MINERALIZATION OF SOIL ORGANIC NITROGEN AS INFLUENCED BY ORGANIC AMENDMENTS, NITROGEN FERTILIZER, CROP SEQUENCE AND TIME

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FREDERICK AU

AN ABSTRACT

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ABSTRACT

Soil samples were taken in the fall of 1957 from field plots on which various residue treatments were initiated two to six years earlier. These treatments were initiated prior to corn in a five-year rotation consisting of corn, beans, barley and two years of alfalfa-brome meadow. Five replications of each treatment were established each year on separate blocks of plots in five successive years, beginning in 1951. The four treatments included (1) the check and plots to which the following organic materials were incorporated into the soil prior to corn: (2) All hay grown during the second year of alfalfa-brome meadow, (3) 4 tons of wheat straw, and (4) 35 tons of sawdust. One-half of each plot received supplemental nitrogen with the first three crops in the rotation. The soil was Sims clay loam.

Yields of corn, beans and barley were depressed by the massive sawdust treatment. Supplemental nitrogen did not completely overcome this depression. Six years after sawdust treatment, corn yields were greater than for any other treatment. Carbon and nitrogen retained after six years were greater for this than for any other treatment.

Yields of corn were depressed following the four-ton straw application. Yields of later crops were unaffected. Retention of carbon and nitrogen in the soil was not materially greater than in the check.

Two cuttings of alfalfa-brome returned to the soil prior to corn did not influence the yields of any crop in the rotation. Carbon and nitrogen contents of the soil after six years were only slightly greater than in the check.

Release of nitrate and CO_2 from incubated soil samples reflected microbial immobilization of nitrogen for three years after the 35-ton

application of sawdust. A surplus of energy carbon in these soils was reflected by extremely high microbial activity (CO_2 evolution). These soils were characterized by C:N ratios wider than normal for the soil and by C:N ratios of mineralization wider than the C:N ratio of the soil itself. Such soils released nitrate during incubation in inverse proportion to the quantities of CO_2 produced.

Soils in which surplus carbonaceous energy materials were largely dissipated tended to stabilize at a C:N ratio which appeared to be characteristic for this soil (11:1). C:N ratios of mineralization tended to be equal to or less than the C:N ratio of the soil itself. Nitrate was released from such soils in direct proportion to the quantities of CO_2 produced.

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INTRODUCTION

Much work has been done on methods for estimating the nitrogen supplying power of the soil. Numerous methods proposed have met with limited or local success. The advent of an increased use of nitrogen fertilizers demands as thorough as possible a re-evaluation and/or augmentation of our present knowledge concerning this subject.

In the present study, interest has been centered on the influence of organic amendments, nitrogen fertilizers, crop rotation, and time on biological and chemical properties of the soil and their relation to crop yields. The rotation experiment which was used as the basis for the study was established in 1951. Laboratory investigations were initiated in 1957. The first year's data were the subject of a master's thesis by J. A. Mora (39). The results of a second year's continuation of this research are reported here.

OBJECTIVES

The two objectives of this work are:

1. To obtain chemical and biological data which may be used in later studies to correlate the nitrogen supplying power of the soil with crop yield.
2. To evaluate the effects of residue treatments with and without supplemental nitrogen on chemical and biological properties of Sims clay loam through the course of a five-year rotation.

REVIEW OF LITERATURE

The decomposition of organic materials in soil is a complex process which has engaged the research efforts of numerous investigators for the past two centuries. As experience accrued, it became apparent that decomposition processes were responsible for the dynamic nature of soil as a natural body. The processes themselves and their end products were found to influence the chemical, physical, and biological properties of soils in complex ways. Because of this complexity, the literature in this area is full of apparent contradictions. Some general patterns of behavior have carried through much of this work, however, and have been loosely formalized and accepted as guiding principles of soil management. The review which follows is concerned principally with the relationships between decomposition processes and nitrogen transformations in soil, particularly as these relate to the availability of nitrogen to crops.

Effects on Crop Growth of Organic Materials Added to Soils

It has long been known that the addition of green manure or other organic materials to soil may result in either beneficial or deleterious effects on crops. In 1915 Wright (88), studying the effect of turning under organic materials in an undecomposed form, found that the amount of available nitrogen in soil decreased when mature plant materials were used. On the other hand, no depressive effect occurred when succulent green manure was used. He concluded that, for maintenance of nitrogen supply in soil, green manuring was a good practice. Later workers (72) showed that the incorporation of carbonaceous organic materials, such as sawdust and straw, into soils depressed the level of nitrate in soils. Addition of such materials also retarded tree growth and decreased crop

yields (16,48,61). It was shown that the amounts added and the particle size of these materials governed the degree to which these effects were expressed in a soil (40,58,61).

Explanations for nitrate depression by certain organic materials were not apparent at first. It was known that incorporation of manurial materials into soil produced a more vigorous growth and activity of soil microorganisms (34,81). Smith (62) found that disappearance of nitrates in soil was undoubtedly due to assimilation by microorganisms. He discovered that when straw was added to soil, the rate of assimilation of nitrates by microorganisms was greater than the rate of production, and that crops suffered from an actual lack of nitrates. Allison (2) obtained similar results when he added readily decomposable materials of wide C:N ratios to soils. Although the nitrogen content was maintained at higher levels in soil receiving more resistant materials, very little of the nitrogen was present in the ammonium or nitrate form. Peevy, et al (47) believed that this stabilization of nitrogen by materials more resistant to microbial decomposition was due to the formation of "lignoprotein" complexes.

Processes which result in the tying up of mineral nitrogen in organic combinations which are unavailable to plants have come to be known by the general term, "immobilization." Processes which result in the release of mineral forms of nitrogen which are available to plants are grouped under the term, "mineralization" (7,24). A diagrammatic representation of the recognized processes involved in mineralization and immobilization of nitrogen in soils is presented in Figure 1, Page 5.

Figure 1 shows that the immobilization and mineralization of nitrogen and carbon are associated. The principal agents in the immobilization of

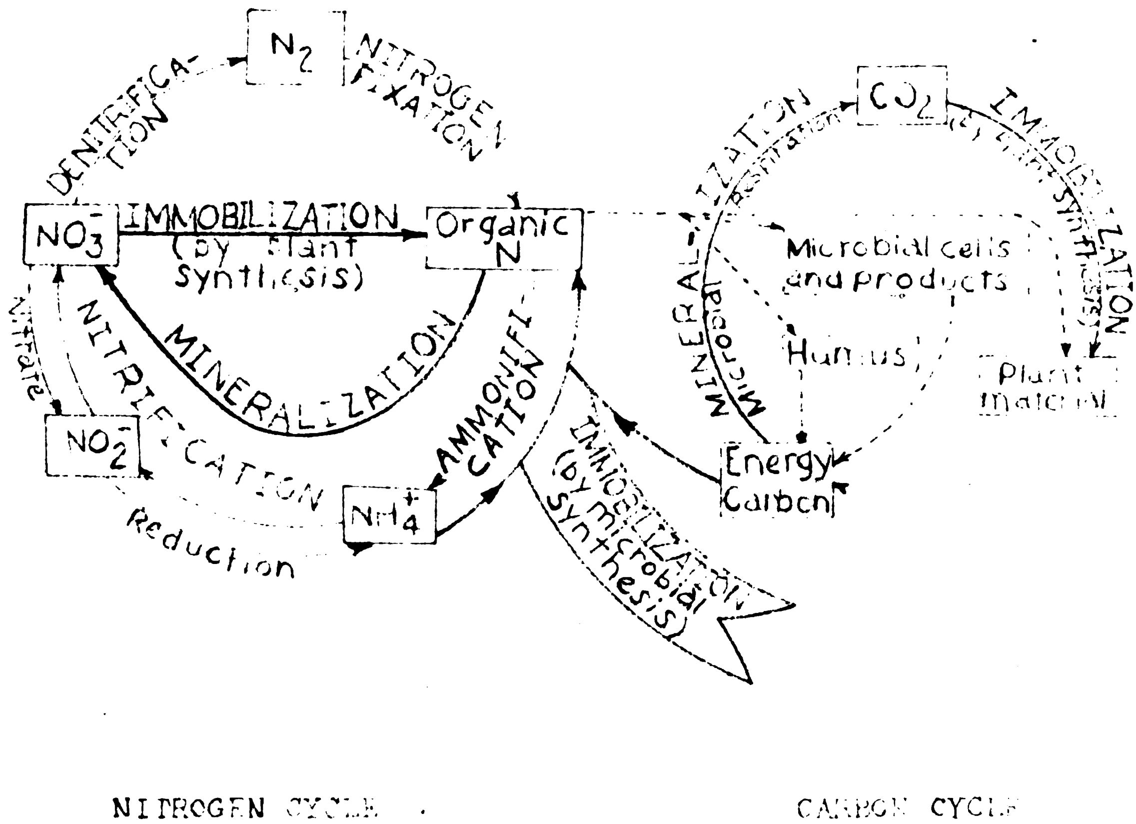

Figure 1. Interlinkage of nitrogen and carbon cycles in mineralization and immobilization processes in the soil.

nitrogen and carbon are the higher plants. Microorganisms are principally responsible for the mineralization or release of nitrogen and carbon as nitrate and CO_2, in which forms they are again available to higher plants.

Microbial immobilization is incidental to the decomposition of plant materials. The absolute quantities of nitrogen and carbon which may be immobilized in the form of microbial cells at any given time is a function of the number of cells, or the size of the microbial population. When plant materials which contain a large proportion of carbon to nitrogen are added to soils, the size of the decay population and the quantity of nitrogen immobilized in microbial cell materials may increase temporarily to levels which seriously deplete the soil of the mineral forms (principally nitrate) which are essential for plant growth.

Stojanovic, et al (67), using nitrate and ammonium salts labelled with N^{15} found that, in the presence of corn leaves, 19 pounds of nitrogen per acre per day was immobilized. During the same period, 27 pounds per acre per day was mineralized, resulting in a net increase in mineral nitrogen of 8 pounds per day. In the presence of wheat straw, 95 per cent of added ammonium, or 38 pounds per acre per day, was immobilized while 18 pounds per day was mineralized from soil organic matter. This resulted in a net immobilization of 20 pounds per day. From their calculations, it was apparent that fertilizer nitrogen can be absorbed by soil organisms at much faster rates than by growing crops. However, where soil conditions are favorable, net immobilization is a rather transient phenomenon, and nitrogen immobilized by microorganisms is again released very rapidly. They found that nitrogen utilized by

plants came partly from soil organic matter, even when fertilizer nitrogen had been added to the soil.

Carbon-nitrogen ratios of organic materials control to a great degree the rate of mineralization of the nitrogen they contain. As a general rule, materials with wide ratios have their nitrogen less immediately available to plants than those with narrow ratios. For example, a fresh plant material with an 80:1 ratio decomposes more slowly than one with a 40:1 ratio because in such materials nitrogen is the first limiting factor in the size and activity of the microbial population responsible for decay. Materials with ratios less than 20 or 30, contain more nitrogen than is required by the microorganisms so that the excess is liberated as ammonia which is later nitrified to nitrates (9). Waksman, et al (80), found, as a general rule, that plant material with a nitrogen content of 1.7 per cent has a sufficient quantity to allow microbial decomposition to proceed rapidly without net immobilization or depression of nitrogen availability.

However, the chemical composition, other than nitrogen content, plays an important role in the decomposability of organic materials and the mineralizability of nitrogen (12). Rubins, et al (55), found that as a rule, materials of low protein content showed correspondingly high proportions of hemicelluloses, celluloses, and lignin. It was the relative ease with which the first two compounds decomposed that rendered the nitrogen of these low-protein content materials relatively unavailable. These investigations showed that the C:N ratio did not always indicate the availability of nitrogen in organic materials. The type of carbon associated with

nitrogen in the materials could influence the release of available nitrogen. For example, castor pomace with a C:N ratio of 9.36 and a lignin content of 32.2 per cent released nitrogen more rapidly than cottonseed meal with a C:N ratio of 5.40 and a lignin content of 5.4 per cent. Since the carbon in lignin is rather resistant to microbial attack, it represents an energy source of low availability and, consequently, plays little or no role in depressing net mineralization of nitrogen. As a result, castor pomace, with its high lignin content, behaved like a material of lower C:N ratio, having a greater availability of its nitrogen.

The most important constituents of natural organic materials added to soil are carbohydrates (celluloses, hemicelluloses, sugars, and starches), tannins, fats, waxes, lignins, proteins and their derivatives. These various chemical constituents are attacked at different rates. Sugars and starches, some hemicelluloses and some proteins undergo a most rapid decomposition by a great variety of microorganisms. Cellulose, certain hemicelluloses, some fats, and oils are decomposed more slowly, and by specific organisms. Lignins, some waxes and tannins are most resistant to decomposition. However, the content of available nitrogen in the soil was found to be the most important factor controlling cellulose decomposition (77).

