QUANTITATIVE AND QUALITATIVE CHANGES IN SOIL ORGANIC MATTER AS RELATED TO RESIDUE ADDITIONS, CROPPING SEQUENCE AND MANAGEMENT

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ABSTRACT

QUANTITATIVE AND QUALITATIVE CHANGES IN SOIL ORGANIC MATTER AS RELATED TO RESIDUE ADDITIONS, CROPPING SEQUENCE AND MANAGEMENT

by Robert Michael Harrison

Effects of residue amendments, time, cropping sequence and management practices on quantitative and qualitative changes in soil organic matter were observed during the course of two field experiments. One involved incorporated additions of plant residues in a 5-year rotation on Sims clay loam. The other involved surface mulches of similar materials in a young apple orchard on an association of lighter textured naturally well-drained soils.

Total soil nitrogen was determined by macro-Kjeldahl procedures and total organic carbon by wet combustion and manometric or gravimetric measurements of CO_2. Selected soils were subjected to an acid hydrolytic fractionation of nitrogenous constituents by an as yet unpublished rapid procedure developed by J. M. Bremner of the Iowa Experiment Station.

After 50 years of cropping, soil nitrogen in the Sims clay loam was still declining at a basal rate of 1.28 percent per year. Large increases in nitrogen above this base line were observed for a massive (40 tons per acre) addition of sawdust. With normal return or moderate (4 tons per acre) additions of alfalfa-brome hay or wheat straw, cyclic fluctuations occurred as nitrogen was depleted below the base line during the years that tilled crops were grown and recovered again to levels above the base line during the two years that alfalfa-brome hay was grown.

It appeared that carbon played a key role in promoting increases in nitrogen. With moderate additions in the rotation experiment, cyclic increases of 100 to 150 pounds nitrogen per acre were preceded by increases in carbon leading to C/N ratios above 10. A 600-pound increase during the first three years after addition of 40 tons of sawdust was associated with C/N ratios of 14 to 16. Increases of 1080, 720 and 680 pounds nitrogen per acre under mulches of alfalfa, grass hay and straw, respectively, were accompanied by increases of carbon in ratios of 10.9, 11.9 and 14.5.

Nitrogen accumulating above the base line in the rotation experiment appeared in chemical combinations which were mineralized more rapidly than indigenous soil organic matter. Nitrogen in indigenous soil materials appeared to have a very stable decomposition rate. A change in the second cycle of the rotation from spring plowing to fall plowing resulted in accelerated losses of carbon, but there was no apparent effect on the basal rate of nitrogen decline. Carbon-nitrogen ratios between 8 and 9 were reached by the end of the experiment.

Increases in nitrogen with incorporated additions of sawdust and with surface mulches of straw greatly exceeded accountable sources of nitrogen in soil, fertilizer or the materials themselves. Extensive non-symbiotic fixation of nitrogen was indicated. Such fixation occurred, however, under conditions which promoted nitrogen deficiencies in trees and crops for 3 to 4 years in both experiments.

Preliminary evaluation of nitrogenous fractions suggests that microbial assimilation and chemical complexing during early stages of decomposition of plant residues may be reflected by disproportionate

increases in α-amino nitrogen and nitrogen in the non-hydrolyzable fractions. With further oxidation, a transfer of nitrogen from the non-hydrolyzable fraction to ammonium or unidentified forms in the hydrolysate may occur. Humus formed from leguminous residues may be disproportionately low in α-amino nitrogen because of more rapid and extensive degradation of protein constituents.

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by

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INTRODUCTION

A general and direct relationship between soil organic matter content and productivity has long been recognized. Beneficial effects of organic matter on soil structure, water-holding capacity, nutrient supply and cation exchange capacity, have been demonstrated. Numerous organic compounds with specific antibiotic or growth regulating properties have also been isolated from soil organic matter and from plant and microbial residues and products. However, it has been impossible to sort out the specific contribution of each of these effects to crop growth. The only quantitative correlations between soil organic matter levels and crop response for which general validity has been shown in the field have been in terms of nitrogen supplying power.

It has been found that the release of nitrogen from soil organic matter has the characteristics of a first order reaction. In other words, the annual rate of release is a function of organic matter present. Useful correlations between soil organic matter and crop yields have been obtained when soils differing rather widely in organic matter content have been compared.

Within groups of soils differing less widely in organic matter content, however, large differences in crop responses to nitrogen appear to be related to differences in quality, or composition, of the soil organic fraction. These differences are related to differences in texture and clay content of the mineral fraction, to topographical and management factors influencing drainage and aeration, and to the nature and rate of return of organic residues.

The objectives of the present investigation were:

1) To evaluate quantitative changes in levels of carbon and nitrogen in soils as related to time or cropping sequence where surface and incorporated additions of widely varying rates and types of plant residues had been made.

2) To evaluate associated changes in quality of soil organic matter as reflected by C/N ratios and, in a preliminary manner, by the distribution of certain important chemical forms of soil nitrogen.

REVIEW OF LITERATURE

Soil organic matter is described by Bollen (11) as being a product of environment. It is a complex mass of partially decomposed or more or less humified material resulting from microbial action on dead organisms of all kinds.

Plant constituents form the major source of energy for soil organisms and of raw materials for the production of soil organic matter. In addition to oxygen and hydrogen, organic matter is essentially composed of the elements carbon, nitrogen and smaller amounts of phosphorus and sulfur. In many soils the ratio of carbon, nitrogen, phosphorus and sulfur is of the order of 100:10:1:1, respectively. Since carbon and nitrogen are the main elemental constituents, the carbon-nitrogen ratio is often used to describe organic matter in a general way. The carbon-nitrogen ratio is helpful in assessing the influence of organic matter on plant growth as well as in the gross characterization of the quality of organic matter in the soil (68).

It is a well known fact that a few percent of organic matter in the soil has profound effects on soil fertility. These effects may include improvement of soil structure, drainage and aeration, increased water holding capacity, buffer and exchange capacity and the supplying of nitrogen, phosphorus and other elements. Under some conditions organic matter also affects the availability of inorganic phosphorus and nutrients such as manganese and copper. Some of the low molecular weight, water soluble components may be responsible for the mobilization and transport of sesquioxides in the processes of gleying and podzolization (19). Some of these soluble components of plant or microbial origin have antibiotic or growth

regulating activity which is readily demonstrable in controlled laboratory experiments (51, 53, 72).

For several hundreds of years, observations have been made on the beneficial effects on plant growth following the incorporation of plant and animal residues into the soil. Only in the last 80 years have laboratory analyses and controlled experimentation been employed for studying the physical, chemical and biological bases of these plant responses. Such investigations have been concerned with the nature of organic constituents of plants, their rates of decomposition in or on the surface of soils, the role of soil microorganisms, and the characterization of processes accompanying decomposition, such as the immobilization and mineralization of nitrogen and other nutrient elements, the nature of decomposition products, and the formation of humus.

Enzminger and Pearson (33), in reviewing the contributions of several investigators, discuss the origin and depletion of soil organic matter, nitrogen and carbon. For virgin temperate soils, equilibrium levels of these components are established according to the conditions prescribed by the five soil forming factors. Climate and vegetation are deemed more influential than topography, parent material or time. Such an equilibrium is disturbed upon cultivation, and declines in organic matter levels result. Jenny (46) points out that various agricultural practices can differentially influence this rate of decline. Beginning with organic matter equilibrium levels under virgin conditions, the initiation of agronomic practices involving the use of perennial legumes and lime without tillage are found to slightly increase organic matter. Tillage operations promote rapid declines from virgin levels, as does

drainage of imperfectly or poorly drained soils, and lime accelerates such declines in soils initially acid. Depletive effects of tillage are directly proportional to the frequency or intensity of cultivation. Thus, the rate of decline increases with the proportion of tilled to untilled crops in the rotation, and is greater with a cultivated crop such as corn than with cereal grains. The rate of decline is inversely related to the annual or rotational rate of residue return. The effectiveness of different residues in opposing depletion varies, livestock manures being particularly effective.

Based on the assumption that with a given type of culture a certain fraction of total organic carbon and nitrogen will be lost in a year of cropping, Salter and Green (70) used the equation $C_t = C_oK^t$ to mathematically express this loss. C_t and C_o represent the levels of carbon or nitrogen at any time (t) and at zero time, respectively. K represents the percent of the element remaining after growing the crop a single year. In long term field experiments, it was found that one year of corn resulted in an annual loss of 3 percent of both carbon and nitrogen. Oats and wheat contributed approximately $1\frac{1}{2}$ percent losses of both elements. When Timothy hay or clover were included in rotations of corn and small grains their individual effects for the year in which they were grown were to increase the levels of soil carbon and nitrogen. Clover was about three times as effective as timothy.

Woodruff (96) and Dawson (30) have demonstrated that organic matter decline in soils has the characteristics of a first order chemical reaction and that the equation of Salter and Green applies to field observations when allowance is made for additions of carbon or nitrogen from

residues or other sources, and when observations cover soils ranging rather widely in nitrogen or carbon content.

Nitrogen additions to soils may occur through biological fixation by certain autotrophic and heterotrophic microorganisms, oxidation by lightning discharge and subsequent water solution associated with rainfall, additions of organic matter and use of inorganic fertilizers. No more than 2 to 3 percent of the total soil nitrogen resides in the inorganic form at any one time, the remainder being associated with organic constituents representing a wide range of structural entities and polymers (16, 33).

Fritschen and Hobbs (35) found continuous losses of soil carbon and nitrogen occurring in a 16 year rotation involving four years of alfalfa plus a corn - wheat - wheat sequence for the remaining 12 years. The same results occurred for a three year corn - soybean (hay) - wheat rotation. The greatest losses were on those plots having initially high carbon and nitrogen levels. Row crops caused the greatest decreases of carbon and nitrogen and losses were influenced little by fertilization treatments. Stevenson (81), examining some of the Morrow plot soils at the University of Illinois, found that after 50 years the percent of soil carbon and nitrogen associated with continuous corn or corn - oat rotations were about one-half the amounts found in the grass border or where clover had been included in the rotation. Hobbs and Brown (41) and Haas (37) found similar decreases in nitrogen and carbon contents of Great Plains soils during 30 to 43 years of cropping. Small grains or small grains and fallow were mostly employed. Karrager (49) observed a total nitrogen increase over a three year period when a rotation included red clover. No increase was noted if soybeans or orchard grass

replaced clover. After 45 years, a continuous blue grass - white clover sod had 77 percent more nitrogen within an 18 inch depth than when tobacco was continuously grown.

Mineralization of nitrogen from organic forms involves the processes of ammonification and nitrification, whereby nitrogen in soluble forms is made available for use by plants and microorganisms. Mineral forms of nitrogen may be lost from the soil by crop removal or leaching. Other pathways of nitrogen loss, dependent upon soil physical and chemical conditions, include gaseous losses through ammonia volatilization, microbial denitrification under anaerobic conditions and the chemical decomposition of nitrite to gaseous products at pH's below 5.0 to 5.5 (7, 19, 25, 44, 79). Such losses are unpredictable, but they may average 15 percent of the annual additions of fertilizer nitrogen to agricultural soils in the humid regions (3).

The fact that most cropping practices result in declines of soil organic matter, carbon and nitrogen has led to investigations concerning how such declines may be reversed, stabilized or lessened in rate. One such approach considers the effects of extraneous sources of plant residues and their incorporation into or their placement on the surface of soils.

It is found that when carbonaceous organic materials are added to the soil, microbial populations increase as a result of the new energy source. The initial high carbon to nitrogen ratios rapidly decrease with the evolution of CO_2. By the time organic residues have been decomposed to more or less stable forms comparable to humus, approximately 75 percent of the carbon will have been lost as CO_2 (4, 9, 36, 63).

When carbonaceous materials are added to soils, that portion of soil nitrogen which was present in the mineralized form and considered available for crop utilization is directed into immobilized organic forms by the microbial population. The extent of immobilization is dependent upon the amount of residue added and its nitrogen content. As decomposition continues and soil carbon-nitrogen ratios approach approximately 15:1, some of the organically combined nitrogen becomes mineralized into plant utilizable forms (40, 45, 60, 63, 68, 73, 89).

When inorganic forms of nitrogen are supplied to soils along with carbonaceous residue incorporations, the intensity of microbial activity and the amount of immobilization of soil nitrogen is temporarily increased. At the same time, however, more nitrogen is available for crop use. There is controversy as to whether the addition of supplemental nitrogen with organic residues leads to the stabilization of carbon during decomposition, and an increase in the amount of humus formed. An increase in levels of humus may be more indirectly related to higher amounts of crop residues being returned to the soil, resulting from the nitrogen having stimulated crop growth (4, 19, 62).

Investigations indicate that organic matter, nitrogen and carbon contents of soils are temporarily increased by the incorporation of organic residues. The nature of such increases are dependent upon the environmental conditions of the experiment and the composition of the residue employed. Decomposition rates and soil changes in carbon and nitrogen levels are more rapid for incubation studies than under field conditions. Carbon contents in soils are initially high for such carbonaceous residues as straw, grass hay and sawdust. Loss of carbon with

decomposition results rather quickly in soil carbon levels comparable to the control. Total soil nitrogen is increased when manure or leguminous residues are used and the soil is then allowed to remain under fallow conditions. When crops are grown in conjunction with these residues, soil nitrogen levels decline (1, 29, 36, 54, 66, 74).

