STUDIES ON THE CHEMICAL, PHYSICAL AND BIOLOGICAL PROPERTIES OF SOIL ORGANIC MATTER

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This is to certify that the

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R L Cook
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STUDIES ON THE CHEMICAL, PHYSICAL AND BIOLOGICAL PROPERTIES OF SOIL ORGANIC MATTER

by

Harry H. Johnston

AN ABSTRACT

Submitted to the School for Advanced Graduate Studies of
Michigan State University of Agriculture and
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DOCTOR OF PHILOSOPHY

Department of Soil Science

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H. H. Johnston

ABSTRACT: Studies on the chemical, physical and biological properties of soil organic matter.

Ph. D. thesis submitted to the School for Graduate Studies, Michigan State University, by Harry H. Johnston. 1958

In a greenhouse experiment, 12½ and 25 tons per acre of sawdust, acid-extracted ("lignified") sawdust, corn stalks, wheat straw and alfalfa hay were added to Oshtemo sand. Three levels of supplemental nitrogen added as urea were used with non-leguminous materials. One series of pots was not cropped during a 40-week decomposition period. Separate series were cropped to wheat or alfalfa. Periodic soil samplings were made for estimation of microbial numbers and pH. All pots were planted uniformly to wheat after 40 weeks. Nitrogen taken up was determined on the harvested portions of all wheat and alfalfa crops.

Soil samples were taken at the end of the 40-week decomposition period. Samples were also taken from a field experiment 1, 3 and 5 years after addition of 35 tons per acre of sawdust to Sims clay loam.

Laboratory determinations on field and greenhouse soils included total carbon and nitrogen, water-floatable materials, water-soluble nitrate, fractionation of nitrogen in acid hydrolysates and alkali extracts, and release of carbon and nitrogen during controlled incubation.

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Microbial assimilation of nitrogen during early stages of decomposition was reflected by suppressed nitrogen uptake of wheat, increased microbial numbers, increased microbial activity (CO_2 evolution), and suppressed mineralization of nitrogen during incubation. Large increases in acid-hydrolyzable amino nitrogen were found in the field soil and in the greenhouse where microbial numbers were unusually high in soils to which nitrogen was added as alfalfa hay, or as urea with readily decomposable materials such as corn stalks.

Subsequent mineralization of microbially immobilized nitrogen was reflected by increasing uptake of nitrogen by successive crops of wheat and was closely associated with declining microbial numbers, declining microbial activity and narrowing soil C:N ratio. Release of microbially immobilized nitrogen occurred earlier with more readily decomposable residues such as corn stalks and wheat straw than with sawdust. Earlier release also occurred when nitrogen was added with carbonaceous materials.

Associated with the inferred dissipation of energy materials as decomposition progressed was an increase in nitrogen not accounted for in acid hydrolysates. The quantities of non-acid-hydrolyzable nitrogen found were directly related to the level of nitrogen treatment, the expected lignin content of the different organic amendments and their degree of decomposition. It appeared that these acid-resistant nitrogenous materials represented products of oxidative complex formation between lignaceous substances and ammonia or proteinaceous nitrogen.

The quantities of non-acid-hydrolyzable nitrogen increased as a continuous geometric function of decreasing soil C:N ratio. Resistance to microbial decomposition of residues in the soil after 40 weeks increased as a continuous geometric function of increasing acid-resistant nitrogen content and as an inverse linear function of soil C:N ratio.

Nitrogen taken up from a majority of treated soils by the last crop of wheat was a sigmoid function of the sum of water-soluble nitrate plus nitrate released during incubation. Soils with high levels of non-acid-hydrolyzable nitrogen released nitrogen to the wheat at a rate in excess of the function described by soils with lower levels of acid-resistant nitrogen.

Additional studies are reported involving the application of infrared and ultraviolet absorbance phenomena, paper electrophoresis and high frequency titrations in soil organic matter research.

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GENERAL INTRODUCTION

Characterization of individual soil components is one of the major goals of the soil scientist. With the help of X-ray, differential thermal analysis, and the electron microscope, clay components can now be fairly well identified and their structure written with a reasonable degree of accuracy.

Organic matter and clays together comprise the greater percentage of the "active fraction" of the soil; therefore it is logical that an expanded program of research on these fractions be initiated. Knowledge of the organic fraction of soils is extremely limited. Its structure is mere postulation and as yet no easy or simple way has been devised to separate it from the soil. The degree to which various organic fractions are altered during extraction is not known with any certainty.

Organic residues added to the soil are changed because they are a source of food and energy for microorganisms. Materials resistant to decomposition and by-products of decomposition remain in the soil and impart to the soil some of its most important physical and chemical properties.

The fact that no single method of isolation has been found for the separation and characterization of soil organic matter indicates its complexity. Hydrolysis of soil organic matter with acids or bases has given the best information regarding its composition thus far. Extraction of soil organic matter with alkali or neutral reagents permits the study of some of its physical properties as well as its chemical properties. Even if unaltered organic matter could

be isolated from the inorganic fraction of the soil, its structure would be so complex that no one tool or instrument could identify its structure and characteristics.

Studies on soil organic matter must then be dealt with in a stepwise manner until enough information is available to reasonably account for its structure, characteristics, and properties. Two definite approaches can be made: (a) Synthesis of postulated model compounds and comparison with natural soil organic matter fractions, and (b) isolation of the organic soil constituents by new and improved methods.

Objectives of this study on soil organic matter include the characterization of some of its properties as influenced by organic amendments, soil type and nitrogen supply, with special emphasis on nitrogen transformations; and a detailed study of individual components of certain organic matter fractions.

LITERATURE REVIEW

The literature on soil organic matter is so extensive that a separation of the various investigations into related sections should give a clearer and more understandable presentation.

Soil Organic Phosphorus

Soil organic phosphorus has proven difficult to determine quantitatively because of it complexity, and the large amount of inorganic phosphorus present in some soils. Present biochemical methods, especially the use of exchange columns, have permitted more detailed study of specific phosphorus containing organic compounds, including inositol phosphates (91),

Several methods have been devised for the gross determination of organic phosphorus in soils. As yet no direct method has been introduced. One of the first methods used was that of Pearson (78). The organic phosphorus is extracted with alkali and determined by the difference between total and inorganic phosphorus content of the extract. Another method based on extraction is that of Mehta and Legg (67) where NaOH is used for extraction instead of NH_4OH. A more recent method uses ignition (56) to determine the amount of phosphorus before and after heating the soil to 240°C. Only the more labile organic phosphorus compounds would appear to be broken down at this temperature, however.

There still exists considerable controversy about each of the mentioned methods. Legg found 20 per cent more phosphorus extracted with the Wrenshall and Dyer (118) method than by the method of Pearson.

Black et al (13) have prepared a comprehensive review of soil organic phosphorus. Further work is needed on procedures for the determination of organic phosphorus.

Chang (28), working with pure cultures of microorganisms, found that between 0.3 and 0.4 per cent phosphorus was assimilated in organic form per unit of cellulose decomposed. Kaila (53) reported similar results with the use of pure cultures and the use of other organic materials as sources of carbon. If the phosphorus content of the organic material was below 0.3 per cent phosphorus, then mineral phosphorus was taken from the soil and incorporated into organic form. Kaila found that the ratio of organic carbon to organic phosphorus in mineral soil was about 100:1 to 150:1. The ratio of organic nitrogen to organic phosphorus was about 8:1 to 10:1.

Predominant evidence indicates that most of the organic phosphorus exists in the form of nucleic acids and phytin (15) with as much as 40 to 50 per cent in the latter form. The availability to plants of these two forms has been tested (16, 12) but no definite conclusions have been reached as to their effectiveness when compared to inorganic forms. The presence of large amounts of organic phosphorus in some soils would certainly warrant further investigation of these phosphorus compounds and their relation to plant nutrition.

The Uronide Or Polyuronide Fraction Of The Soil

Based on the method of Lefevre and Tollens (55), which depends on the liberation of carbon dioxide when uronic acids are boiled in 12 per cent HCL, many investigations have been conducted on the uronide or polyuronide fraction of soil organic matter. Shorey and Martain (89), Norman and Bartholomew (74), Waksman and Reuszer (111), and Fuller (41)

have shown that from 10 to 40 per cent of the total carbon may be present in this form. Bremner has criticized the large portion of the total carbon attributed to this fraction, since no proof has been given that uronides are stabilized in the soil to such a large extent.

Lynch (59) used a chromatographic technique to separate some of the sugars present in the fulvic acid fraction of soils. The same carbohydrates were found following various treatments of the fraction, but mild acid hydrolysis gave higher yields of these materials.