The composition of organic materials varies with the degree of maturity of plants, and maturity influences the rate of decomposition. Green plants are rich in soluble sugars and soluble nitrogenous compounds; mature plants are rich in hemicelluloses, celluloses, and lignins (73). Waksman, et al (80), found that mature plants decomposed more slowly than younger plants due to differences in the proportions of their chemical constituents.

Under normal soil conditions, the initial decomposition of plant material such as straw or hay is more or less rapid, depending upon species and age and the relative proportions of easily and difficulty decomposable constituents. As decomposition progresses and the more readily decomposable constituents disappear, the process becomes much slower until a certain level is reached, when the residual mass changes from brown to black (74). This residue is humus. It includes the slowly decomposing resistant constituents of the original plant materials and of successive past generations of microbial cells. The formation of chemical complexes between lignaceous and nitrogenous constituents may contribute to the low decomposability of humus(11, 36, 47, 78). In spite of its resistant nature, humus is decomposed slowly and continuously. Because of its high nitrogen content and low energy availability, decomposition of humus is accompanied by net mineralization of nitrogen. This slow but continuous release of nitrogen from humus represents the principle nitrogen supply for plants in nature and for most crops under cultivation.

Environmental factors which may affect decomposition and availability of nitrogen in soils can be briefly mentioned. Russell, _et al_ (57), pointed out that temperature, moisture and dissolved oxygen in rain water were important in the biochemical decomposition of soil organic matter. They noted that decomposition did proceed noticeably below 5° C., and that rainfall was unusually effective in initiating decomposition. Temperature effects on soil were studied by Panganiban (43) who found that ammonification took place between 15° and 60° C., the rate increasing with rise in temperature. Nitrification occurred between 15° and 40° C. The optimum temperature for nitrification was 35° C. or slightly higher. More recently, Rothwell, _et al_ (54), have found that nitrification proceeds at a low but significant rate at temperatures as low as 5° C. On the other hand,

microbial immobilization of nitrogen may become increasingly important at low temperatures: Greaves, et al (22), showed that larger numbers of of microorganisms developed in soils stored at 10° C. than at higher temperatures.

Bollen (9) observed that 60 percent moisture saturation capacity was optimum for ammonification and nitrification. However, 75 per cent of saturation capacity was found to be the optimum moisture content for carbon dioxide evolution in prolonged respiration experiments. Waksman, et al (79), found that soil moisture equivalent to 50 per cent moisture-holding capacity was optimum for carbon dioxide evolution.

Noteworthy work on the effects of oxygen and carbon dioxide concentrations on soil nitrification and ammonification has been reported by Plummer (49). He found that the relationship between carbon dioxide concentration and nitrate production was governed by the partial pressure of oxygen. When the oxygen in the soil atmosphere was reduced below two per cent, denitrification occurred.

The extensive changes which occur when a soil is subjected to drying and remoistening have been noted by numerous workers. Allison (1) concluded that air-drying caused a decided decrease in bacterial numbers.

Lebedjantzev (33) observed that air-drying of soil brought about an extremely large increase in ammonium and amide nitrogen, a sharp decrease in numbers of microorganisms, and a large increase in solubility of organic substances. Waksman (75) reported an increased liberation of ammonium when any change was imposed upon physical, chemical or microbiological equilibria of a soil. Among the treatments which were found to bring about such changes was drying, followed by moistening.

Birch, et al (8), found evidence that clay played a role in the effects of drying and remoistening. They noted that the drying effect was one of liberation from the clay of rapidly decomposable material which, under steadily moist conditions, was protected by the clay from microbial attack. The kind and amount of clay and the amount of organic material associated with it were also important.

An adequate supply of nutrients, notably phosphorus, is also needed for rapid decomposition. Kaila (30) concluded that phosphorus equivalent to 0.1 to 0.4 per cent of the dry weight was required for decomposition of natural organic material. As an average, 0.2 per cent phosphorus appeared to be the critical level below which decomposition was retarded and immobilization of mineral phosphate occurred. Chang (14) reported a marked increase in rate of decomposition of straw when dipotassium phosphate was applied to mature straw compost.

Soil reaction is another factor that influences the patterns of nitrogen transformation in soil. Potter, et al (50), showed a greater gain in soil nitrogen in limed than unlimed soils. Fraps, et al (19), found that addition of $CaCO_3$ stimulated nitrification. Jensen (28), studying the mineralization of organic nitrogen, observed that the critical C:N ratio of added materials was strongly influenced by soil reaction. In an acid soil, pea pod meal with a C:N ratio of 13.3:1 was the only material that showed an increase in inorganic nitrogen. No mineralization occurred in an alkaline soil where the C:N ratio was 26:1 or above. Below this, the release of nitrate increased rapidly with decreasing C:N ratio.

Seasonal variations in carbon dioxide production, nitrate accumulation and bacterial numbers have been reported in soil. Russell, et al (57), found very little activity during the winter months. Bacterial numbers, carbon dioxide production and nitrate accumulation all increased with

temperature above 5° C. In general they observed successive periods of spring activity, summer sluggishness, autumn activity, and winter inertness.

The presence of roots of growing crops and the sequence of crops in a rotation have been reported to affect mineralization of nitrogen and soluble phosphorus content. Kubota (32) found that a rotation which included alfalfa reduced soluble phosphorus content of soil more than a comparable rotation without a legume. Brown (13) observed that rotation of crops resulted in greater numbers of soil organisms as well as greater ammonifying and nitrifying powers in soil than continuous cropping to corn or clover. Carbonaceous matter exuded or abraded from roots of growing plants has been shown to favor development of nitrate consuming organisms in soil with a consequent transformation of nitrates to insoluble organic forms (35). Lyons, et al (35), pointed out that certain plants differ in their ability to take up nitrogen from the soil because of characteristic differences in the amount or composition of the organic matter liberated by their roots. Goring, et al (21), investigating the influence of crop growth on mineralization of nitrogen in soil, concluded that less mineral nitrogen accumulated in cropped soils than in fallow soils. They believed that nitrogen unaccounted for in the cropped soils was immobilized in the soil and was not lost to the air.

The depressive effects of plant materials on crop growth are principally due to depletion of the available soil nitrogen, and sometimes phosphorus, by microbial assimilation, rather to toxic constituents of these materials (4).

Crops may be benefited in a number of ways by the addition of plant

materials to soils. Murray (40) found that incorporating green manures into a soil, not too low in humus, brought about a priming action which promoted an intensified mineralization of soil humic nitrogen. Birch et al (8), attributed this priming action, not to an increase in microbial activity, but to an exchange displacement of organic compounds, already associated with and protected by clay, by similar organic compounds released during the decomposition of the freshly added organic materials. Hallam, et al (23), concluded that green manure crops served the most useful purpose as immobilizers and conservers of plant nutrients, or, in the case of legumes, as possible contributors of nitrogen.

Incorporation of materials with narrow C:N ratios can have immediate beneficial effects on crop growth. However, microbial immobilization of nitrogen by materials with a wide C:N ratio has been reported to have useful residual effects. Allison (2) noted that an immediate harmful effect resulted from adding materials of a wide C:N ratio to soil. However, the ultimate effect of this action was beneficial, provided sufficient time was allowed for the nitrate supply to return to normal. He attributed these results to a temporary increase in biological activity, followed by a slowing up of this activity until a point was reached where the proteins assimilated in microbial cells were made available to plants through their death and the subsequent ammonification and nitrification of the microbial remains.

Plants take up from the soil through their roots a number of different elements in the form of mineral salts. One way in which these elements are added to the soil is through plant residues. Essential plant nutrients, including sulphur, phosphorus, magnesium, calcium, copper, zinc, boron,

manganese, are held in plant residues largely in insoluble forms which are unavailable to the succeeding crop. These are released in soluble mineral forms by microbial mineralization in a manner similar to that previously described for nitrogen and carbon. Tottingham, et al (69), found that, when manure was added to soil, the rapidly growing bacteria caused an impressive decrease in the water-soluble phosphorus of the manure and transformed it into organic phosphorus. Eventually, this was released in an available form as a result of bacterial action on dead microbial cells, after the more available energy materials were used up.

Inorganic acids, such as H_2CO_3, H_2SO_4, HNO_3, and organic acids are formed during the decomposition of plant residues. These serve to solubilize soil minerals, releasing essential nutrients in forms available to plants.

Plant materials are sources of carbon and nitrogen for the maintenance of soil organic matter or humus. The latter displays typical properties of hydrophilic colloids: Its ability to absorb considerable quantities of water makes it an important factor in determining the water-holding capacity of the soil; it takes part in base exchange reactions; and it is subjected to dispersion and flocculation phenomena which play an important role in crumb formation and aggregate stabilization (56). As has been noted (p. 9) the slow but continuous release of nitrogen through decomposition of humus represents the principle source of nitrogen for many crops in most soils.

Residue Management and Maintenance of Humus

It is generally accepted that equilibrium levels of soil organic matter are much lower under cultivation than in virgin soils under forest or grass vegetation (6,56). This is due to the fact that all soil

management practices which promote optimum crop growth also promote higher numbers of microorganisms, higher levels of microbial activity, hence more rapid decomposition of soil organic matter (44). Livestock manures and legume grass sods have proven most effective in maintaining soil organic matter levels in the face of the depletive influence of cultivation (56).

Livestock systems of farming are being replaced by cash crop systems on many farms. This has prompted renewed interest in research designed to re-evaluate other organic materials in terms of their usefulness in maintaining soil organic matter. Some of these investigations are concerned with normal cash crop residues, such as corn stalks, or cereal straw,- materials which can be grown without sacrifice of cash return in any year of the rotation (37). The use of catch crops and cover crops falls in this same category (52,53). Other investigations have involved the use of extraneous organic materials, such as sawdust, large quantities of which are frequently produced as waste by-products in areas where transport distances might not prohibit their economic transfer onto agricultural land (4,10).

Among the concepts which have been and are being tested in such studies is the role of supplemental nitrogen in stabilizing carbon and promoting greater retention of resistant materials in the form of humus. In general, such increases in organic matter level as have been observed as a result of nitrogen fertilization have been attributable to increased residue production due to increased crop growth rather than to any sparing action of nitrogen on carbon loss (3). However, it does appear that such a sparing action may be expressed with materials high in lignin or at advanced stages of decomposition of other materials (29). Such a result

would be expected on theoretical grounds, considering the high degree of resistance to microbial attack which has been shown for chemical complexes of ammonium and proteins with lignin or soil humic materials (11). Numerous investigators have shown increasing retention of carbon in soils with increasing nitrogen treatment (10,27,59,71). It is known that soil organic matter can fix large quantities of ammonia by strictly chemical processes (36,63). To what extent this phenomenon influences the efficiency of ammonium fertilizers and the stability and properties of humus is not known.

Estimating Availability of Soil Nitrogen

Under intensive systems of culture, as in the greenhouse, it has been found possible to relate crop growth to the level of nitrate in the soil (64). Under field conditions, however, nitrates are subject to unpredictable losses due to leaching, crop removal, denitrification and biological or chemical immobilization. As a result, nitrate revealed by chemical test at any given time may or may not bear a relation to crop performance.

Approximately 98 per cent of the nitrogen in soil is present in organic materials,-plant residues, microbial cells or humus. The soil's nitrogen supplying power is primarily a function of the quantity of this combined nitrogen which is present and the rate at which it is released or mineralized by microbial decomposition. Procedures for estimating nitrogen supplying power in soils have, therefore, been based on methods for estimating either total nitrogen or mineralization rate, or both. These procedures may be classified into three general categories: 1. Estimation of total organic nitrogen; 2. Measurement of labile fraction of soil

organic nitrogen; 3. Microbiological methods for estimating mineralization rate or mineralizability.

Gainey (20) observed that a very close and direct relationship existed between nitrogen content of soils and their nitrate accumulating ability. He obtained a correlation of 0.990 ± 0.012 for a "nonfertile" series of soils and 0.988 ± 0.0006 for a "fertile" series. Fraps (18) concluded from results with pot experiments that the average size of the crop, and the nitrogen withdrawn from the soil, increased with the total nitrogen content of the soil. Allison, et al (5), showed that a positive correlation existed between total soil nitrogen and nitrate formed from soil organic matter at all incubation periods for limed and unlimed soils. Woodruff (87) was able to estimate the rate of nitrogen delivery to crops from a chemical determination of soil organic matter. The organic matter determination was actually an estimate of total nitrogen, since a uniform nitrogen content was assumed. An empirical mineralization factor was calculated using yield data from long term fertility experiments.