The use of sawdust as an organic amendment serves to depress crop yields during the first years after incorporation. Such depressions may be overcome through the use of supplemental nitrogen. Slight improvements in soil structure are sometimes noted. Other observations indicate that sawdust does not affect soil acidity nor does it result in the formation of toxic substances (12, 57, 69).

Several investigators (24, 26, 38, 56, 76) have observed the accelerated decomposition of native soil organic matter upon the addition of organic residues to soils. In explanation, the addition of such residues promotes enhanced microbial activity by reason of increased energy supply. The attack on native organic matter is enhanced more or less in proportion to the increase in microbial activity. Pinck and Allison (65) maintain that no marked accelerative oxidation of native carbon occurs, stating that carbon released from soil sources is independent of the amount of extraneous organic carbon added. They found no accelerated oxidation of soil carbon when one gram of millet was added to 100 grams of soil or sand and clay mixtures. They concluded that a priming effect on decomposition of soil organic matter would not occur with rates of residue addition used in actual farming practice.

The practice of using organic materials as mulches has been prevalent for many years. Mulches are known to have many effects on the surface

layers of the soil and in consequence are often employed for the benefit of shallow rooted plants. Mulches are known to maintain lower temperatures in soil surface horizons, reduce evaporation rates, reduce surface water run-off, reduce erosion, maintain soil organic matter and nitrogen levels, maintain a more permeable soil surface, check weed growth, and under high moisture conditions and high decomposition rates may reduce soil levels of nitrates. By producing favorable soil conditions and providing an organic energy source, mulches serve to support a large microbial population. Improvements in soil structure and permeability and the release of plant nutrients from organic forms lead to increased growth and vigour of higher plants (12, 42, 59, 68, 75, 92). White (95) found no influence on microbial populations when sawdust was used as a mulch on corn, lima beans and tomatoes. Increased yields of these crops were attributed to higher soil moisture levels induced by the mulch. Turk and Partridge (90) and Stephenson and Schuster (78) report that soil organic matter levels were increased through the use of mulches.

Since soil organic compounds are of plant, animal and microbiological origin, complex heterogeneous mixtures and polymers in various stages of decomposition and synthesis are present. For this reason, great difficulty is encountered in attempting to isolate specific nitrogen containing compounds. Fractionation procedures are often employed to separate soil organic constituents. Separations of organic matter are frequently based on differential solubility in water, alkali or salt solutions, and inorganic or organic solvents or on differential resistance to hydrolysis by acids or bases. Various analytical techniques and procedures are applied to these fractions to identify specific organic forms in which nitrogen is present (22, 68).

On the basis of resistance to acid hydrolysis, organic nitrogen forms can be separated into hydrolyzable and non-hydrolyzable fractions. As much as 30 percent of soil nitrogen is resistant to acid hydrolysis. Such nitrogen is likely to be of a non-protein nature, much of it possibly in the form of heterocyclic nitrogen compounds, although some may also be present as "fixed" ammonium within the lattice structure of clays (14, 15, 48). Approximately 70 to 90 percent of the soil nitrogen is released by acid hydrolysis. Of the nitrogen in soil hydrolysates, α-amino acids account for about one-third, although these appear to be present in "protein-like" combinations which behave like chemical complexes rather than true proteins. Approximately one-fourth of the hydrolyzable nitrogen is present as ammonium nitrogen. The origin of this ammonia is not clearly understood. It may result from deamination and deamidation reactions involving aromatic amines, hydroxyamino acids, purines, pyrimidines, uric acid and amino sugars, through the release of clay-fixed ammonium, and possibly from the amine or ammonia moities of condensation products of carboxyl and amino derivatives likely formed by mechanisms proposed for "browning" processes (14, 20, 85, 87). Amino sugars are found to comprise from 5 to 10 percent of the total nitrogen and are found in soil hydrolysates (19, 77, 80, 82, 94). The remaining forms of nitrogen in soil hydrolysates (approximately one-fourth to one-third) have not as yet been identified.

Fractionation on the basis of solubility in alkali or salt solutions provides an additional method by which soil organic nitrogen compounds may be characterized. Upon extraction of soil organic matter with alkali or salt solutions, two fractions are obtained, that which is solubilized and that which is not extracted. In the latter, called humin, organic nitrogen forms which are present may be partially extracted with acid to comprise an acid soluble humin nitrogen fraction. This fraction may comprise about 5 percent of the total soil nitrogen according to Chang and Kurtz (27). Organic matter which is soluble in alkali and salt solutions is usually fractionated into two fractions upon acidification. That portion which is precipitated upon acidification is referred to as the "humic acid" fraction and that portion remaining soluble is called the "fulvic" fraction. It is important to note that the amount of organic matter, the maintenance of its original structural integrity and its distribution within the fractions obtained are dependent on the nature of the extractant and the conditions under which it was used. Pyrophosphate solutions solubilize a much smaller proportion (10 to 20 percent) of the total soil nitrogen than do alkali (30 to 60 percent). It is considered that the smaller quantities of organic matter removed by neutral or buffered pyrophosphate extractants are more nearly representative structurally of the original humic materials in soils than are materials removed by alkali. The latter are likely altered by extensive hydrolytic and oxidative changes promoted by high pH (14). Gross differences in humic acids were noted by Savage and Stevenson (71) using sodium hydroxide and sodium pyrophosphate solutions as extractants.

From one-fourth to one-half of the soil organic matter occurs in the "fulvic" fraction. Of the nitrogen contained in this fraction, 20 to 30 percent is associated with amino acids. Over 50 percent of the nitrogen is associated with compounds which can be deaminated by acid hydrolysis to form ammonia. Such ammonia is associated primarily with compounds other than amino acid amides and hexosamines and may be associated with amine or ammonia moieties of sugar-amine condensation products resulting from mechanisms similar to those in "browning" reactions (16, 85). Humic acids contain 1 to 5 percent nitrogen, 60 to 70 percent of which is acid soluble upon hydrolysis with $\underline{N}$ HCl. Of this acid soluble nitrogen, approximately 40 percent is associated with α-amino groups, 15 percent appears as ammonia-nitrogen and about 5 percent is related to amino sugars. Oxidation of humic acids results in dialyzable and non-dialyzable intermediate products. Higher molecular weight compounds under continued oxidation are degraded into low molecular weight compounds and ultimately to ammonia, carbon dioxide and volatile acids. The low molecular weight materials have high nitrogen contents of about 10 percent. Of this nitrogen some is accounted for as amino acid nitrogen and the non-amino nitrogen is thought to be associated with azo-, diazo or heterocyclic nitrogen compounds. Small amounts of purine and pyrimidine bases have been found in association with humic acids (8, 18, 34, 71).

In general, of the organic forms of nitrogen in soils, Bremner (19,22) states that 30 to 40 percent occurs as amino acid, 5 to 10 percent as hexosamines, less than 2 percent as purines and pyrimidine bases and 50 percent has not been identified. Of the unidentified nitrogen, it is postulated that it may exist in complexes involving lignin-ammonia, quinone-ammonia, quinone-amino acid and carbohydrate-amino acid condensation products. Stevenson (79) places soil organic nitrogen in four separable groups: 1) basic and non-basic amino nitrogen, 2) basic and non-basic non-amino nitrogen, 3) amide or ammonia nitrogen, and 4) humin nitrogen.

In consideration of the inorganic forms of nitrogen in soils, a certain amount of nitrogen in the ammonium form is found to be "fixed" within clay crystal lattices. The proportion of the total soil nitrogen which is present as fixed ammonium increases with increasing soil depth and is dependent upon the kind of clay present. Such "fixed" ammonium may account for 3 to 8 percent of the total nitrogen in surface soils and up to 40 percent or more in subsoils (20, 39, 58, 83, 84, 86, 87, 88).

In the studies cited above the identification of specific nitrogen compounds was accomplished by the use of chromatographic and analytical procedures which are time consuming and poorly adapted to extensive studies directed toward correlating qualitative differences in soil organic matter with management factors on the one hand, or with plant responses on the other. Bremner (22) has recently proposed methods whereby several chemical forms of nitrogen, including mineral forms and α-amino and hexosamine nitrogen may be determined by sequential conversion to ammonia. The ammonia is recovered by distillation into boric acid and may be converted after titration to N_2 for partitioning of isotopic forms of nitrogen in tracer studies.

EXPERIMENTAL METHODS AND PROCEDURES

The soil samples examined in this study were obtained from two field experiments. One experiment concerned the effects of various organic mulches on an apple orchard. The second experiment dealt with the effects of various incorporated organic residues applied during a crop rotation sequence. These experiments together with sampling and statistical procedures are described below.

Mulching Studies at the Graham Station

This experiment located at the Graham Agricultural Sub-station at Grand Rapids will be referred to as the mulching experiment throughout the remainder of this study. It was established in 1954 on rolling topography and a rather complex association of well-drained soils representing the more fertile very fine sandy loams to loams in the fruit growing areas of the state. The site itself was marked by rather wide variations in texture and vertical development of surface horizons in the soil profile. The experiment had, as its purpose, to investigate how various mulches may affect tree performance, soil properties, and nutrient content of apple leaves during the first eight years of growth of an apple orchard. The determination of carbon, nitrogen, and carbon-nitrogen ratios at increasing soil depths in the present study is in partial fulfillment of the experimental goals set forth at the time this field experiment was established in 1954.

In the spring of 1954, uniform nursery grown stock of the Red Delicious variety was planted in a systematically arranged block design experiment with four replications. In mid-summer, mulches of alfalfa hay,

grass hay, wheat straw, and hardwood shavings were applied at rates of 100, 200, and 300 pounds per plot with the exception of the check plot. Wood shavings were not applied at the low rate. Each plot, containing a single tree, measured 23 x 26 feet. All mulches except the wood shavings were annually replenished to maintain the prescribed quantity of mulching material on each plot. Weeds were controlled with herbicides and by hand. No inorganic fertilizers were applied and all plots received the same insecticide sprays.

Soil samples were taken in the summer of 1961, seven years after the experiment was initiated. Six to eight cores were composited from depths of 0 to 6, 6 to 12, 12 to 18, and 18 to 24 inches for each plot. Composited samples were forced through a 4-mesh screen and thoroughly mixed before aliquots were withdrawn for transporting to the laboratory.

Although four replications of each treatment had been established in systematically arranged blocks in 1954, attrition of trees by wind damage left only 37 of the original 48 plots for sampling in 1961. Because of the large number of missing values, analytical data were analyzed statistically in accordance with a completely random design with unequal replication (28). Ranges of equivalence among equally replicated means were determined according to the method of Duncan (32).

The Rotation Residue Experiment

The rotation residue experiment was on the Lee Ferden farm approximately 35 miles northeast of Michigan State University and was initiated in 1951. The soil was classified as a Sims clay loam. The 5-year rotation sequence was corn, followed by white field beans, barley and two

years of alfalfa-brome hay. Each crop appeared on one of five tiers during each year. The following residue treatments were replicated five times on each tier:

1) Two years alfalfa-brome hay (two cuttings per year, removed).
2) Two years alfalfa-brome hay (two cuttings per year; removed the first year, weighed but not removed the second year).
3) One initial hardwood sawdust application plowed down before corn at the rate of 35 to 40 tons per acre. Two cuttings per year of alfalfa-brome hay were removed as in treatment 1.
4) Wheat straw, at 4 tons per acre, applied each cycle of the rotation after second year alfalfa-brome hay and plowed down for corn. Two cuttings per year of alfalfa-brome hay were removed as in treatment 1.

Each residue plot, measuring 14 x 90 feet, was further divided longitudinally, with one-half receiving supplemental nitrogen on corn, beans, and barley, although nitrogen top-dressings were discontinued on barley in the second cycle of the rotation. Table 1 shows the basic fertilization and the supplemental nitrogen application rates for each crop.

Soil samples for the rotation experiment were composited for each unit treatment (residue x nitrogen) from each tier of plots in late September or early October for five consecutive years (1956 through 1960). Ten cores to an 8-inch depth were taken randomly from each plot. The cores for the five replications of each unit treatment within each tier were combined, passed through a 4-mesh metal screen and thoroughly mixed before removal of representative aliquots to be taken to the laboratory.

The residue treatments were initiated on a different tier in each of five consecutive years (1951 through 1955). Thus, the soil samples taken during the years 1956 through 1960 actually represented a sequence of nine years after initiation of residue treatments. The relationships

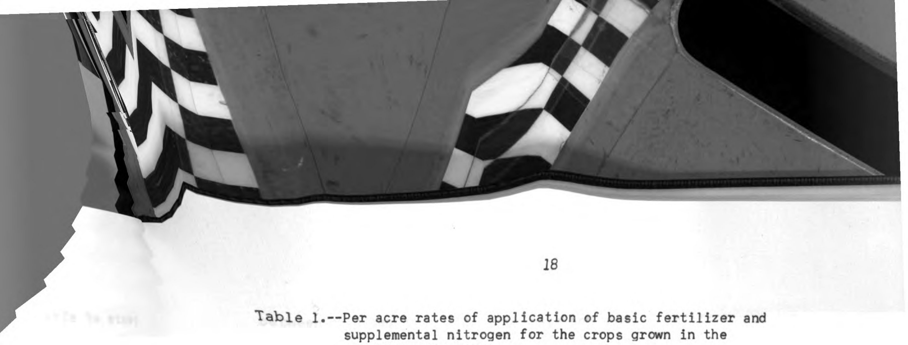

Table 1.--Per acre rates of application of basic fertilizer and supplemental nitrogen for the crops grown in the rotation experiment.