Fuller (42), doing work on the uronic fraction, concluded that this fraction is of microbial origin because of the high proportion of CO_2 produced when compared to plant uronides. He also reasoned that if uronides were not intimately combined with other fractions, then their isolation would be easily made. Fractionation of the soil, however, was found to bring into solution various other materials in the presence of which positive identification of uronides was impossible.

Mattson and Kouttler-Andersson (63) found that in beech lignin the content of uronic anhydride increased 2 to 10 per cent as a result of auto-oxidation in alkali. This would show that sources other than uronic acids are capable of releasing CO_2 under the conditions of the Lefevre-Tollens determination for uronic carbon.

From the existing evidence it would seem that some of the uronides are of microbial origin, but the method that has been used for the determination of this particular fraction is too empirical. The method in its present application gives no assurance that only uronides release CO_2 under these conditions, even though uronic acids are decarboxylated quantitatively by this method.

Composition of Soil Organic Matter

Literature references to the gross organic fraction are rather difficult to evaluate, since no uniform method has been accepted for the isolation of the humus or humic acid fractions.

Previous to 1900, much work had been done on the characterization of the dark colored material present in the soil but little work was done on the chemical compounds present in the organic fraction. Schreiner and Shorey (90) brought to the attention of investigators the fact that specific organic constituents could be isolated from the soil. Robinson (86) found that acid hydrolysis of soils liberated amino nitrogen. The amount of amino nitrogen released increased up to a point and then decreased with further hydrolysis. Gortner and Morrow (70) fractionated the nitrogen present in mineral and organic soil according to a protein hydrolysis. Their pioneering work (Table 1) showed that a large part of the organic nitrogen present was in the form of protein or proteinaceous compounds. Other work of this kind led several workers to conclude that a large part of the protein present in soil was in the form of yeast and bacteria. Gortner found that ammonia and sodium hydroxide do not extract the same substances from the soil, and that humus does not consist of a black colored compound alone, but that a portion of almost colorless products is masked by it. Little interest was shown in this work until recently when similar studies on soil organic matter have received considerable attention.

Hobson and Page (50), and Page (77) performed numerous studies on soil organic matter and concluded that the humic materials contain

Table 1. - Average nitrogen distribution in per cent of total soil nitrogen.

Data obtained from Gortner (45)

	Sphagnum	Muck	Fargo clay loam	Fargo silt loam	Prairie covered loess	Forest covered loess
Ammonia N	28.69	19.49	24.00	26.56	30.53	28.69
Humic ppt. by $Ca(OH)_2$			9.21	13.26	5.19	4.84
Amino N	45.71	42.95	36.21	40.16	33.72	32.74
Basic N	9.73	13.55	9.58	12.11	12.68	13.98
Insoluble humin in soil	26.38	27.61	28.27	22.93	24.35	26.92
Amino N of bases	5.26	9.13	6.44	7.52	7.48	7.57
% Recovery	102.28	99.46	103.77	100.00	98.33	101.64
Basic N set free by 50% KOH	2.98	3.10	3.23	3.20	3.27	3.69

a complex of non-nitrogenous humic acids and protein. A smaller proportion of the total nitrogen extracted from soils with cold soda was found to be in the amino form than in proteins of animal or vegetable origin. From this they concluded that the protein was of a different source than plant of animal protein. They also found that humic acids prepared from sucrose and furfural did not behave as acids but did give a dark color.

At about the same time Waksman and Iyer (107, 108, 109, 110) postulated that protein existed in soils in the form of a resistant ligno-protein complex, and that this accounted for its apparent stability in soil. From synthetic preparations it was found that such complexes were more resistant to microbial decomposition and possessed a higher exchange capacity than the original protein or lignin. However, other workers (77, 61) have failed to observe an increase in exchange capacity during formation of such complexes. Because of the great influence of Waksman's work on other workers in this area of investigation (105, 113, 114), it was not until recently that the ligno-protein complex theory was subjected to serious criticism.

McGeorge (64) found that the exchange capacity of highly organic soils is approximately a linear function of the per cent of carbon in the soil. He attributed the exchange activity to lignin or lignin-hemicellulose.

The mechanism of clay-organic combination was studied with pure clay systems by Gieseking (43) and Ensminger and Gieseking (33). The organic molecules and proteins used interacted with the clays and resisted hydrolysis to a much greater degree than the substances

themselves. There was some question as to whether the clay absorbed the enzyme and caused its inactivation. Allison, et al, (4) found that inorganic colloids exerted an influence on the decomposition of some organic materials. Montmorillonite was most effective in stabilizing carbon, and kaolinite the least. Lynch et al, (60) studied some carbohydrate-clay complexes and found that complex sugar molecules were absorbed by clays.

McLean (65) reported that the activities of clay-humic systems were not decreased too much from those of the clay itself. He stated that the activities of ions on the clay could still be projected to the whole soil. He attributed the decrease in exchange capacity of the complex to reactions between the clay and the organic material and not to the mechanical covering of the exchange sites on the clay. Gillman (44) used acetylation and methylation to study the exchange reactions of humic acids. Humic acids were methylated and acetylated and showed reduction of exchange capacity by these treatments. The reduction was less than equivalent to the increase in acetyl and methyl content. More recent work along these lines was conducted by Broadbent (26), with the use of diazomethane and dimethyl sulfate. He found that, in soil freed from the inorganic fraction by hydrofluoric acid, diazomethane reduced the cation exchange capacity to a greater extent than methyl sulfate. From this he concluded that the exchange activity in humic materials resides in carboxylic, phenolic, or enolic groups.

Gottlieb and Hendricks (46) tried hydrogenation and alkaline nitrobenzene reduction as a method for isolation of compounds from the

soil organic matter without too much success. They concluded that the lignin molecule undergoes condensation to form a fused ring structure more resistant than native lignin.

More recent work on acid hydrolysis of soil organic matter by Kojima (54), Stevenson (96, 97, 98) and Bremner (17, 18, 19, 20, 21, 22) showed that: (a) About 25 to 35 per cent of the total soil nitrogen is present as alpha amino nitrogen; (b) that a large part of the ammonia in acid hydrolysates could come from hydrolysis of protein or amides so that the above figure is a minimum for proteinaceous nitrogen; (c) compounds like amino sugars are present to some extent and may account for 7 to 10 per cent of the total nitrogen; and (d) that 65 to 80 per cent of the total nitrogen is hydrolyzed in acid.

Hock (51), along with other workers, tried to use the color of alkali extracted material as an index of the amount of humus present. Bremner (21) showed that color is a poor index of organic matter present in solution. Hock separated out various soil organic components on a starch column and used ultra violet light for comparison of the extracts from different soils. This method appears worthy of further investigation, especially with new equipment available for such separations.

Forsyth (38, 39) characterized the fulvic acid fraction by selective adsorption and found at least four components, depending on the solvent used. He selected the polysaccharide fraction and studied it in further detail. Five sugars were isolated, along with galacturonic acid. The molar ratio of the sugars isolated after hydrolysis seemed to be a constant for this fractional component of fulvic acid in the

different soils tested.

In hydrolyzed soil humates, Sowden and Atkinson (93) found the proportion of amino nitrogen to total nitrogen to be lower than would be expected if the soil nitrogen was combined largely in proteins. Sowden and Parker (94) treated soils with 2, 4-dinitro-fluorobenzene prior to hydrolysis, according to Sanger's method for complexing terminal amino acids, but no free amino groups were found in the whole soil or the humic fraction.

Puustjarvi (83), working with humic acids of peats, found no appreciable differences in the humic acids isolated. The smallest possible humic acid molecule had the formula, $C_{17}H_{20}N_2(COOH)_4$. The tentative postulation was made that humic acids are cyclic compounds coming from oxymethylfurfural.

Flaig and his group in Germany (35, 36, 37) have worked with synthetic substances and with natural materials. From these studies they postulated a degradation of lignin to phenols, followed by synthesis to humic acids. Nitrogen content of humic acids could arise from nicotine and indolic compounds which were found to be products of fungal metabolism.

A great many workers have concerned themselves with the hypothesis that the resistant lignin in plant residues accumulates in the soil as part of the humic acid molecule (31, 57). It was been found by Broadbent (24), Peevy and Norman (72), and Waksman and Hutchings (106) that generally the methoxyl content of lignin decreases as lignin is decomposed, it darkens in color and increases in nitrogen content. Mattson and Koutler-Andersson (62) found that alkaline auto-oxidation

of lignin changes its solubility, and causes it to fix ammonia against hydrolysis by strong acids. Bennet (11) confirmed Mattson's work on oxidative nitrogen fixation by lignin. By subsequent methylation he showed that this process most probably involves phenolic groups.