Truog (70) proposed a method for extracting a labile fraction of the nitrogen from soil organic matter by partially oxidizing the latter with alkaline permanganate. It was presumed that the solution attacked the readily oxidizable portion of the soil organic matter. By this oxidation, nitrogen is released as ammonia and is measured together with exchangeable ammonia. Thus, it appeared that the method would provide a direct measure of the two most immediately available sources of ammonia for the nitrification in soils. These, in turn, should be related in a straight-forward manner to a soil's ability to release nitrate for crop use.

Fitts, et al (17), found that under Iowa conditions nitrate produced during incubation under standardized conditions provided a basis for

predicting the nitrogen requirements of corn. They obtained a negative correlation between nitrifiable nitrogen and yield response of corn to nitrogen fertilization on Iowa soils. Saunder, et al (60), used this technique as a means for estimating available nitrogen on some Southern Rhodesia soils. They found that the nitrogen mineralized in laboratory incubated soils, sampled towards the end of the dry season, appeared to provide a good index of the nitrogen likely to be available for crop use under field conditions during the subsequent growing season. The authors noted a time-lag between the start of ammonification and the start of nitrification. This lag was still noticeable after 5 weeks of incubation. Thus, it was necessary to determine the time when nitrification began for each soil type and for different environmental conditions before a reliable estimate of mineralization or nitrification rate could be made.

Waksman, et al (77), believed that the cellulose-decomposing power of a soil could yield information on the total availability of soil nitrogen. Whereas nitrification was found to be a good index of soil fertility, ammonification was not (76).

Since a principal end-product of aerobic heterotrophic microbiological processes is carbon dioxide, the evolution of carbon dioxide from incubating soils has been used as an index of the respiratory activity of soil microorganisms. Waksman, et al (79), obtained data which indicated that the determination of the amount of carbon dioxide evolved from incubating soils, as well as estimates of the number of microorganisms and of the nitrification yield of the soil, could be used as indices of soil fertility (76). Stoklasa (68), in 1912, stated that where there was greatest nitrification, there was found the greatest production of carbon dioxide. However, Patrick (45), attempted to find out why crops following

red clover should yield more than those following timothy or corn. He found that high carbon dioxide evolution was correlated closely with nitrate depression. These conflicting results were probably due to differences in composition of the crop residues remaining in the soils.

Oxygen uptake may be used to measure microbiological activities, and current attempts are being made to use it as a basis for estimating soil fertility levels (31).

MATERIALS AND METHODS

Field Treatments and Cropping History

Forty soil samples, composited by treatment and by year of establishment from the "Michigan rotation" plots at the Ferden farm in Saginaw County were used in this experiment. The soil is classified as Sims clay loam.

The "Michigan rotation" was originally established to determine the effect on crop yields of the addition of large amounts of sawdust in comparison with more normal quantities and types of residues. The five-year crop sequence for the rotation was corn, beans, barley, and two years of alfalfa-brome.

Fertilizer treatments were as follows:

Corn: 100 pounds 5-20-10 per acre

Beans: 200 pounds 0-20-10 per acre

Barley: 240 pounds 5-20-10 per acre

Alfalfa-brome: no fertilizer either year

This crop sequence had been in effect for three complete cycles of the rotation before the following residue treatments were initiated:

1. Two-year-old alfalfa-brome (two cuttings of hay removed), followed by corn, beans, barley and two years of alfalfa-brome.
2. Same as treatment one, except that neither cutting of the second year of alfalfa-brome was removed.
3. Same as treatment one, except that 35 tons of sawdust per acre was applied after removal of the second cutting of hay on the second year of alfalfa-brome.

4. Same as treatment three, except that four tons of wheat straw was applied instead of sawdust.

Residue treatments were initiated on a complete block of plots in each of the following years: 1951, 1952, 1953, 1954, and 1955. The soil block which was cropped in 1956 was the same as that on which treatments were established in 1951. On this block, in 1955, two cuttings of alfalfa-brome hay were returned on treatment two, and four tons of wheat straw was applied on treatment four, as was done in 1951 before the first cycle of the rotation was initiated. However, the application of sawdust on treatment three was not repeated in 1955 preceding the second cycle of the rotation which began on this soil block in 1956.

Through the first cycle of the rotation, one-half of each plot planted to corn received a supplemental side-dressing of 40 pounds per acre of nitrogen, except for the sawdust treated plots which received 120 pounds. Beginning with the second cycle of the rotation in 1957, one-half of all corn plots received 100 pounds of supplemental nitrogen per acre. One-half of each plot planted to beans has consistently received 40 pounds of nitrogen per acre. On one-half of each plot of barley, 20 pounds of supplemental nitrogen was applied as topdressing, except in 1952 when 40 pounds was used and in 1956 and 1957 when the supplemental application was discontinued due to lodging which accompanied a change in the variety planted.

The treatments were replicated five times on the soil block which was established each year. Soil samples were taken from each of these treated plots. Replicates were then composited by treatment and by year of establishment. The samples for the present study were taken in September

1957. They were obtained from plots which represented the second, third fourth, fifth and sixth years after the initial residue treatments were made.

Laboratory Determinations

The following laboratory determinations were made on air-dried soil samples:

A. Soil pH was determined with a glass electrode, using a 1:1 soil-water suspension.

B. Ammonium and nitrate nitrogen were measured in Morgan's extracting solution using procedures described by Peech and English (46). Ammonium determinations were made by direct nesslerization. Nitrates were determined by the phenoldisulfonic acid method (15).

C. Alkaline permanganate oxidation, as described by Truog (70), was used to determine "available organic soil nitrogen," including ammoniacal soil nitrogen.

D. "Nitrifiable nitrogen" was estimated as nitrate formed during a two-week incubation period, following a slight modification of the procedure developed by Stanford, et al (66). Ten-gram soil samples were used. The soil was mixed with an equal volume of vermiculite. The mixture was placed on another layer of vermiculite over a glass wool pad in a carbon filter tube. A covering layer of vermiculite was placed on top. Nine ml. of a 0.2 per cent water solution of synthetic soil conditioner[1] was added and allowed to remain in contact with each sample for

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[1]"Loamaker," a proprietary product of the Monsanto Chemical Co. was used.

15 minutes before beginning the initial leaching. This was done to assure clear leachates. The moisture was adjusted by applying suction. The tubes were stoppered with one-hole rubber stoppers. Samples were incubated at 35° C. in a chamber adjusted to a constant relative humidity of 98 per cent. The latter was achieved by exposing shallow pans filled with 2 per cent H_2SO_4 in the bottom of the incubator. Nitrate produced was determined after two weeks by the phenoldisulfonic acid method (25) in water extracts cleared of colored organic materials by filtration in the presence of dry $Ca(OH)_2$.

E. Total carbon was calculated from the weight lost during ignition, according to the procedure described by Mitchell (38). Duplicate 25-to-40 g. samples were ignited in a muffle furnace at 380° C. for 8 hours. Total carbon was also determined by dry combustion in a carbon train on duplicate samples of 17 soils. The error regression of total carbon on ignition weight loss for the paired duplicates of this group of 17 soils was used as the basis for calculating total carbon from weight loss for all soils: $y = 0.30 + 0.415X$; $r = 0.94^{**}$.

where: Y = total carbon

X = ignition weight loss

r - correlation coefficient (significant at 1 per cent)

F. Total nitrogen was determined by standard Kjeldahl procedure as modified by Prince (51). The catalyst was a mixture of $CuSO_4$, HgO and K_2SO_4. Methyl red (0.1 per cent in 95 per cent ethanol) was selected as the indicator.

G. Rate of respiration was measured by the simultaneous CO_2 absorption method of Norman, et al (41). One-hundred-grams of soil was placed in two quart Mason jars, and moisture adjusted to 70 per cent of

water holding capacity. The soils were incubated for two weeks at 35° C. The soils were aerated every four days to supply sufficient oxygen for maximum CO_2 production. Vials containing 0.5N NaOH were placed in each jar to collect CO_2 at one-to four-day intervals, depending on rate of CO_2 evolution. Carbon dioxide produced was calculated after titrating the contents of each vial with standard HCl in the presence of an excess of $BaCl_2$. Phenolphthalein was used as indicator.

H. Reserve phosphorus (P_2O_5) was determined in soils by the method developed by Spurway and Lawton (65).

All determinations were in duplicate, except for incubation CO_2 measurements.

EXPERIMENTAL RESULTS

In the experimental design of the present work, three factors were confounded in such a way as to make it impossible to relate experimental results directly to initial treatments in a cause and effect relationship. The time elapsed since treatment was confounded with soil differences between blocks of treatments which were established in different years. Both of these factors were systematically related to the sequence of crops in the rotation. Thus, a single year's data provide no basis for distinguishing between: (a) Soil effects; (b) effects of residues from immediately preceding crops; and (c) the changing intensity with time of specific effects associated with the initial treatments.

The comparison of one year's data with another, however, does permit some separation of these confounded effects. This will be true, particularly, when data for a complete 5-year rotational sequence are available. To facilitate such comparisons, all data have been tabulated so as to permit ready identification of soil blocks with years after treatment and the crop grown during the sampling year. In the discussion, a tentative comparison is made with the previous year's data as reported by Mora (39).

Laboratory Determinations

Soil reaction (PH)

Table 4 (Appendix) contains the results of determinations for soil reaction. The lowest PH was found the second year after treatment. In the previous work (39), this soil block was found to be lower in pH than the others. Thus, the principle differences in soil reaction observed in Table 4 appear to have been related to soil differences rather than to treatment or the immediately preceding crop sequence.

Reserve phosphorus (P_2O_5)

Determinations for phosphorus extracted with 0.13N HCl (65) are presented in Table 5 (Appendix). The smallest amounts recovered were from the second year and the largest from the fourth year after treatment. The same soil blocks were low and high in the previous year's data. The results tend to indicate that soil variation was more of a factor than treatment or cropping sequence in determining the level of reserve phosphorus in these soils.

Total carbon

The results of total carbon determinations by the ignition method are recorded in Table 6 (Appendix). The actual values obtained were of the order of 30 per cent greater than those obtained the previous year by Mora, who used the wet combustion method (51). The values reported here for total carbon are undoubtedly erroneously high because an undetermined quantity of water of hydration was included in the weight lost during ignition. In the group of 17 soils for which carbon determinations were made using both dry combustion and loss on ignition, the ratio of ignition weight loss to total carbon was approximately 2.11:1. This is considerably greater than the factor 1.72 which is conventionally used to convert total carbon to organic matter.

Loss on ignition was selected as the method for determining carbon in the present study because it was felt that the use of large samples of soil would minimize sampling error, particularly in the sawdust-treated soil. However, this expectation was not fulfilled. Using 25-to 40-g samples, a standardized ignition temperature of 380°C. and a standard 8-hour ignition period, the precision of the determinations was such as to yield a relative standard deviation between duplicates of 6.5 per cent of the mean. Using one-to two-gram samples in the carbon train the relative standard deviation was 1.8 per cent of the mean.

The rather low precision and the erroneously high carbon recovery of the loss-on-ignition determinations were not recognized early enough to permit the use of a different procedure. The results are presented here because they are the only data available. Some interpretations can be drawn from the relative differences between treatments and years.

The total carbon data are presented graphically in Figure 2. As would be expected, the highest levels of total carbon were found in the sawdust-treated plots. Two years after sawdust application, there were seven to ten tons more carbon in the sawdust-treated plots than in the checks. By the sixth year after treatment, this difference had been reduced to three to five tons.

However, there was no evidence that the level of total carbon in the sawdust-treated plots had declined from the second to the sixth year, as would have been expected from progressive decomposition of the large sawdust application. On the contrary, there was a marked tendency for carbon to increase. A similar increasing tendency was observed for all other treatments.