Crop	Basic fertilizer	Supplemental nitrogen
Corn	100 lbs. 5-20-10	100 lbs.
Beans	300 lbs. 0-14-7 or 210 lbs. 0-20-10	40 lbs.
Barley	240 lbs. 5-20-10	40 lbs.*
Alfalfa-Brome hay	None	None

* First rotational cycle only, discontinued in second cycle.

Table 2.--Relationships between crop sequence, sampling years, and year of initiation by tiers in the rotation experiment.

Years after initiation	Crop grown	Treatments sampled	Sampling years 56	57	58	59	60	Tier	Year initiated
1	* Corn_1	4 residues x 2 N levels = 8	8						
2	Beans_1	"	8	8					
3	Barley_1	"	8	8	8				
4	Alfalfa_{11}	"	8	8	8	8			
5	Alfalfa_{12}	"	8	8	8	8	8	E	55
6	Corn_2	"		8	8	8	8	C	54
7	Beans_2	"			8	8	8	B	53
8	Barley_2	"				8	8	A	52
9	Alfalfa_{21}	"					8	D	51

*First subscript refers to rotational cycle, second subscript refers to first or second year alfalfa-brome.

between crop sequence, sampling years and year of initiation are shown in Table 2.

It is apparent in Table 2 that the degree of replication available for evaluating unit treatment effects varied from one in the first and ninth years after initiation to five in the fifth year. There was also a confounding of replications (tiers) and sampling years. For purposes of statistical analysis, variations between tiers was cancelled out by converting all 40 values for a given tier to relative values. Within each tier, the observed value for percent carbon or nitrogen or carbon-nitrogen ratio for the control (treatment 1) without supplemental nitrogen in the fifth year after initiation was set at 100. This, in effect, adjusted all values to a common base in the fifth year after initiation. This adjustment appeared to be valid, since soil samples were available from all five tiers for this year in the cropping sequence.

The relative values were subjected, initially, to analysis of variance in accordance with an unequally replicated, completely random factorial design, involving four residue treatments, two levels of nitrogen, and seven crop years (omitting the first and ninth years for lack of replication). This initial analysis revealed no significant effects of nitrogen or of residues other than sawdust. Succeeding analyses, using data for all nine years, made use alternately of nitrogen treatments or residue treatments other than sawdust to increase replication of the earlier and later years of the cropping sequence. The significance of differences between the means for various combinations of treatments and crop years was tested by calculating ranges of

equivalence by the method of Duncan (32), as modified by Kramer (52) to allow the use of sample means containing varying "n" populations.

Analytical Procedures

In the laboratory, all field samples were quickly air dried and crushed on sheets of heavy wrapping paper. After again thoroughly mixing, 40 to 50 g. aliquots were ground to pass through a 0.5 mm. sieve. All chemical analyses were performed on these finely ground laboratory samples.

Total nitrogen

Total nitrogen for all samples was determined by the macro-Kjeldahl procedure as described by Jackson (43), with slight modifications according to Bremner (21). Ten gram aliquots of soil were used. Copper sulfate and mercuric oxide were used as catalysts. Digestion was continued for one hour after visible disappearance of carbon from the digest. Distillation was carried out directly without removal of mineral soil residues, using coarsely ground pumice to eliminate bumping. Sodium thiosulfate was added with the alkali to reduce mercuric salts during distillation. Distilled ammonium was collected in 4 percent boric acid and titrated against standard acid in the presence of methyl red-bromecresol green mixed indicator to a grey end point. Percent nitrogen was calculated after subtracting the titration value for a blank determination, as follows:

$$\%N = \frac{(\text{ml. acid for sample} - \text{ml. acid for blank})(\text{normality of acid})(1.4)}{\text{sample weight}}$$

All determinations were in duplicate, and reruns were made when replicates differed by more than 5 percent of the lower value.

Total carbon

All soil samples prior to organic carbon determination were pretreated with reagent grade sulfurous acid (assay 8.9 percent SO_2) to remove carbonates (13). Soil samples were saturated with this acid, then placed under vacuum over calcium chloride for two to three days at room temperature. When dry, the soils were ground before analysis.

The carbon determinations for the rotation experiment were conducted manometrically according to the procedures of Van Slyke and Folch (91) and Peters and Van Slyke (64), involving the Van Slyke blood gas apparatus. Soil aliquots of 50 to 70 mg., accurately weighed, were taken for analysis and placed into 25 ml. digestion flasks. Wet combustion, with heat, was accomplished in a mixture of chromium trioxide, phosphoric and fuming sulfuric acids, in the presence of activated alumina and potassium iodide. Visible release of I_2 from the latter was considered to indicate complete oxidation of carbon contained in the soil sample and the end of digestion. Two to three minutes of boiling were required. Carbon dioxide was absorbed in an alkaline solution of hydrazine sulfate in the manometric chamber of the apparatus. After expulsion of other gases, excess 2 N lactic acid was admitted into the manometric chamber and the pressure at standard volume of the released CO_2 was measured in mm. on the mercury manometer. This reading constituted the P_1 reading. The CO_2 was reabsorbed by the addition of excess 5 N NaOH and the pressure of the system at standard volume was again measured on the manometer. This second reading constituted the P_2 reading. Carbon contents were calculated by multiplying the difference in pressures by a tabular factor dependent upon the temperature of the

manometric chamber. Prior to running a consecutive group of samples, the apparatus was equilibrated by running as many blank determinations as needed to obtain constant blank values. These constant blank values were used in calculating a first approximate carbon content according to the following equation:

$$\%C = \frac{(P_1 - P_2)(\text{factor})_{\text{sample}} - (P_1 - P_2)(\text{factor})_{\text{blank}} \times 100}{\text{sample weight}}$$

Dextrose samples of 4 to 7 mg., accurately weighed, were occasionally run to check techniques and the efficiency of the apparatus. A correction factor based on the percent carbon recovered from the dextrose was then applied to the percent carbon found by the above equation to obtain a final value for percent carbon for each sample. All determinations were in duplicate or were repeated until agreement within 5 percent was reached among triplicate determinations.

The wet combustion procedure of Allison (5) was used to determine carbon contents for the mulching experiment soils. Wet combustion was effected with heat, using 25 ml. of a sulfuric-phosphoric acid combustion mixture. Soil aliquots of 1.0 to 1.2 g., accurately weighed, were analyzed. A continuous stream of CO_2-free air was used to carry CO_2 evolved in the digestion flask through a series of traps to remove volatile halides and water. The CO_2, sorbed by "ascarite" in the terminal absorption bulb, was quantitatively measured by gravimetric procedures. Heat was applied initially so as to achieve boiling in 3 to 5 minutes. Boiling was continued for 10 minutes. Aeration was continued for an additional 10 minutes after the flame was removed. Percent manometric chan[illegible] samples, the apparatus w[illegible] the determinations as needed t[illegible] constant blank values were used in [illegible] according to the follow[illegible]

$$\%C = \frac{[illegible]}{[illegible]} \times 100$$

Dextrose [illegible] were occasion-ally run to [illegible] of the apparatus. A correction [illegible] recovered from the dextrose was then applied [illegible] by using the above equation to obtain a [illegible] for each sample. All deter-minations were [illegible] repeated until agreement within 5 percent was [illegible] among [illegible] determinations.

The wet combustion procedure of Allison (6) was used to determine carbon contents for the various experiment soils. Wet combustion was effected with heat, using 20 ml. of a sulfuric-phosphoric acid combustion mixture. Soil aliquots of 1.0 to 1.2 g., accurately weighed, were analyzed. A continuous stream of CO_2-free air was used to carry CO_2 evolved in the digestion flask through a series of traps to remove volatile halides and water. The CO_2, sorbed by "ascarite" in the terminal absorption bulb, was quantitatively measured by gravimetric procedures. Heat was applied initially so as to achieve boiling in 3 to 5 minutes. Boiling was continued for 10 minutes. Aeration was continued for an additional 10 minutes after the flame was removed. Percent carbon was determined by the following calculation:

$$\%C = \frac{(\text{weight of } CO_2 \text{ in sample} - \text{weight of } CO_2 \text{ in blank})(27.27)}{\text{sample weight}}$$

Dextrose samples, accurately weighed, approximating 75 mg. were occasionally run as was done for the Van Slyke carbon analysis. The percentage recovery of carbon from dextrose was applied as a correction factor to soil carbon contents obtained by the above equation. Agreement within 5 percent among duplicates was required, or among triplicates where reruns were made.

The two methods were compared on a group of eight soil samples, in duplicate. Sample means for the two methods agreed within 5 percent, and there was no difference in average recovery of carbon by either method. The manometric measurement was more rapid but also required much more meticulous attention to details of manipulation to attain equal reproducibility.

Carbon-nitrogen ratios for all samples were calculated by dividing the determined percent carbon by the percent nitrogen.

Nitrogen fractionation by acid hydrolysis

Methods commonly used for studying the chemical constitution of soil organic matter are complicated, time consuming and involve a wide variety of analytical techniques. Bremner (22) has recently proposed a greatly simplified approach based on a systematic, stepwise conversion of important categories of nitrogen in acid hydrolysates to ammonia, which is readily determined by steam distillation.

An attempt was made to apply this procedure on a limited preliminary scale to a small group of soil samples selected from the mulching and carbon was dete[illegible]

$$\%C = \frac{(\text{weig}\ldots \text{[illegible]}\ \ldots CO_2 \text{ in blank})(27.27)}{\text{[illegible]}}$$

Dextrose samp[illegible] weighing 10 mg. were occa-
sionally run [illegible] analysis. The
percentage re[illegible] applied as a correction
factor to soil [illegible] above equation. Agree-
ment within 5 per[illegible] required, or among tripli-
cates where rep[illegible].

The two [illegible] of eight soil samples,
in duplicate. [illegible] agreed within 5 percent,
and there was [illegible] recovery of carbon by either
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An attempt was made to apply this procedure on a limited preliminary scale to a small group of soil samples selected from the mulching and rotation experiments. Soils were selected on the basis of wide differences in total carbon or nitrogen associated with treatment or cropping sequence. From the mulching experiment, soils representing the 0 to 6 inch depth from one replication only under the high rates of application of the four mulching materials and from the control plot were examined. From the rotation experiment, sixteen samples were selected from one tier of plots. These represented samples taken after beans in 1957 and after the second year of alfalfa-brome hay in 1960 from plots receiving four different residue treatments with and without nitrogen.

Unpublished details of the procedure were kindly provided by Dr. Bremner (23) for use in this laboratory. Accurately weighed soil aliquots ranging from 5.000 to 5.350 g. were hydrolyzed in 20 ml. 6 N HCl under reflux for 12 hours. Non-hydrolyzable nitrogen was determined on the residue retained after washing with distilled water on Whatman No. 50 filter paper, using the macro-Kjeldahl procedure already described.

The hydrolysate and washings (approximately 60 ml.) were adjusted under ice-bath conditions to a pH of 6.5 ± 0.1 and made up with distilled water to 100 ml. Aliquots of the adjusted hydrolysate were used for estimation of total nitrogen and nitrogen present in the ammonium, α-amino and hexosamine forms.

Total nitrogen in the hydrolysate was determined by a micro-Kjeldahl procedure. Copper sulfate and mecuric oxide were used as catalysts. Sodium thiosulfate was added with the alkali during steam distillation of rotation experi[illegible] results of wide differ-ences in total [illegible] with treatment or cropping sequence. From [illegible] representing the 0 to 6 inch depth [illegible] rates of application of the four [illegible] plot were examined. From the rotation [illegible] selected from one tier of plots [illegible] began in 1957 and after the [illegible] plots receiving four different [illegible] nitrogen.

Unpublished [illegible] kindly provided by Dr. Bremner [illegible] accurately weighed soil aliquots ranging [illegible] were hydrolyzed in 20 ml. 6 *N* HCl under [illegible] non-hydrolyzable nitrogen was determined on the [illegible] after washing with distilled water on Whatman No. 50 filter paper using the macro-Kjeldahl procedure already described.

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Total nitrogen in the hydrolysate was determined by a micro-Kjeldahl procedure. Copper sulfate and mercuric oxide were used as catalysts. Sodium thiosulfate was added with the alkali during steam distillation of ammonia into 4 percent boric acid.[1] A micro-burette was used in the titration against 0.005 N sulfuric acid to the grey end point of the methyl red-methylene blue indicator described by Block and Weiss (10).

Ammonium nitrogen was determined by steam distillation in the presence of MgO. Extensive tests by Bremner indicate that ammonium nitrogen can be determined quantitatively in solutions containing labile organic nitrogen compounds by steam distillation with MgO at pH 10.6 for 2 to 3 minutes.

Alpha-amino nitrogen was determined after first removing ammonium and hexosamine nitrogen by heating for 20 minutes in a boiling water bath after addition of alkali to raise the pH above 11.2, where hexosamines have been shown to be readily deaminated. Conversion of α-amino nitrogen to ammonia was accomplished by reaction with ninhydrin at boiling water-bath temperatures after the pH was lowered to 2.5 by addition of citric acid. Bremner has shown that condensations normally associated with the ninhydrin reaction are suppressed at this pH, and ammonia released is later recovered quantitatively by steam distillation above pH 11.2 for no longer than 4 minutes.