Norman and Peevy (76) oxidized soils with hypoiodite on the premise that lignin or lignin-derived material would be primarily involved in the reaction. Moodie (69) used this method to study various horizons in soils and found some differences in the various horizons. The method is highly empirical. It is difficult to determine precisely what the hypoiodite is oxidizing, or whether lignin is present in amounts to justify the method.

Carbon and Nitrogen Metabolism in Soil

The isotope N^{15} has greatly facilitated the detailed study of nitrogen transformations in soil. Recent studies have shown that denitrification occurs in soils under aerobic, as well as anaerobic, conditions. Wijler and Delwich (115) showed losses of N^{15}, and Arnold (5), by use of infrared absorption, determined the loss of nitrous oxide from soil. Broadbent and Stojanovic (27) and Nommik (72) have found that denitrification is greatly enhanced under anaerobic conditions, resulting in losses up to 80 per cent or more of nitrate initially present within periods of three days to three weeks. Postulations as to the stepwise reduction of NO_3 to N_2 were made by Allen and Van Neil (1), based on N^{15} studies with *Pseudomonas* species.

Bartholomew *et al.*(10), used N^{15} as a tracer to follow the immobilization of mineral nitrogen in soils. They found that 27 to

54 per cent of the N^{15} applied as fertilizer to greenhouse pots was taken up by the plants; the lower plant recoveries of fertilizer nitrogen were found when crop residues were added to the soil.

With an increased need for an accurate nitrogen test, interest has arisen in the nitrifiability of soil nitrogen. Allison (2) found that addition of organic materials caused a tying up of nitrogen, and decreased nitrates. The addition of $NaNO_3$ allowed the plants to grow normally again. Since so many factors can affect the formation of nitrates in field soil, Quastel (84) used the apparatus developed by Audus (6) to study the biochemistry of nitrification under maximally standardized conditions.

Harmsen and Van Schreven (47) have written an excellent review paper on mineralization of organic nitrogen in soil. They summarized an extensive examination of the literature by stating the following well supported principles: (a) Nitrite-nitrogen and ammonia-nitrogen are never found to accumulate in normal soils; (b) under perennial crops, especially under grass cover, the mineral nitrogen content remains low during the entire year; (c) in absence of leaching, nitrates accumulate in fallow soils; (d) with annual cultivated crops, soil nitrates fluctuate seasonally, decreasing during periods of rapid crop removal and briefly or not at all following the incorporation of normal crop residues; and (e) the C:N ratio in organic matter added to the soil is an important factor influencing the course of mineralization, but its influence is not quantitatively predictable when applied to organic materials of widely different origin. A critical discussion of the methods used for determination of nitrogen mineralization is also included in this article.

Carbon metabolism has also been studied by many workers in this field, especially with the rather recent availability of radioactive and stable isotopes for tracer studies. Stotsky and Mortensen (100) used both labeled C^{14} and N^{15} in rye plants to study decomposition of green manures added to Rifle peat.

Waksman (104) found that for thirty parts of cellulose one part of nitrogen was needed for decomposition by a mixed soil population. By using similar studies it was shown that fungi assimilate carbon into cell substance more efficiently than do bacteria, with actinomycetes in an intermediate group.

Corbet (30) considered the evolution of CO_2 more significant than bacterial numbers in measurement of biological activity, and developed an F factor as an index to this activity.

Broadbent (24), and Broadbent and Bartholomew (25) reported that additions of fresh plant residues to soil exerted a "priming" action such that the resistant soil organic matter itself decomposed more rapidly in their presence. The net loss of carbon from the soil was greater with low rates of amendment. Broadbent suggested that larger, less frequent applications of crop residues would be better than frequent, light addition. Pinck and Allison (80) were not able to confirm this work and have taken issue with this concept.

Bollen and Lu (14) studied the evolution of CO_2 from various residues, especially forest products, and showed that the specific origin of the material, in addition to its carbon-nitrogen relationship, was important in decomposition. They also determined that addition of nitrogen to decomposing material resulted in less CO_2 loss

than when no nitrogen was added. In opposition to this, Rothwell and Frederick (87) showed that loss of CO_2 from corn stover tended to be similar with or without nitrogen, provided the incubation time was of sufficient duration. During shorter periods of time, additions of nitrogen resulted in a greater loss of carbon.

Several good review articles on carbon-nitrogen relationships, together with original data were published by Winsor and Pollard (116, 117).

Absorption Spectra of Complex Molecules

The use of infrared and ultraviolet absorption spectra for the study of molecular structure has increased with improvements in commercial instruments.

The cause of absorption in the ultraviolet region is an electronic energy-level shift within the molecule when it is exposed to radiant energy of appropriate wave length. For an organic compound the molecule must include a resonant structure, usually of the type C=C, C=O, N=N, or C=N or other doubly-bonded groups. Ultraviolet absorption apparatus has been widely used for the study of biochemical materials and aromatic compounds. For a further discussion of ultraviolet spectra refer to Friedel and Orchin (40).

While the organic molecule must be capable of resonance to show absorption in the visible and ultraviolet, all organic compounds absorb in the infrared region. The radiant energy absorbed is translated into kinetic energy which may appear in one or both of two forms of movement of atoms in the molecule. One of these is the vibration of

atoms about an equilibrium position in the molecule. The other is a rotational movement of an atom about its axis. The combination of these vibrations with rotation of the atoms gives rise to vibrational-rotational absorbance spectra. The absorption of energy to give a vibrational movement induces an instantaneous dipole moment in the molecule and produces an absorption band in the transmission spectrum. The vibration of the atoms with respect to one another may be resolved into two type of motion:

(1) a "stretching" motion where the atoms move along the direction of their bond axis.

(2) a "bending" motion which produces angular deformation of the interatomic bond (Barnes et al, 7).

Ploetz (82) has used ultraviolet spectra to study polyquinones as possible building blocks for humic acids. A research project report of the American Petroleum Institute (85) presents many infrared spectra for silicate and clay minerals.

PART ONE

CHEMICAL AND BIOLOGICAL STUDIES OF NEW ATWOOD SOILS

INTRODUCTION TO PART ONE

Chemical and biological studies were conducted on two groups of soil samples representing soils previously amended with various plant residues. The first group was taken from a field experiment in which massive applications of sawdust had been made on a Sims clay loam at varying time intervals prior to sampling. The second group was taken from a greenhouse experiment in which various plant materials were added at two rates and with three levels of nitrogen to an Oshtemo sand. Analytical methods which were employed with both groups of samples are described in the following chapter. The experiments themselves are described and the experimental results pertaining to each are presented separately in the next two chapters.

METHODS OF ANALYSIS

Total Carbon

Total carbon was estimated by two methods:

a. The dry combustion method with ignition at 950°C (81).

b. A modified ignition method as described in the appendix.

Total Nitrogen

Nitrogen was determined by the Kjeldahl method using a mercury catalyst.

Nitrates

Nitrates were determined on water leachates by the phenol-disulfonic acid method.

Nitrifiable nitrogen was determined by the Iowa incubation procedure (95).

Hydrolytic Fractionation of Nitrogen

The method used for hydrolytic fractionation of nitrogen was that described by Morrow (70). After removal of nitrate by leaching with water, the soil was hydrolyzed with 6N HCl for a period of 12-14 hours. This hydrolysate was freed from the soil by filtration and washing with hot water. The acid hydrolysate was reduced in volume in vacuum to remove most of the HCl. A measured aliquot was then neutralized with saturated $Ca(OH)_2$ and the ammonia distilled over into a boric acid solution under vacuum. The residual material after the ammonia distillation was collected on filter paper and designated as acid-soluble humin. The filtrate was neutralized with

HCl and concentrated under vacuum to 100 mls. The filtrate contained both the mono-amino nitrogen and basic nitrogen fraction. Alpha amino nitrogen was determined on an aliquot of this filtrate by the Van Slyke nitrous acid method (120). A second aliquot was treated with phosphotungstic acid to precipitate basic nitrogen. In the present study, no attempt was made to separate amino nitrogen in the basic fraction. Total nitrogen in the material precipitated with phosphotungstic acid was determined and designated as basic nitrogen. All distillations were carried out at a temperature less than 50°C and in vacuum.

Alkali-Soluble Nitrogen Fractions

The soil was extracted first with one percent HCl to remove the calcium and then extracted twice with 2 percent NaOH. The extraction was done at room temperature by continuous shaking for a period of four hours each time. The samples were centrifuged, washed and the supernatants combined. To the dark colored supernatants, HCl was added until precipitation occured. This precipitate was then centrifuged, washed, and the supernatants saved for nitrogen analysis. The acid precipitated material was designated as the humic acid fraction and the supernatant, the fulvic acid fraction. The fulvic acid fraction was concentrated by evaporation before analysing for nitrogen. An attempt was made to determine sugars in the fulvic acid fraction but the concentration of sugars was so small that no accurate determination could be made with the methods employed.