Comparison of the data in Figure 2 with the data reported by Mora (39) indicates that the observed trends were more closely related to crop sequence than to soil differences between blocks established in different years. Both years' data reflect a tendency for carbon to be depleted by the three tilled crops in the rotation (corn, beans and barley) and a strong tendency for carbon levels to be restored by the two years of alfalfa-brome meadow. In the present year's data, these effects tend to appear as residual effects which were observed in the year following a given crop. The values for the plots which were in barley (3rd year after

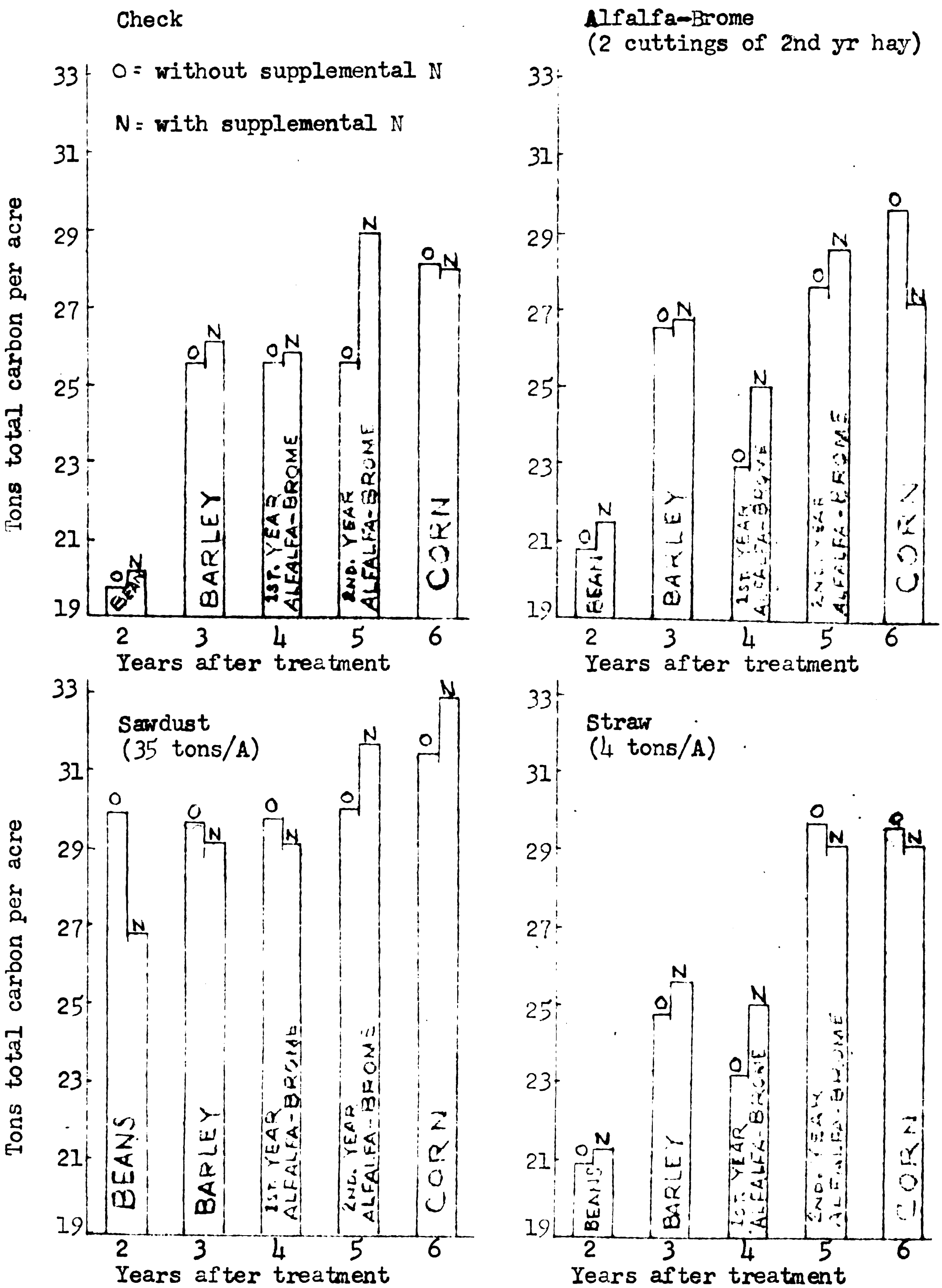

Figure 2. Total carbon in soil at yearly intervals after addition of various organic amendments with and without supplemental nitrogen fertilizer.

treatment) are not consistent with the general trend outlined above. Soil differences might account for this. Until data are available which cover the entire cropping sequence for each soil block, no final conclusions can be drawn regarding these crop effects.

It is of interest to note the effect of supplemental nitrogen on carbon retention in the sawdust-treated plots. As would be expected, supplemental nitrogen hastened the dissipation of carbon from the added sawdust during the early stages of decomposition. Less total carbon was found in the nitrogen supplemented plots through the fourth year after application of the sawdust. Higher total carbon values for nitrogen treatment during the fifth and sixth years after treatment may reflect larger return of carbon in residues from crops grown during the rotation. Another possibility is that added nitrogen exerted a "sparing" action on carbon loss due to the formation of resistent complexes between nitrogen and lignaceous constituents of the sawdust at advanced stages of decomposition, as has been suggested by Johnston (29).

A similar tendency for total carbon to be higher where supplemental nitrogen was used was observed for most years with the other three residue treatments. However, there were exceptions to this general trend, so the evidence cannot be considered conclusive.

Extracted forms of soil nitrogen

The results of ammonium determinations using Morgan's extracting solution are given in Table 1. The values obtained were approximately double those reported by Mora (39). The most probable explanation for this lies in the fact that these samples were held in the air-dry state for nine months prior to extraction, whereas the previous year's samples were extracted shortly after they were taken from the field.

Table 1.--Ammonium extracted with Morgan's solution, as influenced by organic amendments, nitrogen fertilizer, crop sequence and time.

Treatment	Soil block 55	54	53	52	51	Average
	Years after treatment 2	3	4	5	6	
	1957 Crop Beans	Barley	Alfalfa 1st year	Alfalfa 2nd year	Corn	
	NH3-N (pounds per acre)[a]					
Check	60	34	45	33	39	42
Check +N . . .	49	35	38	43	40	41
Alfalfa . . .	45	45	44	53	39	45
Alfalfa +N . .	64	41	43	59	28	47
Sawdust . . .	83	64	45	60	44	59
Sawdust +N . .	90	50	60	71	51	64
Straw	44	38	41	58	47	46
Straw +N . . .	58	37	26	56	50	45
Average for no N	58	45	44	51	42	48
Average for +N	65	41	42	57	42	49
Average for years	62	43	43	54	42	49

[a]Average of duplicate determinations

The largest quantities of ammonium were recovered from the samples taken two years after treatment. Mora had found the samples from this same soil block in 1956 to be much lower in extractable ammonium than the others. The high values for this block in 1957 appear to be related to the fact that this block of plots was flooded for a considerable period during July. How this flooding may have contributed to the high test for ammonium in samples taken in September is not clear, however.

Ammonium levels in the sawdust plots were consistently higher than for the other three treatments. A similar tendency, although not so marked, was observed in Mora's data. There was no apparent relationship between ammonium level and nitrogen treatment.

The data for nitrate-nitrogen are presented in Table 2. The largest quantities of nitrate were found in the flooded block, where beans were planted and removed before maturity. The high nitrate levels in this block were probably due to accumulation during the relatively long fallow period which preceded sampling. In this block, sawdust suppressed nitrate accumulation in the field, which agrees with Mora's data through the second year after treatment.

An effect of supplemental nitrogen on field nitrate levels was observed only in the sixth year after treatment. During this year, 100 pounds per acre of nitrogen applied as a side-dressing of ammonium nitrate on corn was reflected by a substantial increase in the level of nitrate nitrogen in the soil in the fall.

Mora had found no relationships between permanganate-soluble nitrogen and any of the experimental variables in 1956. None was found in the data for 1957. These are recorded in Table 7 (Appendix).

Table 2.--Nitrate extracted with Morgan's solution, as influenced by organic amendments, nitrogen fertilizer, crop sequence and time.

Treatment	Soil block 55; Years after treatment 2; 1957 Crop Beans	54; 3; Barley	53; 4; Alfalfa 1st year	52; 5; Alfalfa 2nd year	51; 6; Corn	Average
	NO_3-N (pounds per acre)[a]					
Check.	23	8	4	8	6	10
Check +N . . .	20	7	5	7	15	11
Alfalfa	18	10	5	10	7	10
Alfalfa +N. . .	18	10	6	10	22	13
Sawdust	7	7	7	11	7	8
Sawdust +N . . .	8	8	7	14	20	11
Straw.	16	8	8	13	8	11
Straw +N . . .	18	9	7	12	19	13
Average for no N	16	8	6	11	7	10
Average for +N	16	9	6	11	19	12
Average for years	16	9	6	11	13	11

[a]Average of duplicate determinations

Total nitrogen

Total nitrogen data are recorded in Table 8 (Appendix), and are presented graphically in Figure 3.

The average value for all total nitrogen determinations was 400 pounds per acre, or 10 per cent, greater than that reported by Mora (39). This difference between samples taken from the same plots in successive years was undoubtedly due to analytical differences, rather than to any substantial increase in level of soil nitrogen from one year to the next.

Relative differences between soil blocks and treatments were very similar in the data for the two years' samplings. In both years there appeared, superficially, to be a marked tendency for total nitrogen to increase with time after treatment. More careful analysis, however, indicated that these differences were due to initial differences in the soils in the blocks of plots which were established in successive years.

On all soil blocks in both studies, total nitrogen was higher in the sawdust-treated plots than in any of the others. In both year's samples, there was 300 to 400 pounds more nitrogen per acre in the sawdust-treated soils than in the checks. The alfalfa and straw treatments tended to promote a greater retention of nitrogen than in the check soils, however this effect was neither as great or consistent as in the case of sawdust.

The effects of supplemental nitrogen were erratic and the differences were not great. In both years, however, there was a tendency for total nitrogen to be lower on nitrogen-treated sub-plots during the early years after treatment and higher during the later years. Supplemental nitrogen may have stimulated a greater removal of nitrogen from the soil by the crops to which nitrogen was applied (corn, beans, barley). The residual

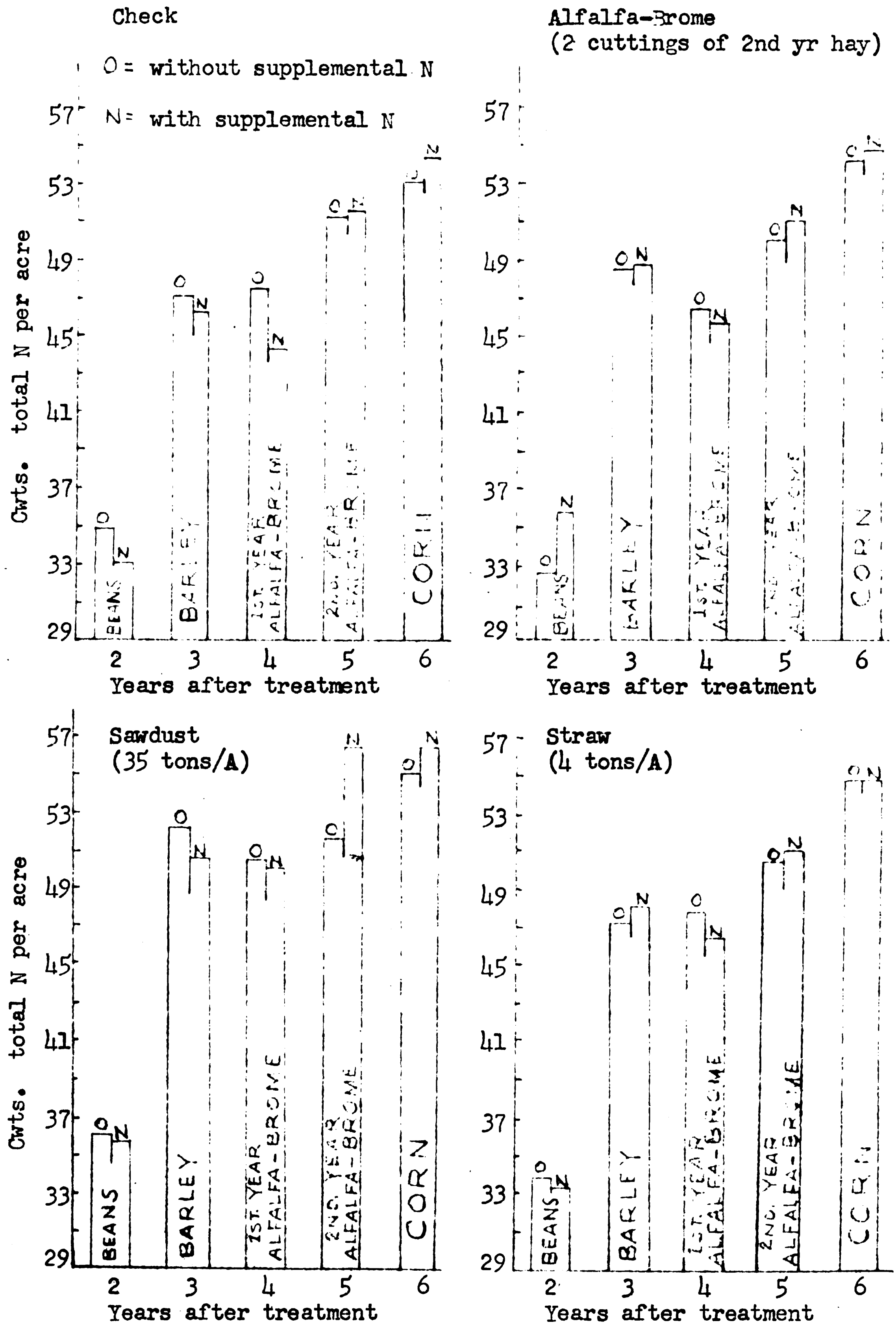

Figure 3. Total nitrogen in soil at yearly intervals after addition of various organic amendments with and without supplemental nitrogen fertilizer.

build-up under alfalfa-brome may have been due to the larger quantities of carbonaceous residues left as roots, stubble and stover from the larger crops of corn, beans, and barley which were produced where supplemental nitrogen was used. (See P. 29.) These could have exerted an immobilizing or stabilizing influence on nitrogen, similar to that exerted by sawdust. The residual build-up of nitrogen late in the rotation, after early supplemental applications of nitrogen, was most marked in the sawdust-treated plots.