By carrying out the ninhydrin reaction at pH 2.5 first, ammonium, α-amino and hexosamine nitrogen were distilled off together as ammonia. This distillation was carried out for 4 minutes in the presence of phosphate-borate buffer at pH 11.2 to avoid breakdown of labile compounds

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[1] The decision to use mercuric oxide rather than selenium as a catalyst in determining total hydrolyzable nitrogen was made in this laboratory. More recent communications with Dr. Bremner indicate that this was an unfortunate choice, since thiosulfate used to reduce mercuric salts during distillation may result in incomplete recovery of hexosamine nitrogen as ammonia.

(other than hexosamines), which have been shown to release ammonia with extended heating at pH's above 11.2. Hexosamine nitrogen was estimated indirectly by difference.

All determinations were performed in duplicate. Additional analyses were performed when the difference between duplicates exceeded 5 percent with respect to the lower value.

RESULTS

Total Nitrogen, Carbon and C/N Ratios

Rotation residue experiment

Preliminary inspection of data from the rotation experiment indicated that variations in carbon, nitrogen and C/N ratio were associated primarily with tiers and the sawdust treatment, as well as with time or cropping sequence. Differences between residue treatments other than sawdust were small. Differences between nitrogen treatments were small or, in the case of sawdust, apparently erratic. Variation between tiers was eliminated by converting all observed values within each tier to relative values, setting the value observed for the control without nitrogen in the fifth year equal to 100.

1. Effects of supplemental nitrogen.

Degrees of freedom for unit treatments were reduced to one in the first and ninth years of the cropping sequence (cf. Table 2). For this reason, significance of effects associated with nitrogen levels was estimated in two ways. In the first analysis (Table 9, Appendix), residue treatments other than sawdust were combined to increase replication afforded by tiers. In the second analysis (Table 10, Appendix), effects of nitrogen within the sawdust treatment were tested, eliminating the data for the first and ninth years. Both analyses supported the conclusion that there was no main effect of nitrogen and no interaction (significant at P_{05}) between nitrogen treatments or crop years in their effects on soil nitrogen, carbon or C/N ratio.

Data for sawdust and the averages for residues other than sawdust, with and without supplemental nitrogen, are presented graphically in Figure 1. Large fluctuations during the first two or three years in levels of carbon or nitrogen in soil amended with sawdust were not statistically significant with the low level of replication available in the first three years, and they may have been due to sampling errors arising from the fact that sawdust and added nitrogen were not uniformly distributed through the soil volumes represented by the samples taken. Fluctuations in soil nitrogen associated with supplemental applications on residues other than sawdust were reasonable in that they were of the same order of magnitude as the quantities of nitrogen applied on corn, beans and barley (180 pounds nitrogen is equal to 0.009 percent of two million pounds soil per acre furrow slice). Changes of this order, although practically important, cannot be measured reliably because they are less than the experimental errors involved in sampling and in the nitrogen determination itself. Standard deviations for nitrogen ranged from 4.1 to 7.2 percent of the mean nitrogen determination (Tables 9 and 10). The 180 pounds of nitrogen applied as a total for corn, beans and barley represented 3.6 percent of this mean.

Soil C/N ratios for sawdust with supplemental nitrogen were consistently lower from the third through the seventh years than for sawdust alone (Figure 1), and suggested that carbon may have declined more rapidly during this period in the plots to which nitrogen was applied. Declines in carbon in sawdust treated plots between the second and eighth years were tested for conformity with the expression which Salter and Green (70) found applicable to organic matter decline in soils under cultivation:

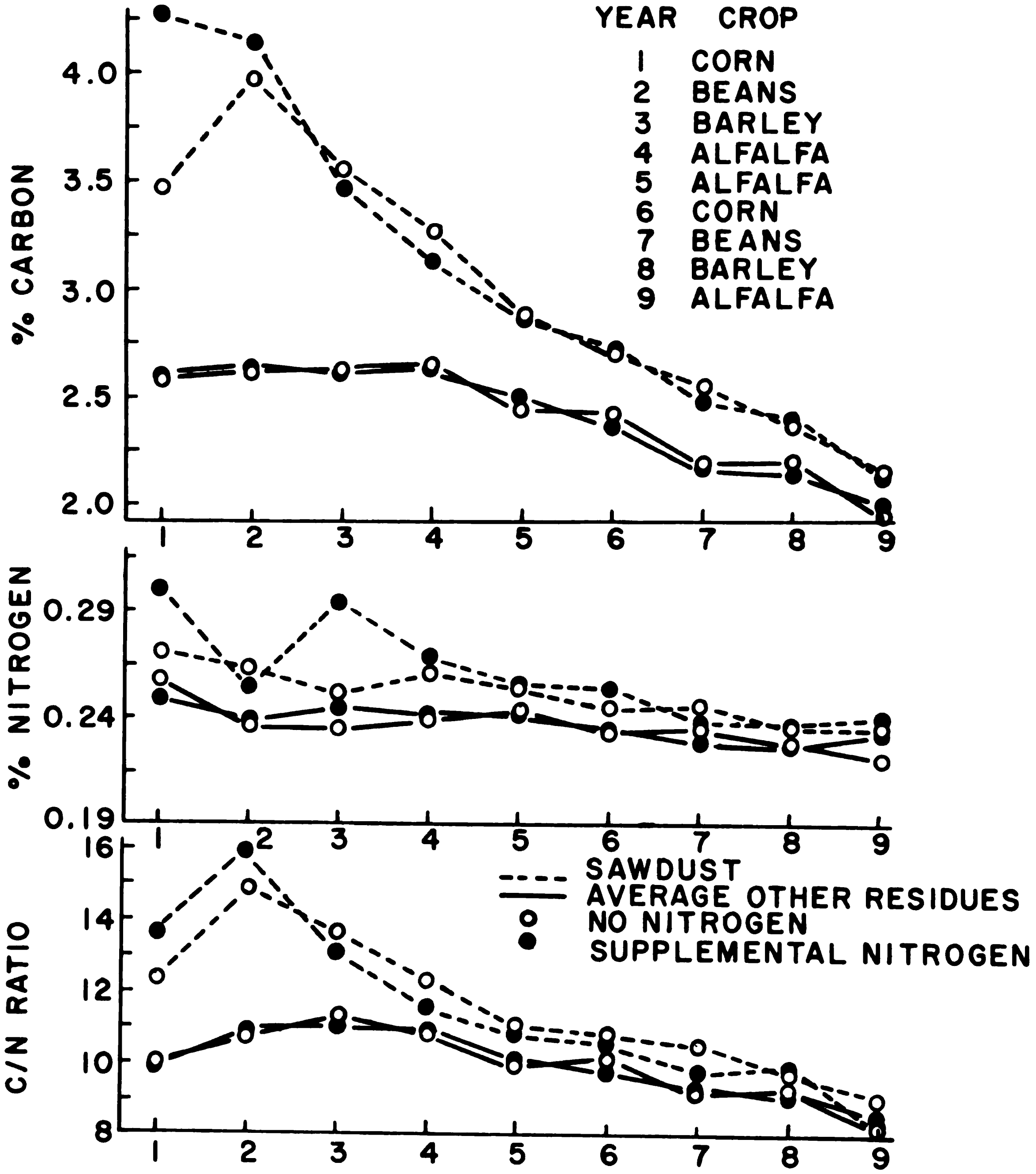

Figure 1.--Changes in soil carbon, nitrogen and C/N ratio for sawdust and the average for other residue treatments, as related to supplemental nitrogen, time and cropping sequence.

$$C_t = C_0 K^t$$

Where: C_t = % C at time t
C_0 = % C at zero time
K = a constant representing residual carbon in any year as a fraction of that present the preceding year.

The correlation coefficient obtained for sawdust alone was 0.989, and for sawdust plus nitrogen 0.977. The slopes of the two curves were not significantly different at P_{05} by Student's "t" test, although "K" value from Salter and Green's equation indicate that the rate of decline with supplemental nitrogen (8.55 percent per year) was somewhat greater than for sawdust alone (8.30 percent per year). Values for C_0 calculated for both equations were not significantly different (4.59 percent for sawdust alone and 4.61 percent for sawdust plus nitrogen). These estimates for total carbon at the time of application of sawdust are realistic. Forty tons of sawdust would have contained carbon equal to about 2 percent in an acre furrow slice. Subtracting this from the 4.60 percent, as the mean for sawdust plots combining nitrogen levels, would leave 2.60 percent in soil organic matter at the time of application. This compares favorably with 2.51 percent found in control plots after corn one year later.

Thus there is a suggestion that the disappearance of carbon from added sawdust may have been hastened somewhat by the addition of nitrogen, at least after the second year. At the rates of supplemental nitrogen used, this effect was not statistically significant, and it is unlikely that it was of any practical significance.

The data in Figure 1 also shows consistency with the view that added nitrogen may have promoted a greater retention of carbon and

and greater immobilization of nitrogen in the presence of sawdust during the first two or three years. The quantities of nitrogen and carbon involved and the patterns of year-to-year fluctuations, however, do not appear to be sufficiently defined to either support or oppose this view.

2. Effects of residues as related to time.

On the basis of the evidence that nitrogen treatments were without significant effect, a third analysis of variance was performed, in which nitrogen treatments were used to increase the replication afforded by tiers, and all data were included in the analysis. This analysis (Table 11, Appendix) showed that significant to highly significant variations in all three measurements were associated with both residue treatments and years. In the case of carbon content, a highly significant interaction between residues and crop years was expressed.

None of the above conclusions were altered by evidence from subsequent analyses in which replications were made equal to tiers and only data from the second through the eighth years were used. These analyses provided no evidence for significant interaction between supplemental nitrogen and either residue treatment or crop year.

Relative values were recalculated to average percentages of carbon and nitrogen, and average C/N ratios. The recovered means for crop years and residue treatments are presented in Tables 3, 4, and 5. In these tables, means for residue treatments within each crop year are given in the first four columns. Differences required at P_{05} for significance between residue means in any crop year are given in the last column.

No significant differences were encountered at any time between the control, alfalfa or straw treatments. Throughout the experiment, carbon,

Table 3.--Rotation experiment: Average percent carbon in soil for crop years and residue treatments, ignoring nitrogen levels.

Year	Crop	Residue treatment				Average for all treatments	L.S.D. at 5% for comparisons between treatments
		Control	Alfalfa	Straw	Sawdust		
				<u>First rotational cycle</u>		*	
1	Corn	2.51	2.46	2.82	3.87	2.91 ab	0.45
2	Beans	2.63	2.55	2.74	4.06	3.00 a	0.32
3	Barley	2.74	2.58	2.53	3.51	2.84 ab	0.26
4	Alfalfa	2.59	2.64	2.70	3.19	2.78 b	0.23
5	Alfalfa	2.51	2.48	2.42	2.85	2.57 c	0.20
				<u>Second rotational cycle</u>			
6	Corn	2.38	2.35	2.41	2.71	2.46 c	0.23
7	Beans	2.12	2.19	2.20	2.50	2.26 d	0.26
8	Barley	2.16	2.18	2.14	2.38	2.21 d	N.S.
9	Alfalfa	1.99	2.00	1.91	2.14	2.01 d	N.S.

Table 4.--Rotation experiment: Average percent nitrogen in soil for crop years and residue treatments, ignoring nitrogen levels.

Year	Crop	Residue treatment				Average for all treatments	L.S.D. at 5% for comparisons between treatments
		Control	Alfalfa	Straw	Sawdust		
				First rotational cycle		*	
1	Corn	0.250	0.253	0.254	0.285	0.261 a	0.025
2	Beans	0.245	0.240	0.234	0.259	0.243 bc	0.018
3	Barley	0.243	0.239	0.237	0.272	0.248 b	0.015
4	Alfalfa	0.240	0.239	0.242	0.264	0.246 bc	0.013
5	Alfalfa	0.240	0.245	0.244	0.257	0.246 bc	0.011
				Second rotational cycle			
6	Corn	0.232	0.236	0.237	0.250	0.239 cb	0.013
7	Beans	0.231	0.234	0.231	0.243	0.234 de	N.S.
8	Barley	0.228	0.230	0.229	0.236	0.231 e	N.S.
9	Alfalfa	0.230	0.230	0.225	0.238	0.223 e	N.S.

* Letters following the figures represent ranges of equivalence. Those figures having the same letters are not signigicantly different from each other.

Table 5.--Rotation experiment: Average carbon-nitrogen ratios in soil for crop years and residue treatments, ignoring nitrogen levels.

Years	Crop	Residue treatment				Average for all treatments	L.S.D. at 5% for comparisons between treatments
		Control	Alfalfa	Straw	Sawdust		
					First rotational cycle		
1	Corn	9.95	9.43	10.99	13.05	10.86 bc	2.14
2	Beans	10.88	10.55	11.59	15.47	11.74 a	1.52
3	Barley	11.68	10.90	10.93	13.31	11.70 ab	1.24
4	Alfalfa	10.70	10.96	11.05	12.00	11.18 ab	1.14
5	Alfalfa	10.39	10.03	9.90	10.98	10.33 c	0.96
					Second rotational cycle		
6	Corn	10.06	9.86	10.09	10.72	10.18 cd	N.S.
7	Beans	9.08	9.29	9.55	10.23	9.54 de	N.S.
8	Barley	9.41	9.40	9.28	9.96	9.52 de	N.S.
9	Alfalfa	8.54	8.62	8.44	8.93	8.63 e	N.S.

* Letters following the figures represent ranges of equivalence. Those figures having the same letters are not significantly different from each other.

nitrogen and C/N ratios were higher with sawdust than with any other treatment, although these differences became non-significant after 8 years for carbon (Table 3), after 7 years for nitrogen (Table 4) and after 6 years for the C/N ratio (Table 5).