Organic Phosphorus

Pearson's method for organic phosphorus was used (78).*

Materials Floatable in Water

Seventy grams of soil was shaken with 200 mls of water and 20 mls of $BaCl_2$ was added to precipitate the colloidal material. Undecomposed residues which floated to the surface were skimmed off. This separation was repeated twice. The water-floatable material was washed and placed in a dish for drying and weighing. Kjeldahl nitrogen was determined on these floatable materials from the field experiment.

Microbial Counts

Microbial counts were made on two different media: Martin's medium for fungi (92) and a modified soil-extract-tryptone-agar (49) for bacteria were used. Formulas for these media are given in the Appendix. Soil samples were weighed and placed in sterile water blanks, with an initial dilution of 1:10. From these, additional dilutions were made so that counts could be made between 20 and 300 colonies per plate for bacteria and 20 to 100 colonies per plate for fungi. All dilutions were poured in duplicate for each of the duplicate greenhouse pots, giving a total of four plates counted per treatment for each group of organisms.

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* The author wishes to thank K. L. Hinra for help in the analysis of the organic phosphorus.

Respiration Rate

One hundred grams of soil was placed in a two-quart glass jar. A vial containing 5 mls of .5N NaOH was placed in the jar to collect the carbon dioxide. The NaOH was then titrated with .1N HCl in the presence of excess $BaCl_2$ and a CO_2 free atmosphere. The vials were changed daily or twice daily depending on the CO_2 production. One empty jar was kept for a blank (75). The jars were aerated with suction every three days to insure an adequate oxygen supply. A constant temperature of 35°C was maintained and the moisture content was adjusted to approximately field capacity at the beginning of the incubation period. Carbon dioxide production was determined for a ten-day period.

FIELD EXPERIMENT

Design of Field Experiment

A field experiment had been started in 1951 by the Michigan Agricultural Experiment Station to test the effects of large applications of hardwood sawdust on crop yields on a Sims clay loam. Five separate blocks had been laid out to accommodate a five-year rotation of corn, beans, barley and two years of alfalfa-brome meadow. Four different plant residue treatments were imposed each year on the block which happened to be in second-year alfalfa brome. The two treatments considered in the present study were the following:

1. Two cuttings of hay removed from both first and second year meadows, no additional plant residues added.
2. Two cuttings of hay removed from both first and second year meadows, plus 35 tons of hardwood sawdust applied in the fall after the second cutting of the second year of alfalfa brome.

All plots received uniform fertilizer applications according to the following schedule:

Corn: 100 lbs per acre 5-20-10
Beans: 200 lbs per acre 0-20-20
Barley: 240 lbs per acre 5-20-20

In addition, the plots of each residue treatment were split, and supplemental nitrogen was applied on one-half of each plot. One-hundred-twenty pounds per acre of extra nitrogen was applied with corn, 40 pounds with beans and 20 pounds with barley. No fertilizers were applied on the two years of meadow.

In the spring of 1956 composite soil samples were taken from the nitrogen-treated half of plots which had received 35 tons of sawdust

one, three and five years previously. A check sample was composited from corresponding plots which had received fertilizer and supplemental nitrogen but no additional plant residues.

Experimental Results

Crop Harvest Data

Data in Table 2 show that crop yields were initially depressed by the sawdust treatment. Yields of corn the first year after sawdust application were reduced 50 per cent. Barley yields three years after sawdust application were also reduced. That nitrogen immobilization was an important factor in crop injury from sawdust is evidenced by the fact that the addition of 120 pounds per acre of nitrogen increased corn yields by 44 bushels per acre. Supplemental nitrogen on sawdust plots raised barley yields to about the same levels as on plots which had received no sawdust. Corn yields with supplemental nitrogen, however, were still much less than where no sawdust was applied.

Bean yields in the second year were lowered by the application of sawdust and showed no recovery when supplemental nitrogen was added. It may have been that some nutrient other than nitrogen was reduced to limiting levels of availability. Phosphorus, in particular, may have been so affected, as is suggested by data to be presented later on changes in forms of soil phosphorus.

Four years after it was applied, sawdust had no effect on the yields of alfalfa-brome.

Total nitrogen, water-floatable material and pH

Table 3 shows some of the gross measurements made on composite

Table 2. - Crop yields in a five year rotation on Sims clay loam with and without a sawdust amendment.*

Sawdust treatment tons per acre	Year after application	Number of crop years in average	Crop	Yield without nitrogen	Yield with nitrogen**
None	one	4	Corn	83.0 bus.	87.4 bus.
35	one	4	Corn	41.6 bus.	55.8 bus.
None	two	3	Beans	33.7 bus.	33.4 bus.
35	two	3	Beans	28.3 bus.	28.3 bus.
None	three	2	Barley	52.5 bus.	55.3 bus.
35	three	2	Barley	43.4 bus.	51.3 bus.
None	four	1	Alf-Brome (1st cutting)	1.22 tons	-------
			(2nd cutting)	1.04 tons	-------
35	four	1	Alf-Brome (1st cutting)	1.32 tons	-------
			(2nd cutting)	1.04 tons	-------

* Unpublished data presented through the courtesy of the Michigan Agricultural Experiment Station.

** 120# N per acre on corn, 40# on beans, and 20# on barley.

soil samples taken one, three and five years after sawdust application and from the checks to which no additional organic materials had been applied.

The initial depression of soil pH is a treatment phenomenon frequently reported when organic materials are decomposing rapidly in soil (3). There was an increase equivalent to 47 tons per acre in materials floatable in water one year after the 35 tons of sawdust was applied. This discrepancy was probably due to the contaminating mineral matter for which no correction was made. By the fifth year after treatment about 7 tons of material light enough to float remained in excess of that on the check plots. Coincident with this reduction in quantity there was a two-fold increase in the percentage of nitrogen in these lighter materials. This might suggest a concentration of nitrogen originally contained or absorbed from soil and fertilizer sources.

Table 3.-Total nitrogen, [illegible] and pH of [illegible] treated soil

Number of years after sawdust application	pH	Material floatable in water		Total soil nitrogen*	
		Grams**	N %	Air dried	Oven dried
Check	6.35	.150	----	.233	.218
1st year	6.35	4.833	.40	.193	.175
3rd year	6.45	1.666	.59	.210	.232
5th year	6.50	.875	.77	.302	.211

*Per 100 grams soil
**Through a 40-mesh sieve

Total soil nitrogen was determined before and after screening through a 40-mesh sieve. The nitrogen percentages were greater in the sieved samples because of the removal of sand and gravel, but their relative values were altered very little. It is difficult to account for the apparent loss of 860 pounds of nitrogen per acre the first year after sawdust treatment. It is possible that anaerobic conditions resulting from rapid decomposition of the fresh sawdust may have promoted denitrification, although such large losses have not been reported from field studies. Comparable rates of denitrification in laboratory studies have involved nitrates as the initial nitrogen substrate. In the absence of confirming data, the low figures for total nitrogen one year after sawdust addition possibly should be ascribed to experimental error.

The increases in total nitrogen shown in Table 3 for the third and fifth years over the check (280 and 460 pounds per acre respectively) are of the order of the amounts of nitrogen added in fertilizer plus that which might reasonably have been fixed biologically over these periods of time. The effectiveness of sawdust in immobilizing and retaining these added increments of nitrogen in the soil is apparent.

Mineralization of Carbon and Nitrogen

Carbon dioxide evolved by these soils during a ten-day incubation period at 35°C is shown in Table 4. Carbon dioxide evolution provides an indirect measure of the energy supply available to the microbial populations which developed in the incubating sample.

There was a six-fold increase in CO_2 evolution over the check in the sample taken the first spring after sawdust application. The

highly carbonaceous nature of the contributing energy materials is reflected in the wide C:N ratio (24:1) for this soil. The high microbial demand for nitrogen to support respiration and growth of microbial cells on these materials is shown by the inability to recover nitrates from the same soil after 14 days of incubation. The field significance of these results was observed in the 50 per cent reduction in corn yields where no nitrogen was applied with sawdust (Table 2).

The failure to detect any nitrifiable nitrogen during incubation in the first year cannot be construed to mean that nitrogen was completely immobilized in a static sense. Rather, the high level of respiratory activity measured as CO_2 was supported by a rapidly circulating pool of metabolic nitrogen which was being drawn on by new microbial cells as rapidly as it was released by the death and decomposition of older cells. In the presence of excess carbonaceous materials the proportion of mineralized nitrogen to organic nitrogen in the metabolic pool may have been very low, as shown by the low level of nitrates in initial extractions of all four samples (Table 4). The nitrate and ammonia produced were available to crops on a competitive basis with the soil microbial population. With the lower temperatures normal to soils in the field, microbial competition would be expected to have been less intense than under the conditions imposed during incubation.