Soil C:N ratio

As has been pointed out (P. 26), the total carbon figures reported here are not quantitatively reliable. Their value lies in the fact that they provide a basis for relative comparisons among treatments or soil blocks. This applies equally to the calculated C:N ratios which are recorded in Table 9 (Appendix).

As far as relative differences are concerned, two similarities were observed in these data and those reported by Mora. The widest C:N ratios were found in the block of plots established in 1955. This was due to the low nitrogen content of the soils in this block. (Cf. Figure 3. and Table 8.) Among the residue treatments, the widest C:N ratios were found during the first two years following the massive application of sawdust. This was due to the higher carbon content of these soils. (Cf. Figure 2 and Table 6.)

There was a tendency for C:N ratios in 1957 to stabilize at a value around 11:1. In Mora's work, this stable ratio, which appeared to be characteristic for the soil and cropping system, was 9:1.

Mineralization of carbon

Carbon evolved during incubation for 14 days at 35° C. is recorded

for the various soil samples in Table 10 (Appendix). The value of this determination is that it provides a measure of the availability to micro-organisms of carbonaceous energy materials remaining in the soil at the time the soil samples were taken. These energy materials include root remains from the immediately preceding crop, as well as true soil organic matter (or humus) and such partially transformed organic materials as may remain from previous crop residues or organic amendments.

The incubation respiration data are plotted graphically in Figure 4. The amount of CO_2 produced from soil two years after sawdust application was approximately double that for any later year or any other treatment. There was little difference among soils sampled three to six years after sawdust was added. These results are essentially the same as those reported by Mora (39). It would appear that surplus energy materials (cellulose, hemicellulose, etc.) in the sawdust were largely dissipated during the first two or three seasons after application. The more resistant materials which remained were relatively stable and did not change appreciably in energy availability during subsequent years.

The quantities of CO_2 produced from soils three to six years after sawdust application were consistently higher than for soils which had received the other residue treatments. This might be due, at least in part, to the fact that residual organic materials from the sawdust treatment may have been qualitatively different than residual materials from the other treatments. However, total carbon was much higher in the soils which received the massive sawdust treatment. (Cf. Figure 2 and Table 6.) The greater production of CO_2 would appear to be more closely related to total quantity of substrates remaining after extensive decomposition. These terminal substrates themselves would appear to be essentially similar regardless of initial treatment. C:N ratios were found to be very similar

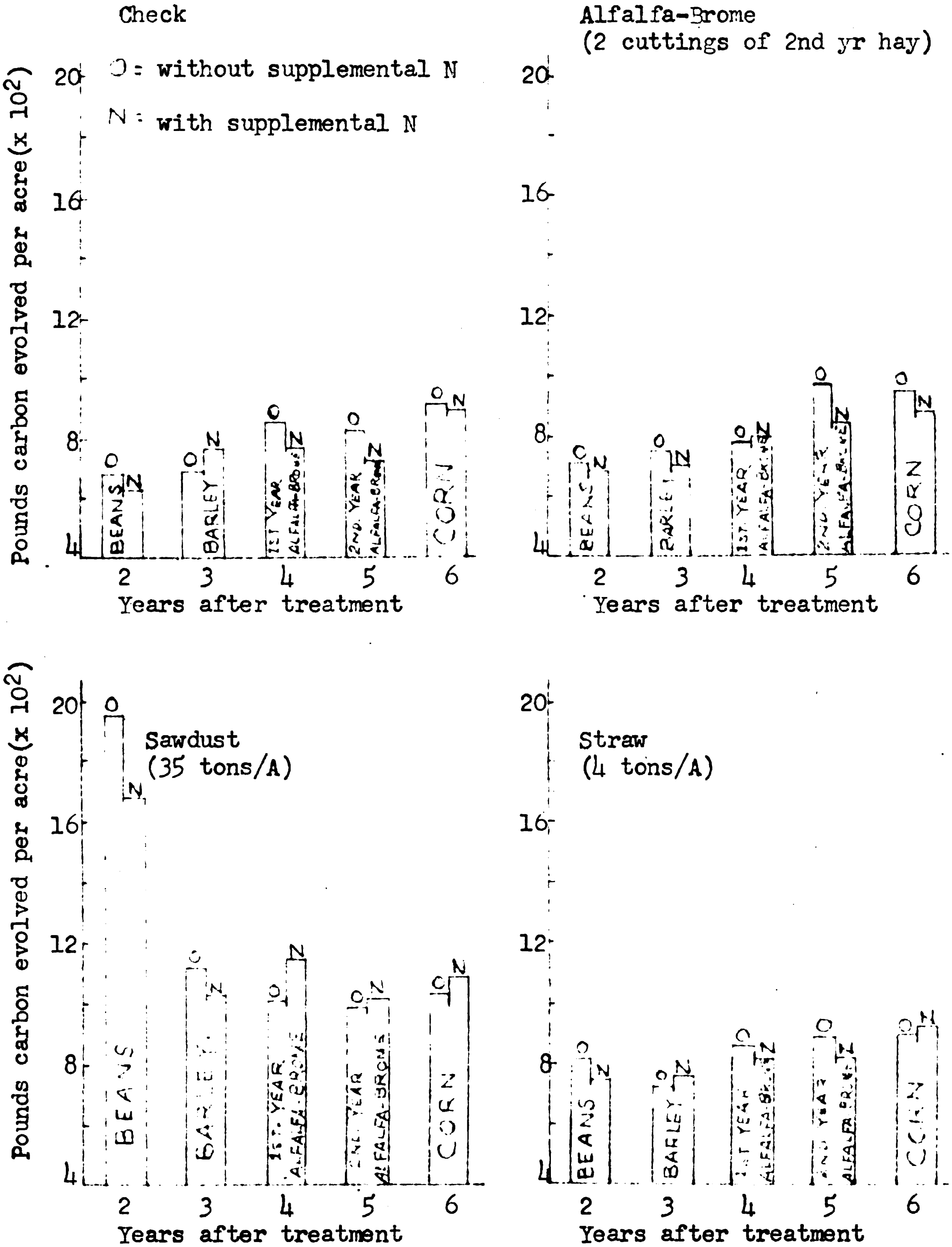

Figure 4. Carbon evolved as CO_2 during a 14-day incubation period from soil samples taken at yearly intervals after addition of various organic amendments with and without supplemental nitrogen fertilizer.

in all soils four or five years after initial treatment.

The CO_2 production from soils following the check, alfalfa-brome, and straw treatments were essentially the same during any given year. The normal rates of application represented by the alfalfa-brome and straw treatments, were not high enough to alter the biological properties of these soils in this six-year period.

Incubation mineralization of nitrogen

Nitrate-nitrogen produced during a 14-day incubation at 35° C. is tabulated by soil blocks and treatments in Table 11 (Appendix). The values are quantitatively of the same order as those reported by Mora (39) for the previous year's soil samples.

The data are plotted in the histograms in Figure 5. The results with sawdust treatment were essentially the same as those for the previous year. There was marked suppression of incubation mineralization rate during the early years after treatment. This was due to microbial immobilization of nitrogen in the presence of excess energy materials contributed by the sawdust. There was a progressive recovery in net mineralization rate, year after year, through the sixth year after treatment. This recovery was enhanced by applications of supplemental nitrogen. By the third year after treatment, nitrate production was essentially equivalent to that for the check soil. In Mora's data this equivalence was not achieved until four years after treatment. During the fifth year, nitrate produced by the sawdust treated soil was substantially greater than the check, and this high rate was maintained through the sixth year.

Mora had found that supplemental nitrogen treatment depressed incubation mineralization of nitrogen during the early years of the rotation in the check soils and those which received the alfalfa-brome and straw treatments.

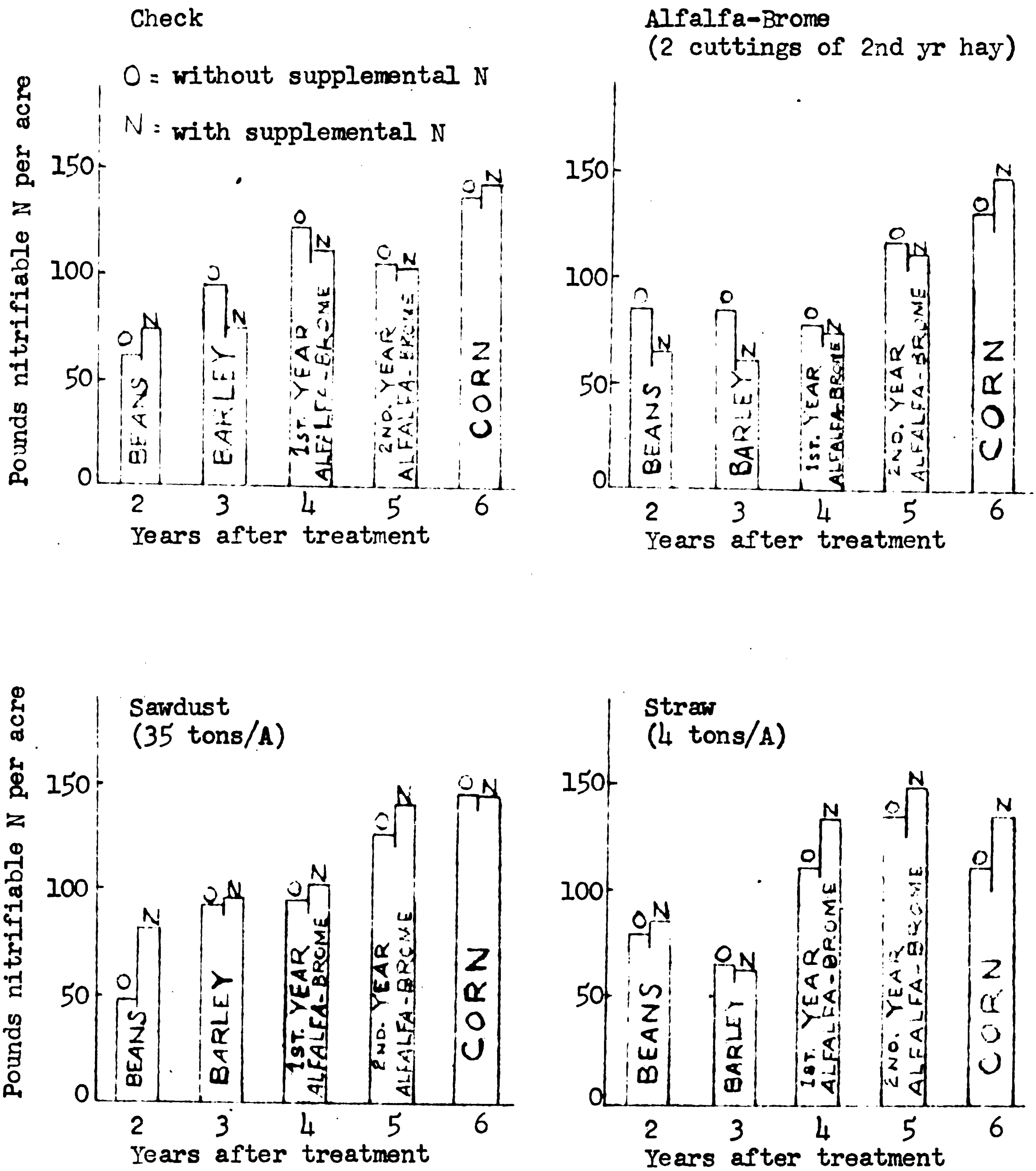

Figure 5. Nitrifiable nitrogen released as nitrate during a 14-day incubation period in soil samples taken at yearly intervals after addition of various organic amendments with and without supplemental nitrogen fertilizer.