In the next-to-last column of Tables 3, 4, and 5 are given crop year averages, combining residue treatments. These means for crop years are identified alphabetically by ranges of equivalence calculated according to the method of Kramer (52) for unequal replication. Significant decreases occurred in all three measurements during the 9-year sequence, most notably during the second cycle of the rotation. In the case of soil nitrogen (Table 4) these overall changes tended to follow a course essentially similar to those observed within individual residue treatments. A highly significant interaction between residue treatments and crop years in their effects on percent carbon (Table 3) was due primarily to a more rapid decline from much higher initial levels in the plots which received the massive application of sawdust.

3. Effects of residues as related to cropping sequence.

Data from Tables 3, 4, and 5 are presented in Figure 2. For purposes of comparison, a base line representing calculated levels of soil nitrogen for the control soil has been drawn on the four graphs in the upper half of the figure. This base line was derived by applying the expression $N_t = N_0K^t$ to the control data for the six years during which cultivated crops were grown (years 1 to 3 and 6 to 8). The value obtained for "K" was 0.9872, which represents a loss of nitrogen at the rate of 1.28 percent per year. This conforms well to K = 0.9894 obtained by Salter and Green for a similar 5-year rotation (70).

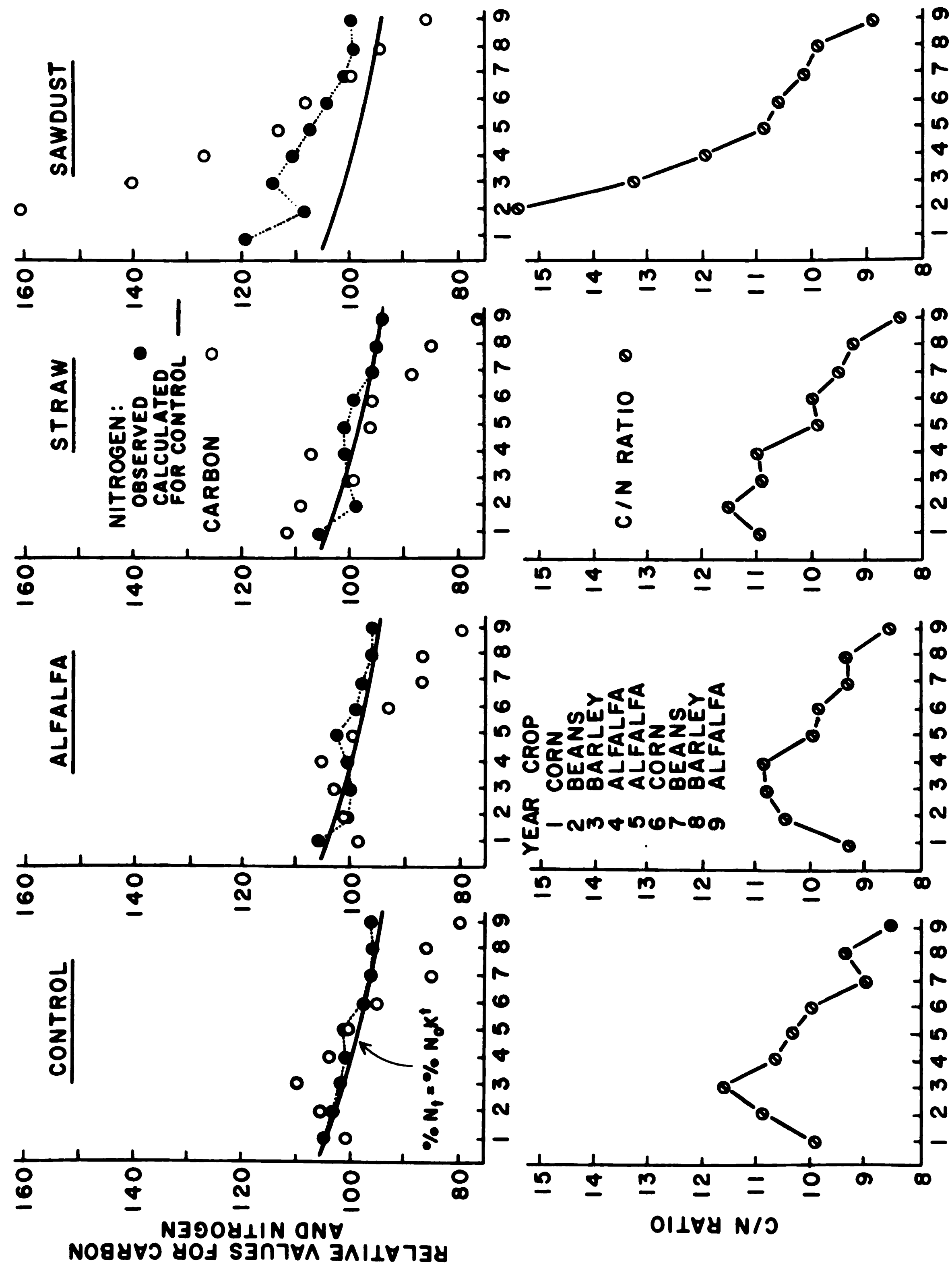

Figure 2.--Changes in soil carbon, nitrogen and C/N ratio as related to residue treatments, time and cropping sequence.

The only statistically significant effect of residue treatment on soil nitrogen was the large increase in the presence of sawdust. This increase over the control in the first year was equivalent to 700 pounds of nitrogen per acre. This would appear to be an extremely unrealistic increase, although, with the exception of the second year, the amount of nitrogen found the first year is in line with the declining levels observed in subsequent years. With respect to the soil as related to the sawdust treatment, nitrogen increases could have resulted from the residue itself as well as from soil related processes. Allison and Anderson (6) state that sawdust generally contains from 0.1 to 0.3 percent nitrogen. Choosing a median value, 40 tons of sawdust could conceivably have supplied 160 pounds of nitrogen per acre. It is unlikely that soil processes were able to supply the remaining 540 pounds of nitrogen, either by the conservation of nitrogen released by mineralization of organic matter or by release of clay lattice "fixed" ammonium. Some absorption of atmospheric ammonia promoted by the high concentration of sorptive organic compounds in sawdust may have occurred, although only as much as 20 to 40 pounds of nitrogen per acre per year is reported for atmospheric ammonia sorption by organic soils (61). In the presence of large amounts of energy sources and low amounts of available forms of nitrogen it is conceivable that a large population of non-symbiotic nitrogen fixing microorganisms may have been promoted (68). Alexander (2), however, states that only 20 to 40 pounds of non-symbiotically fixed nitrogen per acre has been reported for temperate soils. The implications of nitrogen fixation in the presence of sawdust and other carbonaceous residues will be dealt with further in the subsequent chapter on discussion of results.

Regardless of the source of the increased nitrogen levels in the presence of sawdust at the beginning of the experiment, subsequent declines were at a more rapid rate (approximately 2.5 percent per year) than in the control soil (1.28 percent per year).

Differences in nitrogen content between the control soil and soil receiving alfalfa residues or straw were not significant at the 5 percent level of probability and no significant interactions with years were indicated. Nevertheless, it appeared that both alfalfa and straw may have enhanced a cyclic fluctuation in level of soil nitrogen, whereby nitrogen tended to decline during cultivation and increase again during the two alfalfa-brome hay years. It also appeared that the overall rate of decline in soil nitrogen for these two materials was somewhat less than indicated by the base line drawn for the control.

As noted in the previous section, a highly significant interaction between residues and crop years was expressed in their effects on carbon content of the soil. In Figure 2 it is apparent that a major interaction involved carbon being initially much higher and disappearing at a much more rapid rate where sawdust was applied than with any other residue treatment. Additional interactions, however, were derived from the fact that, whereas carbon disappearance in the sawdust plots was essentially a function of time, in other treatments increases as well as decreases in carbon occurred. These fluctuations were related to cropping sequence and less clearly to time.

In control plots and those to which the second year's alfalfa-brome hay was returned, increases in carbon occurred during the first 3 to 4 years. Data for straw were erratic but reflected a tendency for carbon to be maintained at relatively high levels through the fourth

year. In the second cycle of the rotation, however, carbon declined rapidly in all four treatments, with only slight interruptions associated with corn in the sixth year or barley in the eighth year.

Examination of field records revealed that prior to 1959, these plots had been plowed for corn and beans in the spring of the year. Since surface crop residues caused a delay in the drying of soil during the spring, fall plowing prior to corn and beans was initiated in 1959. The data suggest that fall plowing has hastened the loss of carbon without materially affecting the levels of nitrogen in the soil. It is unlikely that this large differential loss of carbon over nitrogen can be maintained over any long period of time, since C/N ratios at the end of the period were already less than 9.0. However, there was no tendency for carbon losses to level off in the period studied.

Relative values plotted for both carbon and nitrogen in Figure 2 indicate that carbon in soil organic matter is subject to much more drastic variations under the influence of cropping sequences and management practices than is nitrogen. Nitrogen appears to be a more valid measure of organic matter content than carbon. The evidence of this 9-year sequence is that organic matter content in this humic gley soil has not reached a new equilibrium level after 50 years of cultivation. Nitrogen is still declining at an average rate of 1.28 percent per year. Although the application of 35 to 40 tons of sawdust resulted in a substantial increase in soil organic nitrogen, this effect appears to have been only temporary. Nitrogen levels under this treatment were rapidly approaching those in the control by the end of the period. More normal residue additions, even though repeated each rotation, promoted

minor cyclic variations in nitrogen content without appreciably affecting the basic pattern of nitrogen decline. Conventional rates of nitrogen additions had no detectable effect on soil nitrogen levels.

The widely fluctuating carbon contents and carbon-nitrogen ratios in Figure 2 indicate that much more can be done by management practices to alter the quality of soil organic matter than its content under a given set of soil and climatic conditions. It would appear that any substantial increase in soil nitrogen content above some characteristic base line must be attended by changes in quality leading to more rapid mineralization and a tendency to return to the base. Conversely, declines below this base line lead to reduced mineralizability and a tendency for nitrogen to accumulate again toward the base. As is shown in this experiment, the base line need not represent a flat equilibrium level.

It is apparent that substantial increases in soil nitrogen above a base line must be preceded or accompanied by substantial increases in carbon from plant residues grown in place or brought in from extraneous sources. However, disproportionate increases in carbon which lead to excessively wide C/N ratios promote competitive uses of nitrogen by the soil microbial population and consequently reduce the availability of nitrogen to crops.

4. Relationships with crop yields.

The extent to which changes in quantity or quality of soil organic matter has influenced the availability of both soil and fertilizer forms of nitrogen is reflected in the crop yields for this experiment. Crop yields averaged over all years of the experiment are summarized in Table 6.

Table 6.--Crop yields through two cycles of the rotation experiment.

Crops and Years	Crop Yields			
	Control		Alfalfa	
	No N	N	No N	N
*				First
Corn 1952-1956	82.3 bu./A.	90.5 bu./A.	84.8 bu./A.	88.1 bu./A.
Beans 1953-1957	34.7 "	35.2 "	32.8 "	35.0 "
Barley 1954-55-57-58	49.8 "	53.5 "	48.9 "	53.9 "
Alfalfa (1st yr.) 1955-1959	3.31 T./A.		3.29 T./A.	
Alfalfa (2nd yr.) 1956-1960	4.41 "		2.39 " **	
				Second
Corn 1957-1961	73.1 bu./A.	97.7 bu./A.	93.4 bu./A.	100.6 bu./A.
Beans 1958-1961	32.6 "	36.7 "	33.0 "	36.2 "
Barley 1959-1961	72.3 "	71.5 "	71.9 "	71.2 "
Alfalfa (1st yr.) 1960-1961	4.17 T./A.		3.97 T./A.	
Alfalfa (2nd yr.) 1961	5.56 "		3.26 " **	

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* First cycle yields are averages for 25 plots (5 years and 5 plots per second cycle, averages for corn, beans, barley and first and second

** First cutting only.

Crop Yields			
Straw		Sawdust	
No N	N	No N	N
rotational cycle			
80.6 bu./A.	83.1 bu./A.	36.4 bu./A.	51.8 bu./A.
33.6 "	35.3 "	27.0 "	32.1 "
51.4 "	52.8 "	36.9 "	45.3 "
3.25 T./A.		3.58 T./A.	
4.40 "		4.36 "	
rotational cycle			
88.8 bu./A.	98.0 bu./A.	98.6 bu./A.	103.0 bu./A.
32.9 "	35.8 "	34.3 "	37.5 "
70.9 "	70.6 "	71.6 "	69.5 "
3.99 T./A.		3.72 T./A.	
5.54 "		5.89 "	

year), except barley which was not harvested in 1956. In the
year alfalfa are for 5, 4, 3, 2, and 1 year, respectively.

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During the first cycle of the rotation, yields of corn, beans and barley were sharply reduced by the sawdust treatment. The addition of 100 pounds per acre of fertilizer nitrogen on corn and 40 pounds each on beans and barley failed to compensate for microbial demand and produce yields comparable to the control without supplemental nitrogen. These yield depressions were associated with greatly increased total quantities of nitrogen in the soil (cf. Figure 2), and also with C/N ratios above 13.0. At this point, the quality factor represented by C/N ratios was more important than the quantity factor in determining availability of nitrogen.