Table 4.-Effects of sawdust (35 tons per acre) and time since application on total carbon and nitrogen and on the mineralization of carbon and nitrogen in Sims clay loam.

Number of years after sawdust application	Total C and N			Nitrate N	Nitrifiable N	Carbon mineralized	C:N of mineralization
	C	N	C:N	(a)	(b)	(c)	(d)
	%	%		#/A	#/A	#/A	
Check	2.40	.218	11.4	3	67	481	7.2
1st year	4.01	.175	24.1	4	0	3121	Inf.
3rd year	3.23	.232	13.9	1	126	895	7.1
5th year	3.05	.241	12.7	1	121	938	7.7

(a) Nitrate-nitrogen in initial water extract of air dried soil.

(b) Nitrate-Nitrogen produced during 14 days at 35°C.

(c) Total carbon evolved as CO_2 in 10 days at 35°C.

(d) Ratio of carbon mineralized to nitrifiable nitrogen.

The long feeding period represented by the growing season of a crop such as corn, would have permitted the crop to accumulate a considerable quantity of nitrogen in competition with an active microbial population. This would have been true particularly in a soil with a relatively high nitrogen content to contribute to the metabolic pool, (about 0.2 per cent total N in this Sims clay loam). This would help to account for the rather creditable 4-year average yield of 42 bushels of corn per acre during the first year after sawdust application.

By the third and fifth years after addition of the sawdust the C:N ratio of the soil had narrowed to approximately one half that of the first year sample (from 24:1 to 13:1). The carbon remaining in residual sawdust materials in these soils was about one third as subject to attack by microorganisms as the sawdust in the first year sample. This was shown by the quantities of carbon mineralized during incubation. On the other hand the residual sawdust and its transformation products were decomposed much more readily than the native soil organic matter in the check; the CO_2 collected from the third and fifth year samples was approximately double that from the check, while the total carbon in the soil was only one third greater.

It is significant that the two-fold increase in decomposition rate in third and fifth year samples over the check was associated with a two-fold increase in nitrifiable nitrogen. The ratio of carbon mineralized to nitrogen mineralized for the check sample was 7.2 to 1, and for the third and fifth years, 7.1 to 1 and 7.7 to 1 respectively. These ratios were somewhat narrower than reported by Thompson and Black (103) because nitrates were determined after 14 days, whereas CO_2 was collected for only 10 days. However the similarity in ratios between the check and the samples containing residues from sawdust decomposition indicates that these residues had reached a stage where the pattern of their decomposition was very similar to that of the native soil organic matter. They represent a substantial addition to the quantity of soil organic matter and an increase in its decomposability. "Nitrifiable nitrogen" as determined by incubation procedures is a measure of both quantity and decomposibility of organic compounds containing nitrogen in the soil.

The incubation procedure showed a two-fold increase in nitrifiable nitrogen by the third and fifth years after sawdust application. The amount found in the third year (126 lbs. per acre) is in the prediction range for no response to nitrogen in the Iowa interpretation scheme of Fitts, Bartholomew and Heidel (34). However, yields of barley this third year were depressed by the residual sawdust, and barley did respond to nitrogen (Table 2). The Iowa interpretation is based on the response of corn. However, the failure of barley to reflect the differences in nitrifiable nitrogen between the check and the third year sample is an example of the inconsistencies frequently encountered in attempts to correlate crop response with incubation tests for nitrifiable nitrogen.

Hydrolytic nitrogen fractions

The soils were sieved through a 40-mesh sieve and subjected to acid-hydrolysis and fractionation of nitrogen according to a modified Hausmann protein analysis (70). The data are presented in Table 5.

As has been pointed out, the large initial decrease in total nitrogen the first year after treatment must be questioned. However, relative changes in the various hydrolytic fractions were consistent with trends observed in the third and fifth years' samples and will be discussed.

Of the total nitrogen in these samples, 70 to 75 per cent was released by acid hydrolysis. This compares with 72 to 77 per cent reported by Gortner (45) and 68 to 87 per cent reported by Bremner (17). The proportion of acid-hydrolyzable nitrogen was greater in the sawdust treated samples than in the check. The increased quantities of

Table 5. - Acid-hydrolyzable nitrogen fractions in a Sims clay loam at different time intervals after incorporation of 35 tons of sawdust per acre.*

Number of years after sawdust application	Total N	Non-acid-hydrolyzable N	Acid-hydrolyzable N	Acid-hydrolyzable fractions in percent of total soil nitrogen				Ratio of humin N to non-acid-hydrolyzable N
				NH_3-N	Amino-N	Basic-N	Acid-hydt. humin N	
	**	***	****					
Check	.233	.069	70.54	20.75	32.31	6.90	10.95	.370
1st year	.193	.051	73.73	17.48	38.50	7.73	9.50	.433
3rd year	.242	.059	75.44	19.14	41.00	8.80	9.66	.381
5th year	.268	.066	74.53	16.76	40.03	7.68	10.90	.385

* Determinations on samples sieved through 40-mesh screen. 40 grams of soil hydrolysed with 6N HCl for 12-14 hours under a reflux column.

** Total Kjeldahl nitrogen as percent of sieved soil.

*** Non-acid-hydrolyzable nitrogen as percent of sieved soil, by difference.

**** Nitrogen in acid hydrolyzate as percent of total Kjelhahl N.

nitrogen in the hydrolysate appeared principally in the amino and basic fractions. The proportion of soil nitrogen which appeared in these two fractions reached a maximum the third year, although absolute amounts were greater the fifth year due to a continued increase in total nitrogen between the third and fifth years. Amino nitrogen and basic nitrogen were determined on separate aliquots of the same hydrolysate after humin precipitation and ammonia distillation. Thus there was some overlapping in the constituents of these two fractions, with some basic amino acids undoubtedly appearing in both.

There was a definite downward trend in the proportion of acid-hydrolyzed ammonia nitrogen, although the relation to the period of decomposition was erratic. A portion of this ammonia may have come from the deamination and deamidation of proteinaceous materials. However, a large part of it could have originated in soil constituents other than proteins (62, 90). Among such non-proteinaceous ammonia sources may have been included oxidative products of lignin decomposition (61,62), as well as ammonia sorbed by clay minerals (98).

The ratio of ammonia to amino nitrogen declined from .642 in the check sample to .454, .466 and .419 in the samples taken one, three and five years after sawdust application respectively. Stevenson (97) found that a widening ratio of ammonia nitrogen to alpha amino nitrogen appeared to be characteristic of the weathering of soil organic matter as reflected in the long term rotation plots in the Morrow experiments at the University of Illinois. Table 4 shows a net relative decrease in ammonia nitrogen of 4.0 per cent between the check and the fifth year's sample. This corresponds to a net increase

reported by Stevenson of 6.0 per cent for continuous corn over continuous sod over a fifty-year period. The relative increase of 7.7 per cent in amino nitrogen five years after sawdust treatment in Table 4 corresponds to Stevenson's net decrease of 9.9 per cent. To the extent that these changes reflect differences in quality of soil organic matter, the transformation products of sawdust decomposition after 5 years represent a drastic reversal of trends associated with normal weathering.

The proportion of nitrogen in the acid-hydrolyzable humin fraction showed an initial decline the first year after sawdust application, followed by a gradual increase. The original level had been established again after five years. The same trends were observed for the total amount of nitrogen which was not released by acid hydrolysis. An essentially constant ratio of .392 mgms of hydrolyzable humin N was recovered per mgm of non-acid-hydrolyzable N in these four samples. This strongly suggests that the hydrolyzable humin N is an equilibrium product of the partial hydrolysis of some organic complex (or complexes), the relatively more resistant core of which appears in the non-acid-hydrolyzable nitrogen fraction. It is generally recognized that both fractions contain a preponderance of aromatic or heterocyclic structures characteristic of lignin or degradation products of lignin, as well as of soil humic materials (17, 61, 62). The difference in refrangibility between these two fractions may be related to differences in degree of oxidation or degree of polymerization or both.