During the last year or two of the rotation, the residual effect of nitrogen applied during the first three years was to enhance the production of nitrate. A similar pattern of fluctuating mineralizability of soil nitrogen associated with nitrogen treatment is apparent in Figure 5 in the data for the check, alfalfa-brome and straw treatments. There were irregularities in this pattern, but the similarity in the trends expressed by soil samples taken during the two years was striking.

Relation of Laboratory Data to Crop Yields

A second objective of this investigation was to provide data which might be used later to correlate various soil tests with crop yields. Some preliminary observations may be made at the present time.

Actual correlations with the data reported here could not be made because the 1958 yield data were not available. Iowa nitrification data were available for soil samples taken in the fall of 1956 from the 1951 block of plots which were planted to corn in 1957 (42). An analysis of covariance of these data and the 1957 corn yields was performed. No significant correlations were found, except within the ten plots which had received the heavy application of sawdust in 1951. Within these plots a positive linear correlation of .978, significant at the 1 percent level of probability, was obtained. Since this highly significant correlation was not borne out by the rest of the data, it cannot be taken seriously unless it is supported by later work.

Some comparisons may be made between the chemical and biological data reported here and the previous history of crop yields. Such a comparison must, of course, be based on the assumption that differences in chemical and biological characteristics which were found to be associated with

certain treatments here were similarly associated in the previous years.

Average yields of the various crops for the various treatments are presented in Table 3 for the period from the start of the experiment through 1957. Three points of interest are worth noting:

1. The return of two cuttings of alfalfa-brome hay preceding corn has had little or no influence on yields of any crop over the rotation. This treatment also had little effect on total carbon, total nitrogen or on the incubation behavior of the soil as compared with the check.

2. A four-ton application of straw tended to depress corn yields but had little or no effect on yields of other crops in the rotation. This effect most likely resulted from a temporary shortage of nitrogen during the early growth of the corn, since the straw was always applied in the fall before corn. The production of CO_2 and nitrate during incubation of soil samples taken in the fall after corn was harvested in 1956 (39) and in 1957 (Figures 4 and 5) showed no residual effect one year after straw treatment on microbial activity or nitrogen mineralization.

3. Yields of the first three crops(corn, beans and barley)following the application of 35 tons per acre of sawdust were reduced. Yields of hay four and five years after addition of sawdust were equal to or greater than the check. Yields of corn six years after sawdust treatment (second cycle of the rotation) were significantly greater at the 5 per cent level than for any other treatment. These results indicate that microbial immobilization of nitrogen in the presence of sawdust was a significant factor in crop yields through the third year after treatment. From the fourth year on, it would appear that release of previously immobilized nitrogen contributed to a supply of available nitrogen in the soil equal to or greater than the check.

Table 3. Average crop yields over a six-year period following the addition of various organic amendments with and without supplemental nitrogen fertilizer.

Treatment	Corn[b]. ('52-56)		Bean[b]. ('53-56)		Barley[b]. ('54-57)		1st yr. Hay[c]. ('55-57)	2nd yr. Hay[c]. ('56-57)
	No N	40lbs N	No N	40lbs N	No N	40lbs N		
Check	82.3	90.5	34.8	35.2	47.9	50.7	3.22	4.15
Alfalfa	84.8	88.1	32.8	35.0	47.8	51.5	3.11	2.17[d].
Sawdust	36.4	51.4	27.0	32.1	39.3	47.2	3.34	4.15
Straw	80.6	83.1	33.6	35.6	49.4	49.0	3.22	4.35

Second Rotational Cycle

Treatment	Corn[b]. ('57)	
	No N	100lbs N
Check	45.3	58.5
Alfalfa	45.3	58.9
Sawdust	52.7	66.7
Straw	36.6	47.8

a. Unpublished data, presented by courtesy of J. R. Guttay, Michigan Agricultural Experiment Station, Department of Soil Science

b. Bushel per acre

c. Tons per acre

d. First cutting only

That nitrogen was immobilized in the presence of sawdust was shown by the fact that total nitrogen was 300 to 400 pounds greater in the sawdust-treated soil than in the check (Figure 3 and Table 8). The CO_2 evolution data (Figure 4 and Table 10) revealed that surplus energy materials were largely dissipated by the end of the third season after sawdust application. The incubation mineralization results (Figure 5 and Table 11) indicate that the ability of the soil to release nitrogen had recovered to a level comparable with the check by the third or fourth year after addition of the sawdust. This level increased in the fifth and sixth years after treatment.

Thus, yield data and the data from the laboratory studies concur in support of the conclusion that suppression of crop yields by massive applications of sawdust to soil under the conditions of this experiment may be expected for a period of three years. Following this initial suppression, decided benefits to crops may be expected as the result of the re-mineralization of nitrogen previously immobilized.

DISCUSSION

The data presented here were consistent in their main features with the data reported by Mora (39) for soil samples taken a year earlier. Some of the discrepancies were clearly related to soil differences between blocks of plots which were established in different years. The extent to which soil variation was a factor cannot be estimated until data are available for the complete rotational sequence on each block of plots.

Exchangeable ammonium levels were generally higher in 1957 than in 1956. This was most likely due to the fact that the 1957 soil samples were held in the air-dry state for a longer period than those taken in 1956. This difference was greater for the sawdust treated soils than for the others. This suggests the possibility that the formation of exchangeable ammonium, which is known to occur during air-drying, may be related to the degree of decomposition or oxidation of soil organic materials.

It was found that the accumulation of nitrate in the field reflected recent additions of fertilizer nitrogen. Accumulation of nitrate was also observed when the soil was not occupied by an actively growing crop for a period prior to the taking of soil samples. However, as was pointed out by Mora, measurement of nitrate in the soil at any given time does not provide a reliable basis for predicting future release. This is because nitrate levels are influenced by such unmeasurable variables as crop removal, leaching losses and continual release through mineralization and nitrification of the nitrogen in soil organic matter.

Nitrate released during incubation was found to reflect the immobilizing potential of surplus energy materials for a period of two to three years after the addition of 35 tons of sawdust. Re-mineralization, during the fourth to sixth years, of nitrogen previously immobilized was reflected by increased release of nitrate during incubation, as well as by increased crop yields. However, attempts to correlate crop yields directly with nitrate released during incubation were successful only when restricted to the data for sawdust-treated soils. This result supports the suggestion of Mora (39) and Johnston (29) that the value of the Iowa incubation test for nitrifiable nitrogen apparently lies in its ability to reflect differences in immobilizing potential of excess carbonaceous energy materials in the soil. Its successful use in practice has been restricted to situations where corn follows a non-legume in the rotation (26).

The Truog permanganate oxidation procedure was proposed as a measure of exchangeable ammonium and of the more labile forms of soil organic nitrogen (70). It was assumed that these would include the forms most readily mineralized in the field and most readily converted to nitrate for plant use. This premise has been questioned by Wolcott, et al (86). They found little correlation in greenhouse studies between acid-hydrolyzable forms of nitrogen and crop uptake of nitrogen from soils. They did find a good correlation between the acid-resistant nitrogen and crop uptake and proposed that the complexing activity of resistant nitrogen compounds controlled the availability of labile forms of nitrogen to microbial attack and mineralization. Regardless of the theoretical merits or liabilities of the permanganate oxidation procedure, the results with the method in the present series of studies have not been encouraging. Quantitative differences between variously treated soils were small and there was no relation to treatment.

Both total carbon and total nitrogen were increased materially by the heavy application of sawdust. These differences were still apparent

six years after treatment, although the differences were small in comparison with the quantities of carbon which were added initially. No attempt was made to relate these values with yields. However, it did appear that the greater release of nitrate during incubation and the increased crop yields during the fourth to sixth years after sawdust application were related to the increased quantity of organic nitrogen which had resulted from microbial immobilization during the first two to three years.

Mora (39) had found that the ratio of carbon to nitrogen in the 1956 samples from these same plots was related in a significant manner to the incubation behavior of the soils. High CO_2 production was associated with wider than normal soil C:N ratios and was observed only in samples taken one and two years after addition of sawdust. Such wide C:N ratios and high microbial activity were associated with marked suppression of nitrogen mineralization. Data for 1957 which are plotted in Figures 6 and 7 show the same coincidence of high microbial activity with low nitrate release at wide soil C:N ratios for the sawdust-amended soils two years after treatment. For the rest of the soils there was little or no relationship between soil C:N ratio and mineralization of either carbon or nitrogen.

These results can only be interpreted in terms of the changing nature of energy substrates which is associated with the progressive microbial decomposition of organic materials. The ratio of release of CO_2 to the release of nitrate during incubation can be used to give some insight into the general nature of the predominant substrates which were being utilized by the soil microbial population (82,83,84,85).

Relationships which were found to exist between the C:N ratio of mineralization and soil C:N ratio are depicted graphically in Figure 8. As was true in Mora's report, wide mineralization ratios were associated

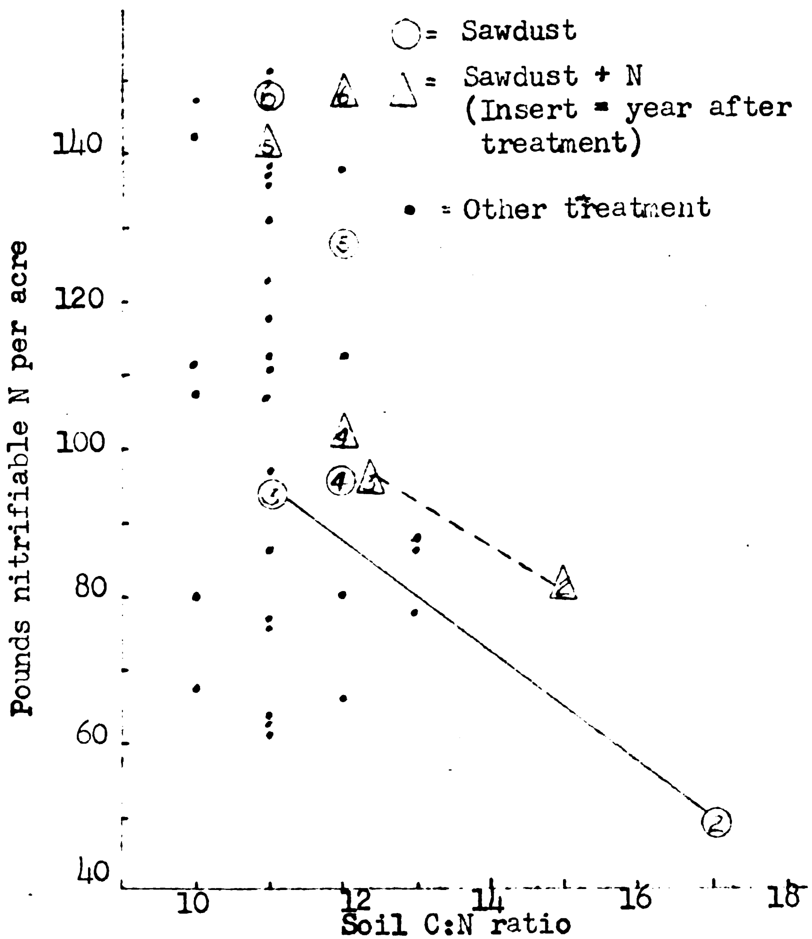

Figure 6. Nitrifiable nitrogen as related to soil C:N ratio and sawdust treatment.

