate. In general, N mineralization estimates were high where soil C and N levels were high. For the system studied, rotations containing 50% corn have the potential to mineralize more N than the Be-B and O-Be-B rotations. The C-B and O-A-Be-B rotations were intermediate in their N supplying potential. Smaller correlation coefficients obtained for the F 1981 samples suggest that it is best to obtain soil samples for this purpose in the spring. Uniform results obtained for the Sp 1981 samples supports this statement and further suggests that a soil sample consisting of 20 probes per plot provides adequate statistical representation of the C and N and N mineralization status of the soil.

The hypothesis that these differences can affect sugarbeet yield parameters in the field was investigated. Only the C-C-Be-B rotation produced significantly lower % S and RST estimates. Since there was no significant linear correlation between estimates of mineralizable N and sugarbeet yield parameters, these differences did not appear to be related to differences in potentially mineralizable N. These results indicate that culturally induced soil N changes are not substantial enough to influence sugarbeet yield parameters at this time.

In the greenhouse experiment there were significant differences between uptake for sequences and N levels in all three cropping periods, as well as significant sequence x N interactions for the cumulative and crop 2 results. The trend in cumulative N uptake at the 0 ppm N level closely paralleled C and N levels and a significant linear correlation between these values was found to exist. As the N level increased, cumulative uptake by the C-C-C-B and C-B rotations decreased. Thus a significant sequence x N interaction resulted possibly due to qualitative differences in organic matter formed under these rotations. Similarly, the relative response to N inversely paralleled trends in cumulative uptake of N at the 0 N level. There was no significant linear correlation between cumulative uptake at the 0 N level and N availability indexes as has been reported in the literature. This lack of correlation cannot be explained.

The highly significant relation between uptake by crop 1 at the 0 N level and cumulative uptake is evidence that one extended cropping period utilizing multiple clippings of a perennial, indeterminate (non N fixing) crop species may be as useful in vegetative tests for N uptake as several consecutive cropping periods utilizing different crops. The former method can save a great deal of time and money.

In conclusion, the greenhouse results indicate that significant differences in uptake have resulted, hence sugarbeet yield parameters may eventually be affected. All other factors being the same (eg. disease, insects, etc.), first order kinetics predict that the 0-Be-B and Be-B rotations (being lower in organic C and N) will be the first rotations in which this effect will be observed. To prevent this effect from being manifested, new N fertilizer recommendations may eventually have to be formulated.

CONCLUSIONS

1. Experimental units were found to be statistically homogeneous upon initiation of the experiment.

2. After 9 years of cropping in specific rotation sequences, significant differences in organic C and N have resulted. Because of the initial homogeneity, it is concluded that these differences have resulted from differences in the amount and nature of crop residue returned to the soil.

3. Organic C and N losses have resulted in all cases but one, and in general organic C losses are paralleled by N losses.

4. For the system studied, rotations containing corn resulted in smaller losses of C and N whereas O-Be-B and Be-B rotations resulted in larger losses of C and N.

5. The amount of N mineralized in the AMN and BMN methods was significantly and linearly related to the initial concentration of mineralizable substrate (WBC and TN).

6. Significant differences in the amount of potentially mineralizable N were shown to exist and in general were high where C and N levels were high and where losses were lowest.

7. The significant differences between potentially mineralizable N estimates were not substantial enough to influence sugarbeet yield parameters at this time.

8. Cumulative uptake in the greehouse at the 0 N level was not linearly related to estimates of potentially mineralizable N as has been observed in the literature. Significant differences in cumulative uptake between sequences were observed at all three N levels.

9. The relative response to applied N indicates that the O-Be-B and Be-B rotations appear to benefit most from applied N while rotations containing corn produced the lowest response. This response appears to follow trends in losses of C and N and trends in estimates of potentially mineralizable N.

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