In the fifth year, before corn was planted again in the second cycle of the rotation, C/N ratios in sawdust-amended soil had been reduced to 11.0. Maximum yields of corn in the sixth year were obtained with the sawdust treatment. The competitive disadvantage to the crop of excessive supplies of energy carbon for the microbial population had largely disappeared. Higher yields of corn at this time were associated with a quantity factor represented by higher levels of soil nitrogen as well as a quality factor reflected in the higher rate of mineralization (Figure 2).

In comparing the second rotational cycle with the first, a further illustration of quantity-quality relationships is indicated by the marked decrease in yields of corn in control plots without supplemental nitrogen. The decrease from 82.3 to 73.1 bushels represents a 7.8 percent decline in yield and corresponds to a 7.4 decline in soil nitrogen calculated along the base line in Figure 2. With the alfalfa and straw treatments, increases in yields of corn in the second

cycle over the first were associated with temporary accumulations of nitrogen in the fifth year above the base line for the control. These increases in quantity of nitrogen were associated with quality factors which led, temporarily, to greater mineralization rates.

Admittedly, these changes in soil nitrogen with alfalfa and straw were small and statistically non-significant. However, their relationship to corn yields serves to illustrate a principle which is more adequately supported by the exaggerated changes in quantity and quality of organic matter afforded by the massive addition of sawdust.

The cyclic fluctuations in nitrogen were clearly expressed only during the first cycle of the rotation when the conservation of carbon was promoted by plowing under indigenous and extraneous residues for corn and beans in the spring of the year. Fall plowing for all three tilled crops in the second cycle of the rotation resulted in rapid declines in carbon, with no evidence that an equilibrium was being approached when the last observations were made. The effect of fall plowing on nitrogen was to reduce the cyclic fluctuations without any detectable effect on the basic rate of decline. What subsequent trends may have been or their effects on crop performance cannot be known, since the experiment was terminated in 1961.

Mulching experiment

Plant materials used in the mulching experiment were similar to those used in the rotation experiment. The essential difference was the fact that residues were incorporated into the soil by tillage operations in the rotation experiment, whereas they were left on the surface in the mulching experiment. In the latter case, contributions to soil organic

matter were limited to soluble materials initially present or released by decomposition at the soil surface and moved by leaching into the soil underneath. Quantitative and qualitative differences in effects on soil organic matter would be expected for a given material applied in these two ways.

The distribution of nitrogen and carbon with depth under the various kinds and rates of mulch seven years after application are presented in Table 7, together with the corresponding C/N ratios. Values shown in parentheses for wood shavings and grass hay were not included in the analysis of variance for lack of adequate replication. No evidence of interaction between rates and materials was encountered, so only means for materials, or rates, or the average for all rates of all materials are given for comparison with the unmulched control.

Significantly higher contents of nitrogen and carbon were found under mulches than in unmulched soil to a depth of 12 inches. At the 300 pound rate of application, significant increases in carbon relative to the control occurred to a depth of 18 inches. The largest increases in both carbon and nitrogen were found under alfalfa, followed by grass hay, straw and shavings. Carbon-nitrogen ratios were not significantly related to mulch material but tended to be higher than the control under all mulch materials, notably straw and shavings. Carbon-nitrogen ratios declined rapidly with depth.

The rates of mulch used (100, 200 and 300 pounds per tree) corresponded to 3½, 7 and 10½ tons per acre. Each summer, the quantity of undecomposed mulch left was estimated by making up and weighing the residue on ¼ of each plot. Fresh mulch material was added to bring the

Table 7.--Mulching experiment: Percent nitrogen and

Table 7.--Mulching experiment: Percent nitrogen and carbon, and carbon-nitrogen ratios in soil for four sampling depths.

	Percent total nitrogen				Percent total carbon				Carbon-nitrogen ratio			
Treatment	0-6"	6-12"	12-18"	18-24"	0-6"	6-12"	12-18"	18-24"	0-6"	6-12"	12-18"	18-24"
					No mulch versus average of mulches for all rates							
No Mulch	.089 b	.036 b	.027	.027	1.011 b	.382 b	.190	.182	11.5	10.3	6.9	6.8
Average Mulch*	.109 a	.056 a	.029	.025	1.302 a	.598 a	.242	.184	12.0	10.5	8.2	7.5
Shavings	(.084)	(.042)	(.029)	(.025)	(1.035)	(.412)	(.246)	(.166)	(12.5)	(9.7)	(9.0)	(6.8)
					Materials, - average for three rates							
Alfalfa	.118 a	.060	.030	.025	1.362	.624	.269	.192	11.6	10.5	8.8	8.1
Grass Hay	.108 ab	.052	(.038)	(.032)	1.284	.540	(.367)	(.271)	11.9	10.3	(8.7)	(8.1)
Straw	.101 b	.057	.028	.025	1.264	.622	.215	.177	12.4	10.7	7.5	7.0
					Rates, - average for three materials							
100 lbs.	.100 b	.050	.024	.022	1.193	.511	.184 b	.179	12.0	10.0	7.7	8.6
200 lbs.	.112 ab	.056	.029	.027	1.316	.632	.217 b	.184	11.7	11.3	7.5	6.7
300 lbs.	.116 a	.064	.035	.026	1.418	.664	.325 a	.191	12.3	10.3	9.3	7.2

a, b Ranges of equivalence. Within each group of comparisons, means noted with the same letter or without letters are not significantly different at 5%.

* Average of mulches other than shavings.

total weight of the mulch back to the initial level on each plot. (This was not done for shavings because of its slow decomposition and the difficulties encountered in getting accurate estimates of the undecomposed residue.)

Recorded quantities of mulch used in the beginning and annually for maintenance of the average mulch application prior to sampling totaled, in per acre equivalents, 34 tons of alfalfa, 31 grass hay and 22 of straw. Representative analyses for nitrogen were 2.49, 2.05 and 0.29 percent for alfalfa, grass and straw, respectively, giving totals for nitrogen applied equivalent to 1690, 1280 and 128 pounds per acre, as an average for the same three materials. Nitrogen actually found in excess of that in the control in the surface 12 inches totaled 1080 pounds per acre as an average for alfalfa, 720 pounds for grass hay and 680 pounds for straw. These represented recoveries of 64 percent of the nitrogen applied in alfalfa and 56 percent of that in grass. The nitrogen recovered in excess of the control under straw was five-fold greater than the calculated total applied. Corresponding increases in carbon for alfalfa, grass, and straw, respectively, were in ratios of 10.9, 11.9 and 14.5 pounds of carbon for each pound of nitrogen.

Thus, it would appear that a part of the differentially higher nitrogen content in mulched soil was due to a conservation of nitrogen more normally lost by mineralization and leaching from unmulched soil, which was maintained under clean cultivation, with weeds removed chemically or by hand. Substantial movement, however, of soluble organic nitrogen compounds from the mulch into the soil is indicated. Their stabilization and retention in the upper 12 inches was likely promoted a

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by the absence of cultivation and by markedly lower temperatures observed under mulches during the summer months. Significant differences in composition, or quality, of the soil organic matter developed under the different materials would be expected, considering their very divergent nature.

Hydrolytic Nitrogen Fractions

The C/N ratio provides only a gross measure of qualitative differences in soil organic matter. An attempt was made to achieve a more detailed characterization of nitrogenous constituents by methods recently described by Bremner (cf. p. 24). Only a preliminary effort was made in this direction, using a few selected soils from each field experiment.

The first step in the procedure was a 12-hour hydrolysis of the whole soil in 6 N HCl. The distribution of nitrogen in hydrolyzable and non-hydrolyzable fractions is shown in the histograms of Figure 3.

Soils selected for the rotation experiment were taken in the fall of 1957 after beans and again in 1960 after the second year of alfalfa in the first cycle of the rotation. Thus, the 1957 samples were taken after 2 years of cultivation and the 1960 samples were taken after a 2-year period during which the soil was undisturbed by cultivation.[2]

The most striking feature of the data for the rotation experiment

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[2] Note: Nitrogen totals in Figure 3 do not correspond to those shown in Table 4 or Figure 1, since all samples for Figure 3 were taken from tier E where nitrogen and carbon levels were markedly lower than in the other four tiers.

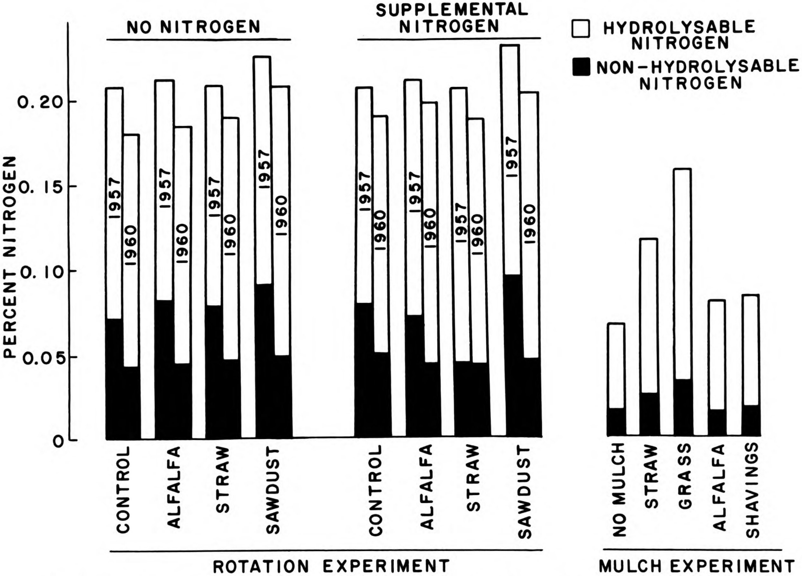

Figure 3.--Distribution of nitrogen between acid hydrolyzable and non-hydrolyzable fractions in soils from the rotation and mulching experiments.

in Figure 3 is the fact that declines in total nitrogen between 1957 and 1960 were associated with decreases in non-hydrolyzable nitrogen, whereas hydrolyzable nitrogen increased, both absolutely and in the proportion of total nitrogen present in this form. The only exception was the straw treatment with supplemental nitrogen. There is no apparent explanation for this exception, which may have been due to an aberrant sample in 1957.

Soils selected from the mulching experiment, involving only the 0 to 6 inch depth of one replication, represented those under the control and the highest application rates of the four mulching materials.

Nitrogen levels in the 0 to 6 inch depth for the mulching experiment in Figure 3 were much lower than for the rotation experiment. This is due to a genetic difference between soils: The Sims clay loam of the rotation experiment is a poorly drained humic gley, suitable for cultivated crops only after tile drainage; the coarser textured fine sandy loams to loams of the mulching experiment are on rolling topography and are well-drained.

Large increases in total nitrogen over no mulch in soil under the high rates of straw and grass were directly related to the nitrogen content of the mulch materials and involved increases in both hydrolyzable and non-hydrolyzable forms. The total for alfalfa is irrelevant, since the soil sample was taken from an aberrant replicate; there was, however, a greater increase in hydrolyzable then in non-hydrolyzable forms. Only a slight increase in hydrolyzable nitrogen was noted in soil under shavings.

The soil percentages in Figure 3 for hydrolyzable and non-hydrolyzable nitrogen are presented again as percentages of total nitrogen in

in the first two columns of Table 8. The proportion of nitrogen in hydrolyzable forms was very similar for the mulching experiment and the 1960 samples taken after two years of alfalfa-brome hay in the rotation experiment. This proportion was distinctly greater than in the samples taken after two years of cultivation in 1957. It would appear that carbon increases promoted by spring plowing in the first two years (cf. Figure 2) led to an increase in resistant forms of nitrogen in the 1957 samples. In the absence of incorporated residue additions during the two hay years and in the presence of the roots of perennial bromegrass and alfalfa, a marked conversion of these resistant forms to less resistant combinations occurred. To the extent that susceptibility to hydrolysis reflects availability to microbial release, a greater proportion of soil nitrogen would have been available for use by corn in 1961 than by barley in 1958.

The distribution of nitrogen within the hydrolyzable fraction is given in the last five colums of Table 8. No attempt was made to evaluate the specificity of procedures and experimental conditions used for estimating α-amino and hexosamine nitrogen. Although obvious discrepancies are apparent in the negative values obtained for hexosamine nitrogen in several samples, attention may be called to two patterns of behavior which appear to be of theoretical significance.

In the 1957 samples from the rotation experiment, the proportions of hydrolyzable nitrogen in soil to which sawdust had been added was markedly lower than with the other treatments, although the proportion of α-amino nitrogen was distinctly higher. At the same time, ammonium and unidentified forms of nitrogen in the hydrolysate were lower with

Table 8.--Percent of total soil nitrogen in fractions obtained by acid hydrolysis.