The non-acid-hydrolyzable nitrogen fraction does represent the

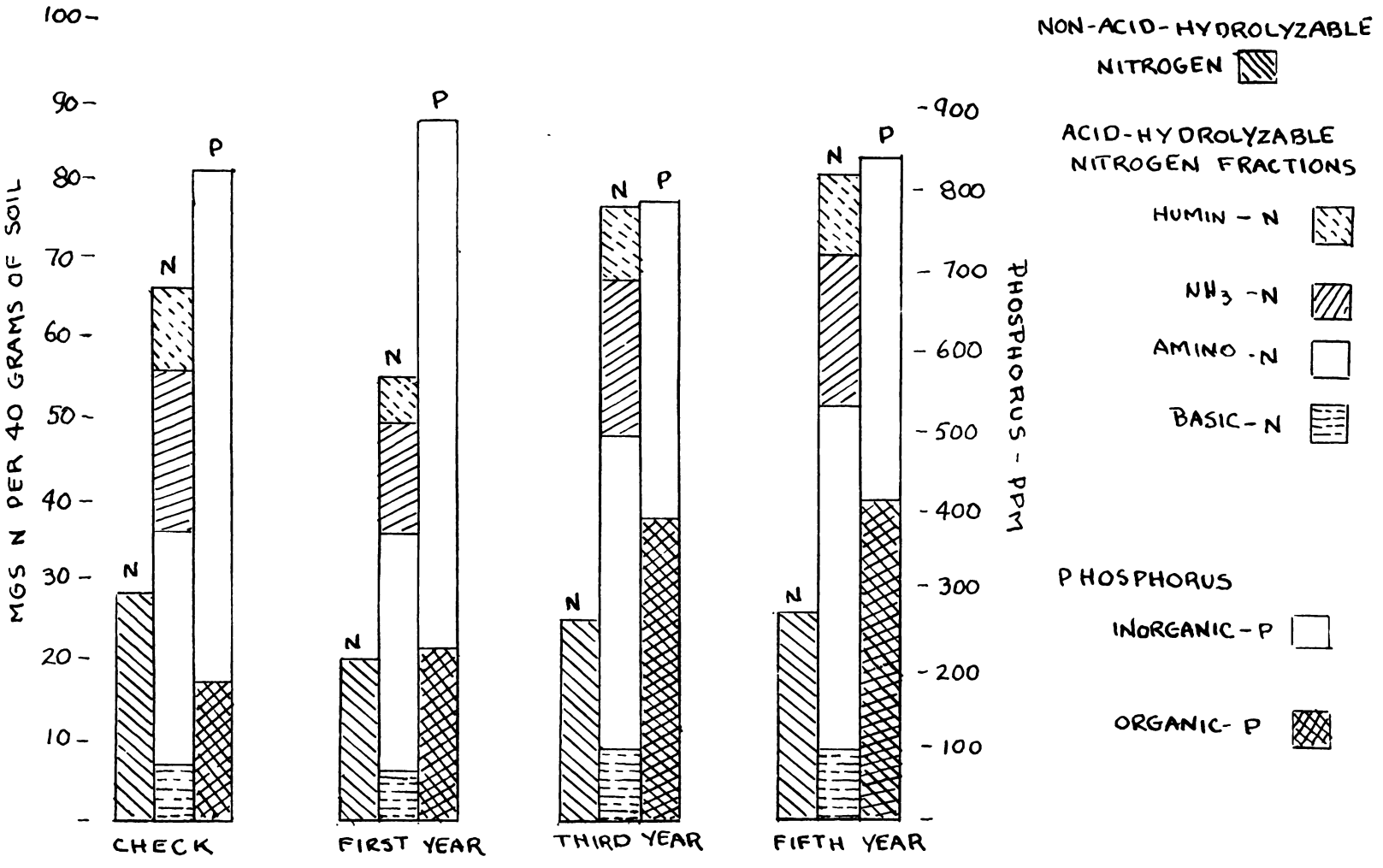
more highly oxidized, resistant humic materials in the soil. The initial decline after sawdust addition in the proportion of nitrogen found in this resistant fraction is analogous to the priming action of fresh residues on decomposition of soil organic matter reported in tracer studies by Broadbent and Bartholomew (25). It would appear that fresh energy materials in the added sawdust made it possible for soil microorganisms to attack these resistant soil humic materials. The concurrent increase in the amino nitrogen fraction suggests that resistant nitrogenous constituents of native soil organic matter were utilized as nitrogen sources by the soil microbial population, resulting in a transfer of nitrogen from one fraction to the other.

The absolute amounts of nitrogen recovered in each fraction are plotted graphically in Figure 1. The data for phosphorus fractions shown in this figure will be discussed later. The initial decrease in total nitrogen the first year involved decreases in all fractions except the amino fraction. Increases occurred in all fractions the third year, but principally in non-acid-hydrolyzable nitrogen and amino nitrogen. Additional increases the fifth year occurred only in acid-resistant nitrogen, amino nitrogen, and humin nitrogen.

Two distinct mechanisms of nitrogen immobilization are reflected in the increases in total nitrogen observed in the samples taken the third and fifth years after sawdust was applied. For the most part, the increases in acid-hydrolyzable amino nitrogen may be considered to represent products of microbial synthesis. The non-acid-hydrolyzable nitrogen and the hydrolyzable humin nitrogen fractions

FIGURE 1

Changes in organic nitrogen and organic phosphorus fractions in a Sims clay loam over a five year period following the addition of 35 tons per acre of sawdust.


KEY
NON-ACID-HYDROLYZABLE NITROGEN
ACID-HYDROLYZABLE NITROGEN FRACTIONS
HUMIN - N
NH_3 - N
AMINO - N
BASIC - N
PHOSPHORUS
INORGANIC - P
ORGANIC - P
MGS N PER 40 GRAMS OF SOIL
PHOSPHORUS - PPM
100
90
80
70
60
50
40
30
20
10
900
800
700
600
500
400
300
200
100
N
P
CHECK
FIRST YEAR
THIRD YEAR
FIFTH YEAR

increased most probably by incorporation of nitrogen into residual lignaceous materials which accumulated as the sawdust decomposed. This latter process is presumably not microbial but is associated with the chemical oxidation of lignin or similar compounds involving benzene ring structures (61, 62, 63).

Alkali-extractable nitrogen

The results of alkali extraction and fractionation of nitrogen in Sims clay loam samples are presented in Table 6. Figures for non-acid-hydrolyzable nitrogen are also shown for purposes of comparison.

Nitrogen extractable in alkali reflected the sharp drop in total nitrogen the first year after sawdust application. However, the upward trend in total nitrogen in the third and fifth years' samples was not paralleled by similar increases in alkali extracted nitrogen.

A somewhat closer correlation was observed between the percentage of total N appearing in the humic acid fraction and the percentage not hydrolyzed by acid, both showing a distinctly downward trend. This might be expected, since insolubility in acid was the basis for separation of both fractions. Humic acid nitrogen in the alkali extract continued to decline in percentage over the whole five-year period, whereas the percentage nitrogen not hydrolyzed by acid definitely leveled off between the third and fifth years. Thus it would appear that certain soil constituents may be common to both fractions, while others are more specifically associated with one or the other of these two fractions.

The fulvic acid fraction showed a sharp increase percentagewise

Table 6. - Effects of sawdust (35 tons per acre) and time after application on the humic and fulvic acid fractions of Sims clay loam.*

Nitrogen fraction	Check	Years after sawdust application 1st year	3rd year	5th year
Total soil nitrogen mgs per 100 gms	233	193	242	268
Total soil nitrogen extracted in alkali mgs per 100 gms	79.5	68.0	69.0	62.2
Percent of total soil nitrogen extracted in alkali	36.3	38.8	28.7	25.8
Humic acid nitrogen mgs per 100 gms	57.2	44.5	51.7	44.4
Percent of total	24.5	23.0	21.3	16.5
Fulvic acid nitrogen mgs per 100 gms	22.3	23.5	17.3	17.8
Percent of total	9.5	12.1	7.1	6.6
Non-acid-hydrolyzable nitrogen-mgs per 100 gms	** 69.	51	59	66
Percent of total soil nitrogen	29.5	26.3	24.6	25.5

*Fifty grams of soil were twice extracted with 2% NaOH for two four-hour periods at room temperature with shaking. The centrifuged supernatant was acidified with HCl until the dark colored material was precipitated. This acid precipitated material is the humic acid fraction and the supernatant the fulvic acid fraction.

**Non-acid-hydrolyzable nitrogen from Table 5.

one year after sawdust was applied. This may have reflected a temporary increase in free, -or loosely complexed, -amino acids or protein moieties resulting from microbial activity stimulated by the large addition of fresh energy materials. The declining level of fulvic acid nitrogen in the third and fifth years parallels that for humic acid nitrogen.

The significance of these changes is not clear. They do indicate that soil organic constituents were being actively transformed as a result of sawdust treatment and that the influence of the sawdust on these transformations was still in evidence five years after treatment. It appears likely that many of the changes noted were related to increased complexing activity of lignin as it was progressively exposed and oxidized during decomposition of the sawdust.

Organic phosphorus

The work of Chang (28) has shown that phosphorus to the extent of 0.3% of added cellulose was assimilated during decomposition. This corresponds to numerous reports that a phosphorus content of 0.2% to 0.3% in organic materials represents the critical level between release and immobilization of mineral phosphorus during decomposition (13).