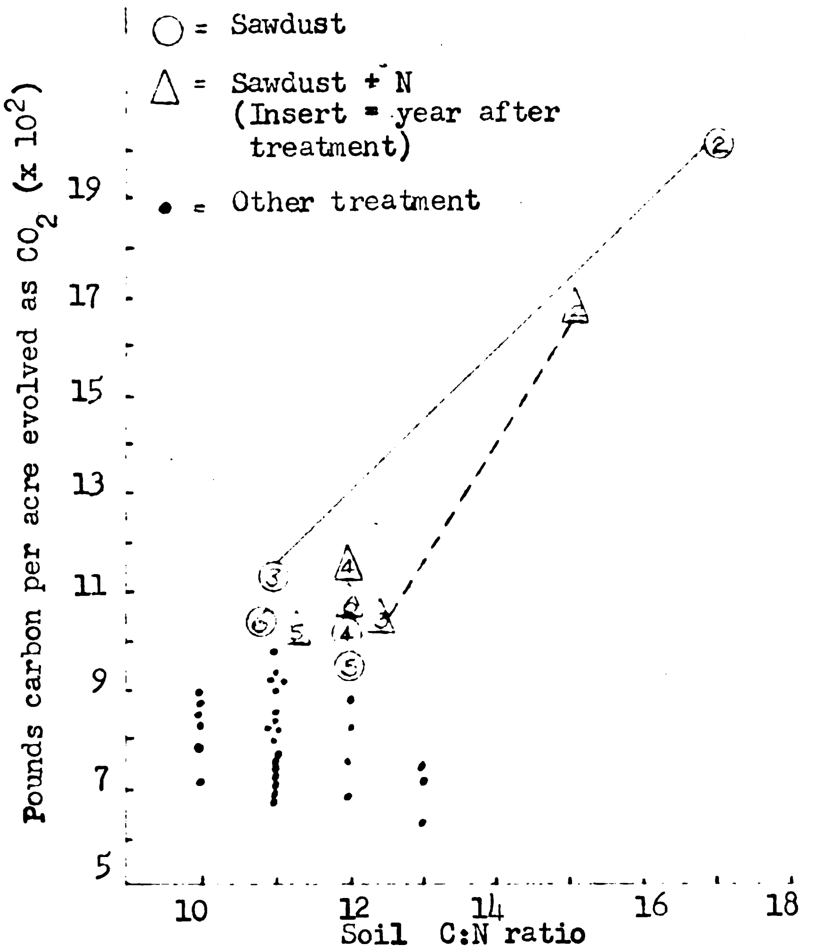

Figure 7. Microbial activity measured as CO_2 evolved during a 14-day incubation period and its relation to soil C:N ratio and sawdust treatment.

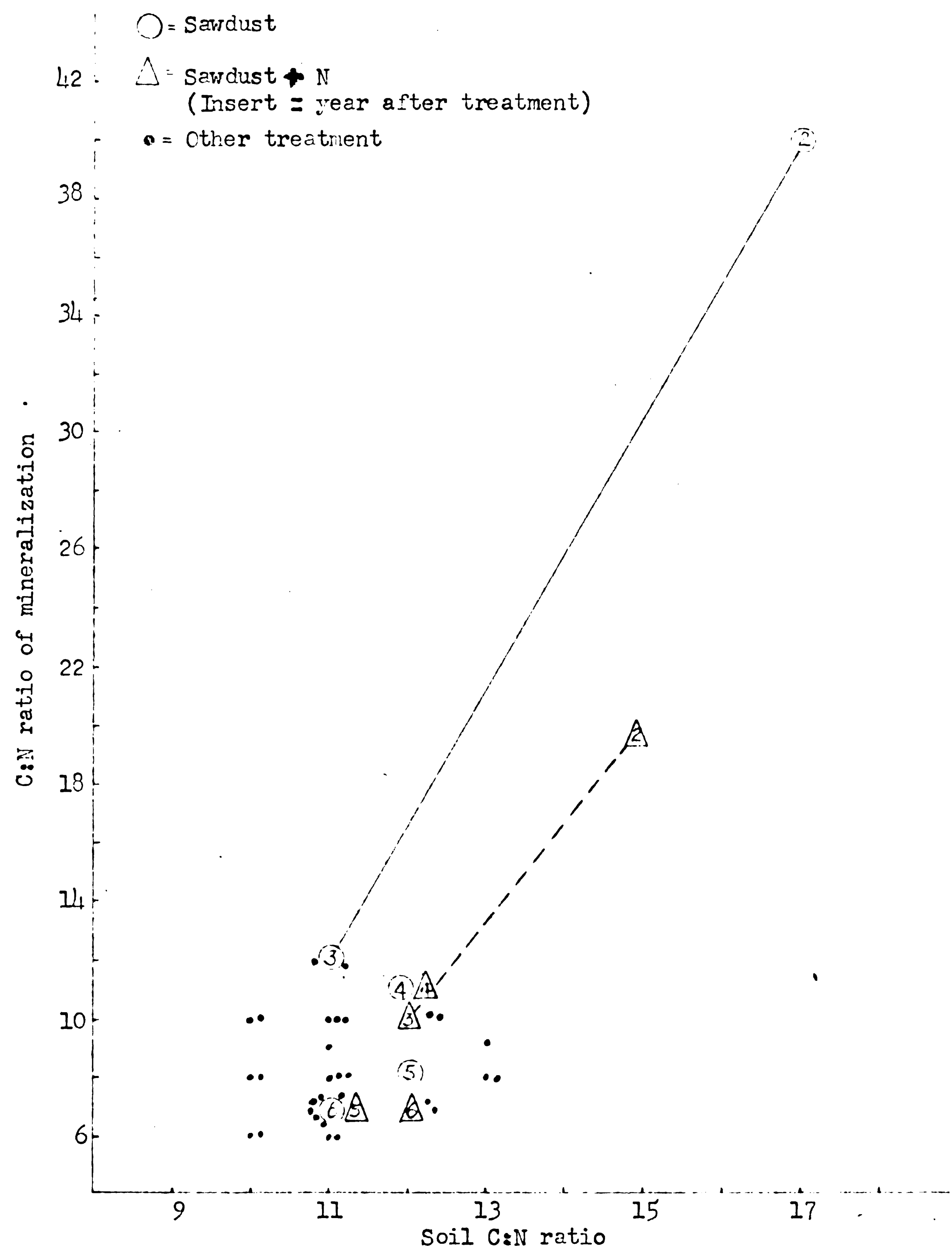

Figure 8. Ratio of mineralization of carbon to mineralization of nitrogen as related to soil C:N ratio and sawdust treatment.

with sawdust-treated soils two years after treatment. Such wide ratios imply that highly carbonaceous energy substrates were being attacked.

Beginning with the third year after sawdust application, mineralization ratios tended to be equal to or less than the ratios of carbon to nitrogen in the soil. This was also true of all other treatments regardless of time elapsed since initial treatment. It may be inferred that organic materials being drawn on for energy by microbial populations in these soils were no longer highly carbonaceous. Instead, energy substrates were predominantly compounds containing both carbon and nitrogen.

These residual nitrogenous compounds were much lower in energy availability than were the constituents of sawdust during the earlier stages of its decomposition, as is shown by the fact that they supported a much lower level of microbial activity during incubation. It may be inferred that microbial numbers and the immobilization of nitrogen in microbial cells were also low. In consequence of this, respiration of carbon was accompanied by net mineralization of nitrogen.

It would be reasonable to expect that the rate of mineralization of nitrogen from such residual nitrogenous substrates would be a function of how rapidly they are decomposed. In other words, there should be a direct relationship between respiratory loss of carbon and net mineralization of nitrogen, instead of the inverse relationship observed where surplus carbonaceous energy materials were present (Figures 6 and 7).

Data plotted in Figure 9 serve to illustrate these relationships for soils varying widely in quantity and kind of initial organic amendment and in their immediate cropping history. Significant to highly significant positive correlations between CO_2 evolved and nitrogen mineralized were

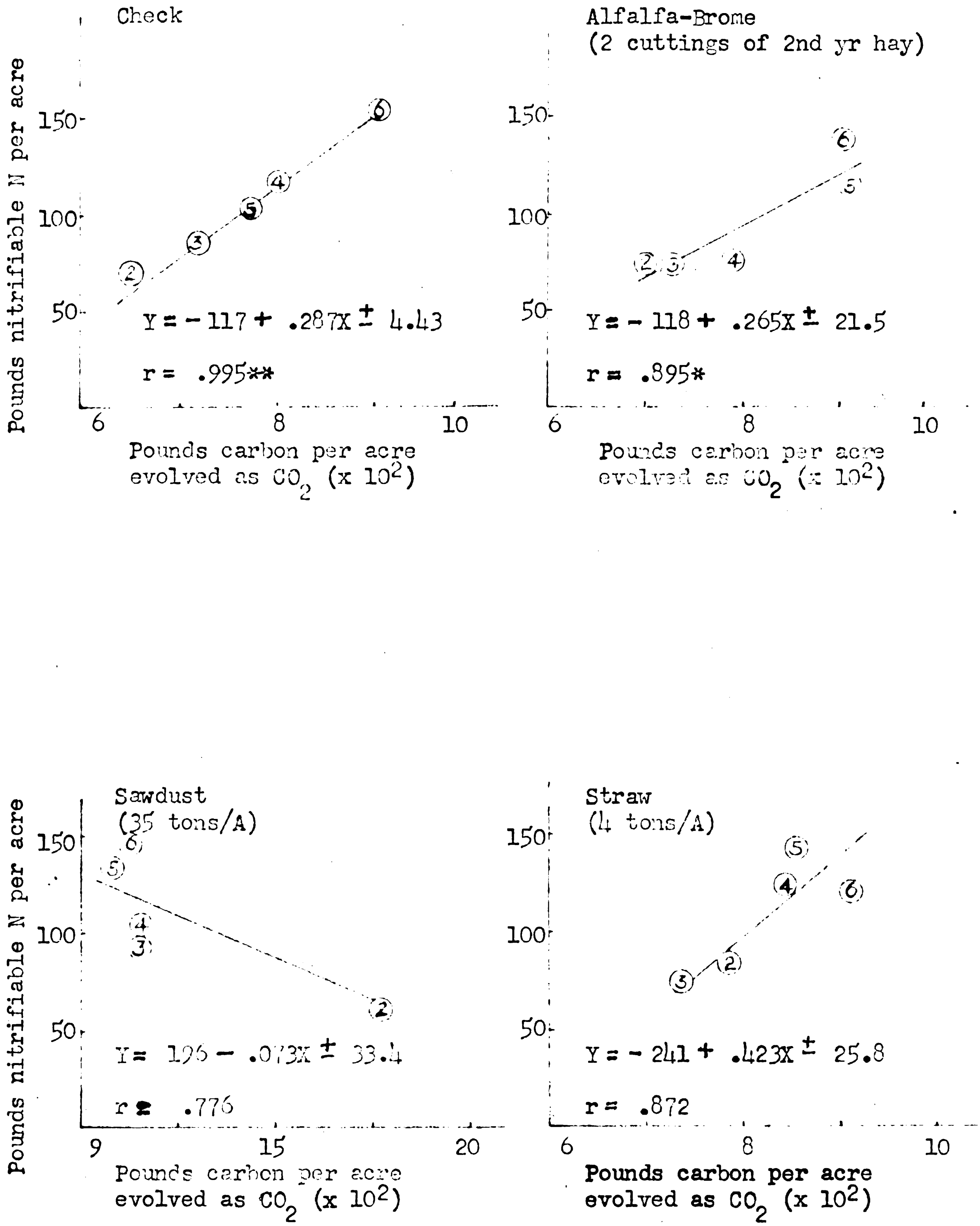

Figure 9. Nitrifiable nitrogen as related to carbon evolved as CO_2 during a 14-day incubation period at 35° C.

obtained for the check soils and those to which alfalfa-brome hay, in moderate quantities, had been added. The positive correlation for the straw treatment closely approached significance at the 5 per cent level. With the massive sawdust treatment, however, a negative relationship was obtained. Although this correlation ($r = -.776$) was not statistically significant, it did reflect the suppression of nitrate release which was associated with extremely high microbial activity in soils one and two years after sawdust application in this and the previous year's work.

These results have a significant bearing on the interpretation of any chemical or biological tests which may be made use of for prediction purposes in soil fertility studies or soil management practices. The immobilizing potential of surplus energy materials in recently returned crop residues or organic amendments cannot be ignored. The specific nature of these residues, as well as the quantities returned to the soil, will influence the degree of immobilization of nitrogen which will occur. With normal quantities of normal crop residues, the residual release of previously immobilized nitrogen appears to follow a rotational, rather than a seasonal, pattern. This rotational cycle of immobilization and mineralization appears to have been influenced in an unexpected way by supplemental fertilizer nitrogen. Addition of nitrogen tended to intensify immobilization or reduce the mineralizability of nitrogen during incubation during the early years of the rotation. Subsequent mineralizability during the later years of the rotation was enhanced as a residual effect of fertilizer nitrogen applied on the first three crops.

Thus, it would appear that the results of chemical or biological tests for nitrogen will vary, not only with the time of year when soil samples are taken, but also with the point in the rotation when they are

taken. The fact that changes in the status of nitrogen occur during air-drying of soils is a further complication.