Treatment		Non-hydrolyzable nitrogen	Hydrolyzable nitrogen					
			Total	Ammonium	α-Amino	Hexosamine	Ammonium + α-amino + hexosamine	Unidentified
				Rotation Experiment - 1957				
No N	Control	33.7	66.3	18.5	21.3	0.6	40.4	25.9
	Alfalfa	38.2	61.8	17.7	17.3	2.0	37.1	24.7
	Straw	37.4	62.6	19.5	20.5	-1.0	39.0	23.6
	Sawdust	40.2	59.8	15.4	24.2	0.2	39.8	20.0
Plus N	Control	37.1	62.8	19.0	20.4	-1.6	37.8	25.0
	Alfalfa	34.0	66.0	18.4	22.1	-1.7	38.8	27.2
	Straw	21.6	78.4	23.3	19.0	0.7	50.3	28.0
	Sawdust	41.4	58.6	17.2	26.4	1.7	37.9	20.7
				Rotation Experiment - 1960				
No N	Control	23.9	76.1	20.3	27.0	-1.3	45.0	31.2
	Alfalfa	24.1	75.9	18.4	22.6	3.4	44.1	31.5
	Straw	24.4	75.6	21.8	31.1	-6.2	50.0	25.6
	Sawdust	22.9	77.1	20.1	31.6	-2.9	45.5	31.6
Plus N	Control	26.1	73.8	20.6	26.1	1.5	48.3	25.5
	Alfalfa	21.9	78.0	21.6	24.6	3.2	49.4	28.7
	Straw	23.4	76.7	20.3	26.8	-1.8	48.2	28.5
	Sawdust	22.4	77.7	19.0	29.0	1.1	46.2	31.7
				Mulching Experiment				
No mulch		22.9	77.1	19.2	24.8	7.3	51.3	25.8
Straw		21.3	78.7	17.5	23.3	3.4	44.1	34.6
Grass		19.3	80.7	18.0	24.4	6.7	49.4	31.3
Alfalfa		17.4	82.6	14.3	16.8	3.7	34.8	47.8
Shavings		20.4	79.6	18.0	22.6	4.8	45.5	34.1

sawdust. These relationships suggest that two distinct mechanisms were operating to immobilize nitrogen in the presence of sawdust. The higher levels of α-amino nitrogen indicate that a larger quantity of proteinaceous materials had been synthesized by microbial populations attacking cellulose and hemicelluloses in the wood. On the other hand, the association of lower levels of ammonium and unidentified nitrogen in the hydrolysate with a larger proportion of nitrogen in forms resistant to hydrolysis may reflect the chemical immobilization of ammonium and organic nitrogen compounds by the formation of complexes with lignin residues exposed as cellulose and hemicelluloses were decomposed (61).

The data suggest that, in 1957, lignin degradation products from sawdust were at a stage of oxidation where their complexing activity was high. It is reasonable to expect that most of the potential complexing sites in the sawdust would have been exposed and their affinities satisfied three years later in 1960, - or after five years exposure to the soil environment. Continued oxidation beyond a point of maximum complexing activity would have led to a weakening of structures formed earlier and an increase in their susceptibility to hydrolsis. It is likely that the large increases in unidentified forms of nitrogen for sawdust treatments between 1957 and 1960 were derived from this source. It would appear likely that much of the unidentified nitrogen in hydrolysates of all soils is present as low molecular weight polymers and heterocyclic compounds formed by complexing of ammonia and amino acids with lignin residues, by "browning" reactions with intermediates of carbohydrate breakdown (61, 85) or a combination of both processes. The increases in hydrolyzable ammonium between 1957 and 1960 with most

treatments, and particularly with sawdust are consistent with an increase in aromatic amines (17).

A second relationship appears noteworthy because it is contrary to what was expected. This is the uniquely low value for α-amino nitrogen obtained for soil under alfalfa in the mulching experiment and again for alfalfa without supplemental nitrogen in the two sampled years of the rotation experiment. Considering the high protein content of alfalfa, a higher level of α-amino nitrogen in soil hydrolysates would have been expected. Conversely, as may be inferred from the quantities of mulch materials required to maintain treatment levels in the mulching experiment (cf. P.47), proteinaceous residues such as alfalfa decompose much more rapidly than do carbonaceous materials such as straw. Thus, more complete degradation of protein constituents can be expected, and since energy sources will be exhausted earlier, microbial cells and products will also be dissipated more rapidly. It is to be expected that "browning" reactions leading to complex formation will also be promoted by the intimate association of amino acids released by proteolysis with intermediates released by the concurrent breakdown of carbohydrates in alfalfa. The unusually high level of unidentified nitrogen in the hydrolysate from soil under the alfalfa mulch supports this view.

Where supplemental nitrogen was used in the rotation experiment, this relative depression of α-amino nitrogen with alfalfa additions was not as clearly expressed. This may have been due to an overriding effect of increased carbonaceous residues from corn and barley associated with the observed yield responses to supplemental nitrogen (cf. Table 6).

The above inferences from the hydrolytic fractionations of soil nitrogen are highly tentative. They do illustrate the potential usefulness of a rapid fractionation procedure for characterizing qualitative variations in soil organic nitrogen as associated with management.

The distribution of nitrogen in the different forms is reasonable when compared with values reported by others, using more involved quantitative techniques. Stevenson (79) found 20 percent of the total soil nitrogen in non-hydrolyzable forms, 79 to 81 percent in hydrolyzable forms and 29 to 39 percent as α-amino acids. Values reported by Bremner (14) and Kojima (50) range from 13 to 31 percent non-hydrolyzable and from 24 to 37 percent as α-amino nitrogen. Stevenson (82) and Sowden (77) reported finding 5 to 11 percent of total soil nitrogen as hexosamines. Values for hexosamines in the mulching experiment approached this range, but negative values for a number of treatments in the rotation experiment are anomalous.

The fractionation data and the data for total carbon, nitrogen and C/N ratios are consistent in supporting the view that the management practice which most drastically and immediately influences the quality of soil organic matter is tillage. Both timing and intensity of tillage may be important. On the other hand, differences in rate or nature of a given residue addition may have subtle influences on quality of soil organic matter that may be projected over a period of years. Where crops respond to supplemental nitrogen, the effect of the fertilizer nitrogen will likely be to enhance quality effects characteristic for residues from the responding crops, whereas actual increases in total soil nitrogen may be small and difficult to maintain.

DISCUSSION

Changes in Total Nitrogen

Russell (68) has pointed out that, where carbonaceous residues are added to soil in excess of a previously established equilibrium pattern of residue return and decomposition, increases in soil nitrogen by both symbiotic and non-symbiotic fixation are promoted. He cites increases of 40 to 60 pounds of nitrogen per acre per year for situations where non-symbiotic fixation appears to have been solely responsible.

There is evidence that extensive non-symbiotic fixation of nitrogen may have occurred where materials low in nitrogen were used in both the mulching and the residue experiments. In the mulching experiment, increases in soil nitrogen in excess of that accounted for in the straw added would have averaged 50 pounds per acre per year over the 7-year period. Where 40 tons of sawdust were incorporated in the rotation experiment, increases the first year were 540 pounds in excess of the nitrogen that could have been added in sawdust containing 0.2 percent nitrogen. If the data for the first two years are ignored because of their variability and inadequate replication (cf. Figure 2), the 600 pound increase over the control in the third year would represent about 400 pounds in excess of the nitrogen added in sawdust. At the observed rate of nitrogen decline in the control (1.28 percent per acre), as much as 180 pounds of this increase could be attributed to soil sources, leaving a balance of about 200 pounds which may have been fixed at an annual rate of approximately 70 pounds per acre per year.

At the average expressed rate of carbon disappearance (8.4 percent per year), roughly 20,000 pounds of cellulose would have been consumed during these first three years. Applying observed _in vitro_ rates of nitrogen fixation (2) for _Clostridia_ and _Azotobacter_ (8 to 20 units of nitrogen per 1000 units of carbohydrate), 160 to 400 pounds of nitrogen could have been fixed theoretically during decomposition of this quantity of cellulose. Actual rates of conversion of cellulose and hemicelluloses during the first years would reasonably have been much greater than indicated by carbon losses in later years. Accordingly, the potential for non-symbiotic fixation of nitrogen could have been even greater.

In the light of the preceding calculations, the apparent non-symbiotic fixation of nitrogen in the presence of sawdust, although unexpectedly large, is reasonable in the light of known capabilities of organisms likely involved.

Non-symbiotic fixation of nitrogen in the presence of cellulose as the principle energy source requires the prior degradation of the cellulose by cellulolytic species to provide the simple sugars and acids required by the nitrogen fixers (47, 55). To the extent that supplemental nitrogen increased numbers and activity of the cellulose decomposers, an increase in production of sugars and acids could also have stimulated nitrogen fixation. Such a response could account for the anomalously large increases in soil nitrogen in the first and third years where fertilizer nitrogen was applied with sawdust (cf. Figure 1).

In an opposing sense, denitrification is also promoted by the presence of large quantities of rapidly decomposing organic materials (2, 31). Losses through microbial reduction of nitrate to N_2 and N_2O

may be extremely rapid during periods of oxygen stress associated with high soil moisture levels (19). Denitrification losses of nitrate formed by continuing nitrification may have contributed to the apparently erratic decrease in soil nitrogen in the second year in the sawdust plus nitrogen treatment (cf. Figure 1). A further factor at this point may have been the exposure of organic groupings in the sawdust which have a strong affinity for nitrite produced during nitrification processes (61). The nature of these active groups and the mechanisms of reaction are not known, but N_2 and N_2O are among the products formed. Losses of ammonium by conversion to these gaseous forms increase with increasing soil organic matter levels and during the degradation of carbohydrates in soil.

The nature of biological and chemical reactions and their known associations with carbonaceous residues leads to the expectation that large and rapid fluctuations in nitrogen content can occur where massive applications of such residues are accompanied by additions of mineral nitrogen and are then subjected to varying conditions of moisture or aeration. The field data support this expectation but need confirmation under more rigorously controlled experimental conditions.

Practical Implications of Data from the Rotation Experiment

From a practical standpoint, it is apparent that larger additions of supplemental nitrogen than were used in the rotation experiment would have been needed to overcome yield depressions during the first three years after the heavy application of sawdust (cf. Table 6). It cannot be known whether a yield depression would have been expressed in the fourth or fifth years if a non-leguminous crop had been grown,

instead of alfalfa and bromegrass. By the sixth year the increased nitrogen supplying power due to the increased quantity and mineralizability of soil nitrogen in sawdust plots was apparent in yields of corn. The calculated release to corn in the sixth year was 150 pounds of nitrogen per acre. This is the theoretical requirement for a 100-bushel corn crop. This appears to have been the maximum yield level imposed by other limiting soil or climatic factors at this location, since response to supplemental nitrogen was negligible, except in the control where no addition of residues from extraneous sources was made.

Assuming a nitrogen requirement of 30 to 40 pounds of nitrogen per ton of sawdust (67), 1200 to 1600 pounds of fertilizer nitrogen would have been required to balance the immobilizing potential of the 40 tons of sawdust and eliminate the yield depressions in corn planted immediately afterward. It is unlikely that the full immobilizing potential would have been expressed the first year, and the data suggest that up to one-half of this requirement would have been met from soil sources and by non-symbiotic nitrogen fixation. Thus, it is likely that only moderate increases in supplemental nitrogen would have been required to overcome the reduction in yield of corn the first year after application. The actual increase in corn yield for the 100 pounds of nitrogen applied with sawdust in the first cycle of the rotation was at the rate of one bushel for each seven pounds of nitrogen. At this rate of response, an additional 280 pounds of nitrogen would have produced the additional 40 bushels needed for the maximum yield of 90 bushels per acre obtained on the

control plots where 100 pounds of nitrogen was applied.

It is likely, therefore, that temporary reductions in availability of nitrogen resulting from massive incorporations of carbonaceous residues can be overcome by rather moderate increases in supplemental nitrogen added with the residues. Increases in the quantity and mineralizability of nitrogen in soil organic matter will result in significant residual increases in the nitrogen supplying capacity of the soil. There are, however, several reasons for questioning the economic feasibility of such a practice: 1) the opportunity for extensive losses of nitrogen by microbial and chemical reduction to gaseous forms is greatly enhanced in the presence of rapidly decomposing residues high in carbohydrates; 2) residual increases in nitrogen supplying power may exceed the capacity of the soil or environment to supply other essential growth factors, as is indicated by the lack of variation in yields of beans and barley in the second cycle of the rotation (cf. Table 6); 3) increases in soil nitrogen above a characteristic base line for a given soil, climate and management situation are accompanied by increases in mineralizability, so that the increases themselves are only temporary in nature and tend to return rather quickly to the base level.

The results obtained with the more normal additions of alfalfa and straw during the first cycle of the rotation, though lacking in statistical significance, are consistent with those obtained with sawdust in that increases in carbon were accompanied or followed by increases in quantity of soil nitrogen (cf. Figure 2). Increases in nitrogen above a characteristic base line were accompanied by increases in mineralizability and a subsequent decline toward the base line. It can be inferred that, if similar additions of carbon had been achieved by increased residues associated with increased yields of the crops grown, similar effects on soil carbon and nitrogen would have been expressed.

It is probably much more efficient to use nitrogen to promote maximum yields of the crops grown than to use it in balancing the immobilizing potential of residues brought in from the outside in an attempt to quickly change organic matter levels. Increases in carbon from increases in crop growth and return of residues will be geared to the productive potential of soil, climate and management. If extraneous additions of carbon (and the necessary nitrogen to avoid harmful effects on crops) result in residual increases in nitrogen supplying power which are in excess of other factors limiting crop growth, this will be as inefficient as using fertilizer nitrogen directly on crops in excess of their actual needs.

Changes in management which result in systematic and sustained increases in the rate of addition of carbon and nitrogen can be expected to reduce the slope of the base line for nitrogen decline, or raise the equilibrium level where such an equilibrium has been established. The data suggest that permanent changes in slope or equilibrium level may be small and expressed only over relatively long periods of time. On the other hand, wide variations in quality, as evidenced by C/N ratios and hydrolytic nitrogen fractions, may occur from year to year in response to cropping sequence or management practices.