Phosphorus in the sawdust was not determined, but analyses of similiar materials reported in the literature (3) indicate that it would have been of the order of 0.01%. An increase in organic phosphorus would be expected where sawdust is allowed to decompose in soils. Changes in total, inorganic, and organic phosphorus with

sawdust treatment and time are tabulated in Table 7. Total phosphorus was maintained at rather constant levels in all soils. Two-fold increases in per cent organic phosphorus were found in the third year and fifth year samples. These increases in organic phosphorus were made principally at the expense of the inorganic phosphorus.

There was a sharp decrease in acid-soluble phosphorus the first year after sawdust applications, at which time 29 ppm (58 pounds per acre) was found. In the Michigan system of fertilizer recommendations based on soil tests, 50 pounds per acre of phosphorus soluble in .135N HCl represents the dividing line between "high" and "low" levels of available phosphorus for soils below pH 6.5. When the combined requirements of a growing crop and the microfloral population supported by the residues is considered, the 58 pounds of acid-soluble phosphorus found in this sample may well have been deficient. This may be one of the reasons that corn and barley did not give larger responses to nitrogen fertilizer. The continued increase in organic phosphorus observed through the third and fifth years suggests that microbial competition for phosphorus as well as nitrogen may have been a significant factor in crop yields over the entire period.

Organic phosphorus increased with amino nitrogen, as is shown graphically in Figure 1 (P. 36). It may be inferred that carbon added to each of these organic fractions came largely from added sawdust by microbial compounding with nitrogen and phosphorus from soil and fertilizer sources. This does not imply that the increased quantities of organic phosphorus found in the third and fifth years' samples were present entirely in the form of microbial cell substance.

Actually, only a small amount of phosphorus could be considered to have been in this form. Rather, a selective accumulation of phosphorus compounds released by the death and decay of successive generations of microbial cells would appear to have occured. The possibility that decomposition products of lignin might form complexes with phosphorus compounds has never been investigated.

Table 7. - The effect of sawdust on the total, inorganic and organic phosphorus in Sims clay loam.*

Years after sawdust application	Replication	Total P ppm	Inorganic P ppm	Inorganic P % of total	Organic P ppm	Organic P % of total	.135N HCl - soluble P ppm	.135N HCl - soluble P % of total
Check	1	832	640		192		57.5	
	2	800	642		138		58.0	
average		816	651	79.77	165	20.23	57.8	7.1
1st year	1	870	664		206		27.7	
	2	900	700		200		30.0	
average		885	682	77.06	203	22.94	28.8	3.3
3rd year	1	840	434		406		52.00	
	2	700	376		324		55.0	
average		770	405	52.60	365	47.40	53.5	6.9
5th year	1	870	400		470		50.00	
	2	800	470		330		40.0	
average		835	435	52.10	400	47.90	45.0	5.4

* Method of Pearson (78)

GREENHOUSE EXPERIMENT

Design of Greenhouse Experiment

An experiment was initiated in the greenhouse to study the effects of decomposition of various organic amendments on the distribution of soil nitrogen. So that relative changes might be more easily observed, a sandy soil low in carbon and nitrogen was selected. It was also considered that the effects of clay minerals on the decomposition would be minimized in a sandy soil.

Oshtemo sand was used. Cation exchange capacity and exchangeable cations were determined by the ammonium acetate method (32). Available phosphorus was estimated as that extracted in 0.1N HCl containing 0.03 N NH_4F, according to the method of Bray (32). The initial pH of the soil was determined with the glass electrode.

The soil was screened and thoroughly mixed. Four thousand grams of soil was placed in each of the one gallon pots used in the study. Calcium hydroxide was added in an amount calculated to bring base saturation to 80 per cent with respect to calcium. Primary and secondary nutrients other than nitrogen were added at double the required rates calculated from soil tests, in an attempt to make nitrogen the only limiting nutrient. Two mls of a minor element mixture suggested by Hoagland (68) was added in solution. The dry mineral amendments which were added to each pot were as follows:

$Ca(CH)_2$	.....	2.0 gm
$CaHPO_4$	.....	1.0 gm
MgO	.....	0.4 gm
KCl	.....	1.0 gm

The following plant materials were used as organic amendments: Sawdust*, lignified sawdust**, wheat straw, corn stalks/, and alfalfa hay. These materials were dried at 70°C before the prescribed aliquots for each treatment were weighed and added to the soil. The materials were added at two rates of application (50 and 100 gms per pot//) and mixed thoroughly with all of the soil in each pot.

Three levels of nitrogen treatment were employed with all materials except alfalfa hay. At the 50-gm rate of organic amendment, the N_o, N_1 and N_2 levels of nitrogen treatment corresponded to additions to each pot of nitrogen from urea as follows: Sawdust (0, .392 and 1.177 gms); lignified sawdust (0, .641 and 1.923 gms); straw (0, .073 and .219 gms); corn stalks (0, .126 and .379 gms). At the 100-gm rate of organic amendment, urea-nitrogen additions per pot to achieve N_o, N_1 and N_2 levels of nitrogen treatment were as follows: Sawdust (0, .785 and 2.355 gms); lignified sawdust (0, 1.281 and 3.846 gms); straw (0, .146 and .438 gms); corn stalks (0, .252 and .757 gms).

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* Hardwood sawdust.

** Acid-extracted sawdust prepared as follows: Hardwood sawdust was treated with 72 per cent sulfuric acid w/v for two hours at 10°C and then hydrolyzed according to the method described by Norman (73). The acid-treated, acid-hydrolyzed material was then washed with water until free of sulfates. This treatment is designed to remove cellulose and other carbohydrates, leaving a residue which, in the case of non-proteinaceous materials such as wood, is considered to be principally lignin.

/ The corn stalks included cobs and hulls in addition to the stalks themselves.

// Equivalent to 12.5 and 25 tons per acre, respectively.

Assuming that the materials used all contained 50 per cent carbon, the N_0, N_1 and N_2 levels of nitrogen treatment represented additions of carbon and nitrogen in the following ratios: Sawdust (353:1, 60:1 and 20:1); lignified sawdust (577:1, 36:1 and 13:1); straw (66:1, 55:1 and 42:1); corn stalks (114:1, 72:1 and 41:1). The ratio of carbon to nitrogen in the alfalfa hay was 18:1.

Two check treatments were included. In one case neither organic amendments nor nitrogen were added. In the other, 1.923 gms of urea nitrogen was added per pot without organic amendment.

Water was added to bring the soils to 10 per cent moisture (approximately field capacity). The soils were incubated for 40 weeks in the greenhouse. One series of duplicated pots of all treatments was planted to wheat, two crops of which were grown and harvested during the first 25 weeks of the incubation period. Soils were removed from the pots and remixed after harvest of the first crop and before planting of the second crop of wheat. The other series of duplicated pots were not cropped during the 40 weeks of the incubation period but were maintained at the same moisture content as the cropped series by periodically adding water to constant weight.

The sawdust and lignified sawdust treatments were also applied in duplicate to a third series of pots which were then planted to alfalfa. Three cuttings of alfalfa were harvested from these pots during the 40-week incubation period.

Daily maximum and minimum temperatures were recorded from January through September, or through all but the first month of the period. Daily maximum temperatures fluctuated moderately in the 70's and 80's

through February, when the first crop of wheat was harvested. From March through May while the second crop of wheat was growing, increasingly extreme fluctuations in temperature were recorded, with increasingly frequent maxima in the 90's, or above. From June through September, mean monthly maximum temperatures were consistently above 100 degrees F, and daily minima ranged between 60 and 70 degrees. Because of these high temperatures, no attempt was made to grow wheat through the summer, although all pots were maintained at the moisture level which had been established in the beginning. Alfalfa was grown continously through September in the pots which were planted to alfalfa at the beginning of the experiment.

During this 40-week period soil samples were taken periodically for determination of pH and estimation of microbial numbers. At the end of the 40 weeks, soil samples from representative treatments were taken for use in the laboratory determinations outlined in Chapter IV.

At the end of the 40 weeks, wheat was planted in all pots as a biological indicator of residual nitrogen availability. Top growth harvested from these plants, as well as from the two crops of wheat and the three cuttings of alfalfa from the two previously cropped series of pots, were dried at 65 degrees C, weighed and ground for analysis for total nitrogen.

A detailed description of all treatments included in the experiment is presented in Table 17 of the Appendix.