For these reasons, it appears that any direct correlation between chemical or biological soil tests and crop response to nitrogen will be difficult to achieve unless ways are found also to evaluate the immobilizing potential of carbonaceous residues in the soil. Mora (39) has suggested that the soil C:N ratio might provide a basis for determining approximately where in the cycle of mineralization or immobilization a given soil might be. Unfortunately, the carbon data reported in the present study were not suitable for a comparable analysis. Johnston (29) has suggested that the acid resistant nitrogen fraction in soils is quantitatively related to the degree of oxidation of soil organic materials and that it might provide an integrated index of the availability of soil nitrogen. These are approaches which should be investigated further.

SUMMARY AND CONCLUSIONS

A number of inferences may be drawn which are consistent with results reported here and with those reported a year earlier by Mora (39). These are tentative and may need to be substantiated or revised as data are accumulated during succeeding years of this rotational study.

1. Total carbon and total nitrogen in the soil were increased by the addition of 35 tons per acre of sawdust. Six years after addition, there remained a surplus over the check soil of three to five tons per acre of carbon and 300 to 400 pounds of nitrogen.

2. Increases in soil carbon and nitrogen from the addition of two cuttings of alfalfa-brome hay or four tons per acre of wheat straw were small and probably not significant.

3. Crop yields were depressed for three years after the addition of 35 tons per acre of sawdust. Addition of supplemental nitrogen at rates of 100 pounds per acre for corn and 40 pounds per acre for beans and barley grown successively in that order did not overcome the yield depression with these crops. Six years after sawdust treatment, yields of corn were greater than the check by 7.4 bushels per acre where no supplemental nitrogen was used and by 8.2 bushels where 100 pounds of supplemental nitrogen were added.

4. The addition of four tons of straw per acre depressed yields of the immediately following corn crop by 8.7 to 10.7 bushels per acre relative to the check, but had no residual influence on the yields of any other crop in the rotation.

5. The return of two cuttings of alfalfa-brome hay immediately preceding corn did not influence the yields of any crop in the rotation.

6. Release of CO_2 and nitrate during incubation of soil samples taken from these plots reflected the immobilizing influence on nitrogen of surplus carbonaceous energy materials in the sawdust through the second or third year after application. Subsequent remineralization of previously immobilized nitrogen was reflected by enhanced release of nitrate during incubation from soils sampled four to six years after sawdust was applied.

7. There was a marked tendency for release of nitrate during incubation to be reduced in soil samples taken in the fall after spring applications of supplemental nitrogen on the first three crops in the rotation. The residual effect of these early nitrogen applications was to enhance nitrate formation in samples taken in the fall of the last two years of the rotation.

8. In this rotation, which included both legumes and non-legumes, there appeared to be cyclic patterns of immobilization and mineralization of nitrogen which were rotational rather than seasonal in character. These were apparent in the incubation data. The differences noted in the incubation data, however, were not correlated with yields, except in the case of soils which had received the massive application of sawdust.

9. Soils which contained a surplus of carbonaceous energy materials were characterized by C:N ratios wider than normal or characteristic for the soil. They were also characterized by extremely high microbial activity and by C:N ratios of mineralization wider than the soil C:N ratio. Such soils tended to release nitrate during incubation in inverse ratio to the quantities of CO_2 produced.

10. Soils in which surplus carbonaceous energy materials had been largely dissipated tended to stabilize at C:N ratios characteristic for the soil. They supported moderate levels of microbial activity. The ratio

of carbon to nitrogen mineralized during incubation tended to be the same or less than the soil C:N ratio. Such soils tended to release nitrate during incubation in direct proportion to the quantities of CO_2 produced.

11. Future studies related to soil tests for nitrogen supplying power of soils should take into consideration methods for estimating the nitrogen immobilizing potential of surplus energy materials in the soil. The significance of changes in status of soil nitrogen which occur during air-drying of soil samples should also be considered in the interpretation of nitrogen soil tests.

12. The alkaline permanganate oxidation procedure has not given results which could be related to treatment or crop performance.

13. Loss on ignition does not provide a valid estimate of total carbon or soil organic matter, particularly in heavy soils which contain substantial quantities of clay.

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APPENDIX

Table 4.--Soil reaction as influenced by organic amendments, nitrogen fertilizer, crop sequence and time.

Treatment	Soil block 55	54	53	52	51
	Years after treatment 2	3	4	5	6
	1957 Crop Beans	Barley	Alfalfa 1st year	Alfalfa 2nd year	Corn
	Soil reaction[a]				
Check	6.1	6.1	6.5	6.6	6.5
Check +N . . .	6.1	6.4	6.4	6.5	6.2
Alfalfa	6.2	6.5	6.5	6.5	6.4
Alfalfa +N. .	6.1	6.4	6.5	6.5	6.3
Sawdust . . .	6.4	6.5	6.5	6.6	6.3
Sawdust +N . .	6.4	6.4	6.4	6.5	6.2
Straw.	6.2	6.6	6.5	6.6	6.4
Straw +N . . .	6.3	6.4	6.5	6.6	6.4

[a]Average for duplicate detеminations

Table 5.—Reserve phosphorus extracted with 0.13N HCL, as influenced by organic amendments, nitrogen fertilizer, crop sequence and time.

Treatment	Soil block 55 — Years after treatment 2 — 1957 Crop Beans	54 — 3 — Barley	53 — 4 — Alfalfa 1st year	52 — 5 — Alfalfa 2nd year	51 — 6 — Corn	Average
	Phosphorus (pounds per acre)[a]					
Check	32	75	81	52	69	62
Check +N . . .	30	80	81	51	70	62
Alfalfa	36	87	74	54	66	63
Alfalfa +N . .	43	82	76	59	64	65
Sawdust	22	74	79	49	66	58
Sawdust +N . .	25	71	75	47	65	56
Straw.	33	76	89	60	63	64
Straw +N . . .	30	76	82	55	64	61
Average for no N	31	78	81	54	66	62
Average for +N	32	77	79	53	66	61
Average for years	31	78	80	53	66	61

[a]Average of duplicate determinations

Table 6.--Total carbon by ignition method, as influenced by organic amendments, nitrogen fertilizer, crop sequence and time.

Treatment	Soil block 55	54	53	52	51	Average
	Years after treatment 2	3	4	5	6	
	1957 Crop Beans	Barley	Alfalfa 1st year	Alfalfa 2nd year	Corn	
	Total carbon (cwts per acre)[a]					
Check	394	512	512	512	564	499
Check +N . . .	414	524	516	578	560	518
Alfalfa . . .	416	530	478	552	592	514
Alfalfa +N . .	430	534	500	572	544	516
Sawdust	598	592	596	600	628	603
Sawdust +N. .	534	584	582	634	658	598
Straw	418	494	464	594	592	512
Straw +N . . .	426	510	500	584	584	521
Average for no N	457	532	513	565	594	532
Average for +N	451	538	525	592	587	539
Average for years	454	535	519	578	590	535

[a]Average of duplicate determinations

Table 7.—Permanganate-soluble nitrogen, as influenced by organic amendments, nitrogen fertilizer, crop sequence and time.

Treatment	Soil block 55	54	53	52	51	Average
	Years after treatment 2	3	4	5	6	
	1957 Crop Beans	Barley	Alfalfa 1st year	Alfalfa 2nd year	Corn	
	Permanganate-soluble N (pounds per acre)[a]					
Check	75	75	75	75	75	75
Check +N . . .	75	75	75	75	75	75
Alfalfa	75	75	75	75	75	75
Alfalfa +N . . .	75	75	75	75	75	75
Sawdust	75	75	75	75	75	75
Sawdust +N . .	75	75	113	75	105	98
Straw	75	75	75	75	75	75
Straw +N . . .	75	75	75	75	75	75
Average for no N	75	75	75	75	75	75
Average for +N	75	75	85	75	94	81
Average for years . . .	75	75	80	75	84	78

[a]Average of duplicate determinations

Table 8.--Total nitrogen (KJELDAHL), as influenced by organic amendments, nitrogen fertilizer, crop sequence and time.

Treatment	55	54	53	52	51	Average
	Soil block					
	2	3	4	5	6	
	Years after treatment					
	1957 Crop					
	Beans	Barley	Alfalfa 1st year	Alfalfa 2nd year	Corn	
	Total N (pounds per acre)[a]					
Check.	3480	4720	4760	5120	5300	4676
Check +N . . .	3300	4640	4420	5160	5440	4592
Alfalfa . . .	3260	4860	4640	5080	5420	4652
Alfalfa +N. .	3580	4880	4580	5120	5480	4728
Sawdust . . .	3620	5220	5060	5160	5500	4912
Sawdust +N. .	3580	5060	5000	5640	5640	4984
Straw	3380	4720	4780	5060	5480	4684
Straw +N . . .	3340	4800	4640	5100	5480	4674
Average for no N . . .	3435	4880	4810	5105	5425	4731
Average for +N	3450	4840	4660	5250	5510	4744
Average for years . . .	3443	4863	4735	5180	5468	4738

[a]Average of duplicate determinations

Table 9.—Soil C:N ratio, as influenced by organic amendments, nitrogen fertilizers, crop sequence and time.

Treatment	Soil block 55	54	53	52	51	Average
	Years after treatment 2	3	4	5	6	
	1957 Crop Beans	Barley	Alfalfa 1st year	Alfalfa 2nd year	Corn	
	Soil C:N ratio[a]					
Check	11	11	11	10	11	11
Check +N . . .	13	11	12	11	10	11
Alfalfa	13	11	10	11	11	11
Alfalfa +N . . .	12	11	11	11	10	11
Sawdust	17	11	12	12	11	13
Sawdust +N . .	15	12	12	11	12	12
Straw	12	10	10	12	11	11
Straw +N . . .	13	11	11	11	11	12
Average for no N	13	11	11	11	12	12
Average for +N	13	11	11	12	11	12
Average for years	13	11	11	12	11	12

[a]Average of duplicate determinations

Table 10.--Carbon evolved as carbon dioxide during a 14-day incubation period, as influenced by organic amendments, nitrogen fertilizer, crop sequence and time.

Treatment	Soil block 55	54	53	52	51	Average
	Years after treatment 2	3	4	5	6	
	1957 Crop Beans	Barley	Alfalfa 1st year	Alfalfa 2nd year	Corn	
	Carbon dioxide evolution (pounds carbon per acre)					
Check	672	682	854	824	912	788
Check +N . . .	636	754	754	726	896	754
Alfalfa	712	738	786	976	934	830
Alfalfa +N. . .	682	710	790	848	870	780
Sawdust . . .	1958	1124	1002	994	1022	1220
Sawdust +N . .	1678	1032	1146	1014	1074	1188
Straw	822	712	852	884	898	834
Straw +N	744	760	826	830	918	816
Average for no N . . .	1042	814	874	920	942	918
Average for +N	936	814	880	854	940	884
Average for years . . .	990	814	878	888	942	902

Table 11.--Nitrate nitrogen released during a 14-day incubation period, as influenced by organic amendments, nitrogen fertilizer, crop sequence and time.

Treatment	Soil block 55	54	53	52	51	Average
	Years after treatment 2	3	4	5	6	
	1957 Crop Beans	Barley	Alfalfa 1st Year	Alfalfa 2nd Year	Corn	
	Nitrifiable nitrogen as NO_3-N (pounds per acre)[a]					
Check	62	96	122	107	137	105
Check +N	77	76	112	106	142	102
Alfalfa	85	85	79	117	131	99
Alfalfa +N . . .	65	61	75	110	146	91
Sawdust	49	94	95	127	146	102
Sawdust +N . . .	81	95	104	140	146	113
Straw	79	67	111	137	112	101
Straw +N . . .	87	64	136	150	137	115
Average for no N	69	85	102	122	132	102
Average for +N	78	74	107	126	143	106
Average for years	73	80	104	124	137	104

[a]Average of duplicate determinations

Table 12.—Ratio of carbon to nitrogen mineralized during a 14-day incubation period, as influenced by organic amendmends, nitrogen fertilizer, crop sequence and time.

Treatment	Soil block 55	54	53	52	51	Average
	Years after treatment 2	3	4	5	6	
	1957 Crop Beans	Barley	Alfalfa 1st year	Alfalfa 2nd year	Corn	
	C:N mineralized in 14 days[a]					
Check.	10	7	7	8	7	8
Check +N . . .	8	10	7	7	6	8
Alfalfa. . . .	8	9	10	8	7	8
Alfalfa +N . .	10	12	10	8	6	10
Sawdust. . . .	40	12	11	8	7	16
Sawdust +N . .	20	10	11	7	7	12
Straw	10	10	8	6	8	8
Straw +N . . .	9	12	6	6	7	8
Average for no N.	17	10	9	8	7	10
Average for +N	12	11	9	7	7	10
Average for years	14	10	9	7	7	10

[a]Average of duplicate determinations