The plowing under of residues in the spring during the first cycle of the rotation resulted in differential increases in carbon

and widening C/N ratios (cf. Figure 2). These were temporary and disappeared rapidly during the two alfalfa-brome hay years. Similar increases failed to appear during the second cycle of the rotation when plowing was done in the fall. The disappearance of carbon from residues is thus, in part, a function of the length of time they are intimately in contact with the soil. Even though microbial activity is greatly reduced, extensive losses of carbon can occur from incorporated residues during the winter and spring, particularly when the ground remains unfrozen under snow cover. Spring plowing, in effect, delays decomposition until the planting season. Such delay would tend to increase the nitrogen required with carbonaceous residues to avoid depressed crop yields associated with microbial immobilization.

A C/N ratio of 10:1 represents an average equilibrium value for soils in the humid region (30). Additions of sawdust and straw and cumulative increases in carbon in the control and alfalfa plots had resulted in C/N ratios distinctly greater than this in the third year when barley was grown. It is possible that the immobilizing potential of surplus carbon may have been responsible for much lower yields of barley in the first cycle of the rotation than in the second cycle when C/N ratios had declined again to 10 or less (cf. Table 6).

On the other hand, C/N ratios of 8 or 9 attained in the second cycle of the rotation reflect a relatively high degree of oxidation and would be expected to result in reduced mineralizability and reduced availability of nitrogen. There was no evidence of this in yields before the time the experiment was terminated. However, if the differentially large losses of carbon during the second cycle were to

continue, either of two results could be expected: 1) organic matter would eventually stabilize at a lower C/N ratio with little change in rate of nitrogen loss, or 2) the rate of nitrogen loss would increase, tending to re-establish an equilibrium C/N ratio, characteristic for the soil and climate. The latter appears much more likely, since the relationships between carbon content and nitrogen content observed during the first cycle of the rotation and with sawdust indicate that carbon additions are essential for the conservation of nitrogen. Any sustained increases in nitrogen availability associated with accelerated losses of carbon must be at the expense of more rapid depletion of soil organic nitrogen.

Practical Implications of Data from the Mulching Experiment

It is known that the rate of decomposition of plant residues increases with the intimacy of contact with soil (9). Surface mulches do not decompose as rapidly as equal rates of the same materials when they are incorporated into the soil. The initial stages of decomposition take place principally in the layers of mulch near the soil surface (42). The most intense immobilization of nitrogen by carbonaceous residues takes place here. Tie-up of mineral nitrogen in the soil itself is less extensive than when the same materials are incorporated into the soil by plowing or discing, although immobilization does occur and nitrogen deficiencies do develop in crops mulched with materials such as straw or sawdust.

In the mulching experiment for which soil data was reported here, trees mulched with shavings were severely deficient in nitrogen and

retarded in growth, with no evidence of recovery at the time samples were taken. Symptoms of nitrogen deficiency and retarded growth were apparent for the first 3 or 4 years in trees mulched with straw. However, by the time soil samples were taken in the seventh year, the straw mulched trees were larger than, and were coming into production well in advance of, trees without mulch. With grass hay and alfalfa, growth from the beginning was more rapid than without mulch, and foliage color reflected a high nitrogen status in the trees.

The C/N ratios for this experiment (cf. Table 7) indicate that soluble organic compounds entering soil under shavings and straw had a higher C/N ratio than those entering from grass hay or alfalfa. The observed reduction in availability of nitrogen to trees under these materials was undoubtedly due to immobilization by microorganisms attacking these soluble materials. This immobilizing effect would have been expressed primarily near the surface of the soil, since C/N ratios declined rapidly with depth. The rapid recovery of trees mulched with straw was associated with appreciable increases in total nitrogen to a 12-inch depth. With shavings, no such increases in total nitrogen occurred, and there appears to have been a continuing release of soluble carbonaceous materials contributing to immobilization of the rather limited soil supplies of nitrogen.

Significance of Hydrolytic Nitrogen Fractions

Although a number of general relationships between C/N ratio and residue composition, management practice and crop response have been shown, the ratio is only a crude measure of organic matter quality.

The preliminary fractionation of nitrogenous constituents led to only tentative conclusions, which need more systematic investigation. Nevertheless, it does appear that the proportion of nitrogen in different fractions obtained by acid hydrolysis of soils will be related more closely to the degree of oxidation of the total organic fraction than to the nature of residues or vegetation which contribute the initial raw materials for humus formation.

The data suggest that the addition of fresh residues will be accompanied initially by increases in α-amino nitrogen arising by proteolysis and microbial synthesis, and by increases in non-hydrolyzable nitrogen complexes involving lignin residues and intermediates of carbohydrate metabolism. With further oxidation, the non-hydrolyzable complexes initially formed, become increasingly susceptible to hydrolysis, and the nitrogen released will tend to appear primarily as hydrolyzable ammonium and unidentified forms of nitrogen. These unidentified forms may, therefore, represent low molecular weight polymers and heterocyclic nitrogen compounds.

It is likely that amino acids may also be complexed initially and may be released again, unaltered, by hydrolysis at later stages of oxidation. However, the very rapid decomposition of proteinaceous materials such as alfalfa may lead to a more complete degradation of amino acids and to a final product relatively low in α-amino nitrogen and relatively high in hydrolyzable forms that are, as yet, unidentified.

The trends noted above were observed in soil materials with C/N ratios of 10 or greater. The soil samples from the rotation experiment represent experimental material for extending the systematic study

of these relationships of narrower C/N ratios representing more highly oxidized organic matter.

The major concern in this study has been in characterizing soil organic matter in terms of factors likely to affect nitrogen availability. It should be emphasized that carbonaceous constituents may be equally as important as the nitrogenous fractions studied here in determining quality of soil organic matter in its relation to responses of plants.

SUMMARY AND CONCLUSIONS

Observations over a 9-year time sequence in a rotation experiment showed that nitrogen levels were still declining in a Sims clay loam fifty years after it was brought under cultivation from the virgin state. The average rate of decline under a 5-year rotation of corn, field beans, barley and two years of alfalfa-brome hay was 1.28 percent per year. The decline conformed closely ($r = 0.9996$) to the expression: $N_t = N_0K^t$.

Temporary increases in nitrogen above the base line represented by the decline were accompanied or preceded by increases in carbon. Increases in nitrogen associated with moderate increases in carbon could be accounted for in terms of conservation of nitrogen from soil and fertilizer sources. Increases of 600 to 700 pounds of nitrogen per acre during the first three years after incorporation of 40 tons of sawdust could only be explained by assuming that extensive nonsymbiotic fixation of nitrogen had occurred, stimulated by the large surplus of energy materials added in sawdust.

The temporary nature of nitrogen accumulations above the base line was due to a concurrent increase in mineralizability. The large initial accumulations under sawdust disappeared at an average rate of 2.5 percent per year and residual nitrogen was not significantly greater than the control after the seventh year. Where alfalfa-brome hay or wheat straw were added at practical rates (4 tons per acre), nitrogen levels tended to fluctuate in a cyclic pattern, decreasing during the years when tilled crops were grown and increasing during the two years of alfalfa-brome.

Marked accumulations of carbon and increases in C/N ratios to values above 10.0 were observed only during the first cycle of the rotation when plots for corn and beans were plowed in the spring of the year. A change in management to fall plowing for all tilled crops resulted in accelerated losses of carbon and rapidly narrowing C/N ratios during the second cycle of the rotation. Although C/N ratios between 8 and 9 were attained by the end of the experiment, there was no apparent effect on the basal rate of nitrogen decline.

The clearly demonstrated role of carbon in conserving nitrogen made it appear that the large differential losses of carbon could not continue indefinitely without reducing the availability of soil nitrogen to crops or increasing the basic rate of depletion of soil nitrogen. The general level of yields in the second rotation was higher than in the first, notably for barley. This suggests that soil nitrogen was being mineralized more rapidly, even though an increased rate of nitrogen loss was not detected.

The key role of carbon in promoting increases in soil nitrogen was demonstrated again in the mulching experiment. Significant accumulation of carbon and nitrogen were observed to a depth of twelve inches under all materials except shavings. The ratio of increase was 1 unit of nitrogen for each 11 to 14 units of carbon. Nitrogen recovered from soil under straw exceeded known additions by a factor of 5, again supporting the view that non-symbiotic fixation of nitrogen is promoted by high concentrations of rapidly decomposing carbonaceous materials. Estimated rates of fixation for straw in the mulching experiment and sawdust in the rotation experiment ranged from 40 to 70 pounds of nitrogen per acre per year. These compare favorably with rates reported by other investigators.

Excessive rates of carbon addition in the form of extraneous carbonaceous residues led to nitrogen deficiencies in trees and crops in both experiments. These were much more severe with shavings than with straw in the mulching experiment, even though heavy annual additions of straw were made, whereas shavings were not replenished after the initial application. This appeared to be due to the fact that the low nitrogen content and slow decomposition of shavings led to no appreciable movement of nitrogen into the soil to balance a continuing downward movement of soluble carbon compounds. Nitrogen deficiencies associated with the continuously maintained straw mulch disappeared after 3 or 4 years. In the rotation experiment, nitrogen deficiencies after incorporation of 40 tons of sawdust were not observed after the third year.

There was evidence in the rotation experiment that extensive losses of nitrogen by reduction to gaseous forms may have been promoted by the addition of fertilizer with sawdust. It also appeared that residual increases in availability of soil nitrogen during the second cycle of the rotation after sawdust application exceeded the availability of other factors limiting crop production. For these reasons, it does not appear to be economically feasible to attempt to increase soil organic matter by large additions of extraneous residues in excess of the potential of soil, climate and management to produce carbon in the form of residues from the crops actually grown.

Values for hydrolyzable and non-hydrolyzable forms of nitrogen obtained by the fractionation used in this study were consistent with values reported by several investigators using more quantitative and time-consuming methods of analyses. The data obtained were preliminary in scope. Nevertheless, suggestive relationships were observed between soil C/N ratios, residues and cropping sequences, on the one hand, and the proportions of nitrogen in the non-hydrolyzable fraction and as ammonia, α-amino nitrogen and unidentified fractions in the hydrolysate, on the other. The rapidity with which these determinations can be made makes the procedure a promising one for correlation studies involving relatively large numbers of samples.

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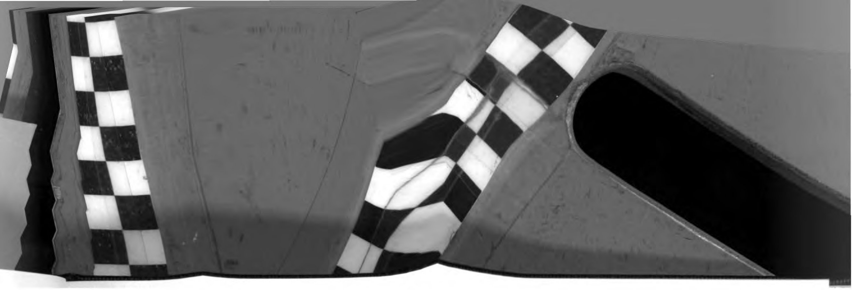

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APPENDIX

Table 9.--Distribution of variance in relative values for carbon, nitrogen and C/N ratios associated with crop years and supplemental nitrogen treatments, combining degrees of freedom for residue treatments other than sawdust with error.

Source	n[1]	df	Total carbon		Total nitrogen		C/N ratio	
			MS	F	MS	F	MS	F
Total	1	149	140.67		20.77		122.40	
Nitrogen	75	1	3.34	0.04	5.11	0.31	12.15	0.15
Crop years	6 to 30	8	1166.14	13.40**	101.53	8.19**	981.38	10.74**
N x C	3 to 15	8	17.20	0.20	12.39	0.75	17.38	0.21
Error		132	87.04		16.50		82.99	
Coefficient of variation			0.096		0.041		0.093	

[1] n= number of observations in each mean.

** Significant at 1 percent.

Table 10.--Distribution of variance in relative values for carbon, nitrogen and C/N ratios associated with crop years and supplemental nitrogen treatments, within sawdust treatment, eliminating data for first and ninth years.

			Total carbon		Total nitrogen		C/N ratio	
Source	n[1]	df	MS	F	MS	F	MS	F
Total	1	45	574.69		73.62		384.24	
Nitrogen	23	1	9.23	0.03	133.96	2.27	52.40	0.25
Crop years	4 to 10	6	2731.75	9.38**	145.66	2.47*	1705.65	8.07**
N x C	2 to 5	6	21.75	0.07	69.65	1.18	44.20	0.21
Error		32	291.59		58.97		211.43	
Coefficient of variation			0.141		0.072		0.130	

[1] n = number of observations in each mean.

* Significant at 5 percent.

** Significant at 1 percent.

Table 11.--Distribution of variance in relative values for carbon, nitrogen and C/N ratios associated with crop years and residue treatments, combining degrees of freedom for nitrogen treatments with error.

Source	n[1]	df	Total carbon		Total nitrogen		C/N ratio	
			MS	F	MS	F	MS	F
Total	1	199	352.13		42.91		220.39	
Residue	50	3	6120.17	72.75**	551.58	3.67*	2210.23	20.33**
Crop years	8 to 40	8	2840.59	33.76**	150.31	3.65**	2365.98	21.76**
R x C	2 to 10	24	646.01	7.68**	41.16	1.44	19.59	0.18
Error		164	84.13		28.63		108.72	
Coefficient of variation			0.130		0.053		0.103	

[1] n = number of observations in each mean.
* Significant at 5 percent.
** Significant at 1 percent.