Results of Greenhouse Experiment

Because of the time-consuming nature of a number of the laboratory

determinations, it was not feasible to make use of soil samples from all treatments in the laboratory. Representative treatments were selected for this purpose. For the most part, samples from the high rate of residue addition (100 gms per pot) were subjected to detailed analysis, since it was felt that maximum variations relatable to treatment would be found at the higher rate. Soil samples from the duplicate pots of each of these selected treatments were bulked and most analysis were performed in duplicate on aliquots of the bulked sample.

The experimental treatments have been described in the preceeding section and in Table 17 of the Appendix. The following code will be used to relate laboratory results with the experimental treatments imposed prior to and during the course of incubation in the greenhouse:

LS: Lignified sawdust
SD: Sawdust
CS: Corn stalks
ST: Wheat straw
ALF: Alfalfa hay
CK: Check

W: The pots were cropped twice to wheat during the first 25 weeks of the decomposition period.
W_o: No crop was grown during the 40 week decomposition period.
N_o: Highest C:N ratio of organic amendment (no supplemental nitrogen).
N_1: Intermediate C:N ratio of organic amendment (lower level of supplemental nitrogen).
N_2: Lowest C:N ratio of organic amendment (higher level of supplemental nitrogen).
CK + N: 1.923 gms of urea nitrogen added per check pot without organic amendment.

Soil pH and nitrates

Data in Table 8 show that the pH of the check soil to which urea

was added had increased to 7.7 two weeks after the start of the experiment. This was due to ammonia released by hydrolysis of the urea. Similar increases over the no-nitrogen check were observed for alfalfa hay and for the uncropped sawdust and lignified sawdust treatments at the N_2 level of nitrogen. Soil pH was depressed at this time by lignified sawdust at the N_0 and N_1 levels of nitrogen. This reflects the high base-binding capacity reported for lignaceous acidoids (61, 62, 63).

Soil acidity after 40 weeks appeared to be closely related to the level of nitrate nitrogen in the soil. Relatively high nitrate levels following addition of alfalfa were less effective in depressing the pH than was the case with the other materials. Of the materials used, alfalfa would have contributed the greatest quantities of mineral cations to neutralize organic and mineral acids produced during decomposition.

The oxidation of lignin is known to result in an increase in acid groups and an increase in cation exchange capacity. This would explain the fact that the increase in acidity after 40 weeks was disproportionately greater for a given level of nitrate with lignified sawdust than with the other materials.

The original organic components of all cropped soils had been augmented by the root residues from two crops of wheat grown during the decomposition period. Evidence will be presented later to show that these root residues contributed greatly to the supply of energy materials and markedly influenced the biological and chemical properties of these soils at the end of the decomposition period. From

Table 8 it is apparent that the net effect of cropping in the case of the lignified sawdust, sawdust and corn stalk treatments was to reduce the level of nitrate and retard the development of acidity following high rates of nitrogen treatment. In the alfalfa-treated soil, cropping had no apparent effect on pH or nitrate level after 40 weeks.

Water-floatable materials and total carbon and nitrogen

The transformation of organic materials added to these soils was probably more rapid and extensive than would have been the case in the field because of the abnormally high temperatures which attained in the greenhouse. A rough indication of the extent to which the various materials were altered in 40 weeks is given by the recovery of materials which floated off when the soils were suspended in water (Table 8). The percentage recovery of materials originally added without supplemental nitrogen decreased in the order lignified sawdust > sawdust > corn stalks > alfalfa. This is the order which would be expected in the light of the original carbon-nitrogen ratios of these materials and what is known about the relative decomposabilities of similar plant materials.

These water-floatable materials accounted for roughly $\frac{1}{2}$ to 2/3 of the increases in total carbon which were observed in all soils to which organic materials were added. This was true in all cases except where the high level of supplemental nitrogen was used with lignified sawdust. Here the greatest increase in total carbon for any treatment ($LS\text{-}W_o\text{-}N_2$) was associated with a very low recovery of water-floatable material. The amount of relatively unaltered lignin

Table 8. - Effects of various soil amendments on soil pH after 2 and 40 weeks and their relation to final levels of nitrate, water-floatable materials and total carbon and nitrogen in Oshtemo sand.*

Treatment	Soil pH		Levels after 40 weeks			
	After 2 wks.	After 40 wks.	NO_3-N lbs. per acre	Water-floatable materials % of original amendment	Total C % of soil	Total N lbs. per acre
				**	***	****
LS-W-N_o	6.7	6.3	1	78.0	1.38	1140
LS-W-N_1	6.5	6.2	32	62.8	1.46	1320
LS-W-N_2	6.9	5.2	79	43.6	1.38	1440
LS-W_o-N_2	7.2	4.8	410	22.4	1.53	1860
SD-W-N_o	7.0	6.7	0	54.8	1.22	1180
SD-W-N_1	7.0	6.3	77	53.2	1.11	1500
SD-W-N_2	7.1	5.6	150	51.2	1.11	1540
SD-W_o-N_2	7.6	5.3	372	61.6	1.18	1840
CS-W-N_o	6.8	6.9	2	48.4	1.02	1260
CS-W-N_2	7.0	6.6	28	30.8	1.00	1460
CS-W_o-N_2	6.8	6.2	98	23.2	1.03	1380
ALF-W-N_o	7.5	6.5	136	13.2	1.00	1580
ALF-W_o-N_o	7.5	6.5	116	15.2	1.01	1580
CK-W-N_o	7.0	6.6	42	--	.84	1060
CK-W-N	7.7	5.3	284	--	.78	1220

* Organic amendments were added at the rate of 25 tons per acre.

** Percent recovery of water-floatable materials was calculated after substracting the amount found in the check from the amounts recovered from the other treatments.

*** Total carbon based on ignition of 50 gm samples of soil. Includes water-floatable materials.

**** Nitrates were not removed prior to determination of total Kjeldahl nitrogen.

recovered in the four lignified sawdust treatments decreased as the level of nitrate nitrogen increased. Thus, increasing the level of mineral nitrogen in the decomposition medium hastened the transformation of lignin, even though the transformation did not always result in proportionate losses of carbon.

The failure of the transformed lignin to float may have been due in part to physical changes which increased its wettability. These changes would appear to have been related to processes of oxidation and complex formation which were enhanced in the presence of nitrogen. Increasing exchange capacity which results from oxidation of lignin would have increased the susceptibility of lignin transformation products to precipitation by the $BaCl_2$ which was added to the water in which the soils were suspended.

Relative values for total carbon and nitrogen in these samples are presented in Table 9. Increases in total nitrogen over the check ranged from 7 to 75 per cent, or from 80 to 800 pounds per acre. The smallest increases were with the lignified sawdust and sawdust treatments which were cropped without supplemental nitrogen. The largest increases occurred with the uncropped, N_2 levels of the same materials. Increases with alfalfa, cropped, were intermediate and of about the same order as the increases with lignified sawdust, sawdust and corn stalks when these were cropped at the N_2 levels of nitrogen. Cropping had little effect on the nitrogen level where alfalfa hay and corn stalks were used.

Except for the lignified sawdust treatments, there was no marked tendency for total carbon to increase with total nitrogen as the

Table 9. - Relative carbon and nitrogen contents of variously treated oshtemo sand after forty weeks in the greenhouse.

Treatment	Relative carbon and nitrogen contents Percent of check	
	C	N
LS-W-N_o	164	107
LS-W-N_1	174	124
LS-W-N_2	164	135
LS-W_o-N_2	182	175
SD-W-N_o	145	111
SD-W-N_1	132	141
SD-W-N_2-	132	145
SD-W_o-N_2	140	173
CS-W-N_o	121	118
CS-W-N_2	119	137
CS-W_o-N_2	122	130
ALF-W-N_o	119	149
ALF-W_o-N_o	119	149
CK-W-N_o	100	100
CK-W + N	92	115

result of nitrogen treatment.

A close relationship was observed between nitrate nitrogen and total nitrogen after 40 weeks. This relationship is shown graphically in Figure 2. The points for all residue-treated soils cluster about the curve established by the values for lignified sawdust and sawdust. The values for the check soils fall away from this curve but show the same upward trend.

Nitrates were not removed prior to the Kjeldahl digestion for total nitrogen. Thus it is possible that some nitrates were included in the determination of total nitrogen. The quantities so included may be inferred to be small. The magnitude of the increases in total nitrogen in residue-treated soils is such as to warrant the assumption that increasing nitrate level was associated with greatly increased quantities of nitrogen in forms other than nitrate.

Hydrolytic nitrogen fractions

The results of acid hydrolysis and fractionation of nitrogen in these samples is presented in Table 10. The addition of sawdust or lignified sawdust alone without supplemental nitrogen resulted in very little change in the levels of total or non-acid-hydrolyzable nitrogen as compared with the check. The addition of supplemental nitrogen with residues low in combined nitrogen and the addition of alfalfa containing a high percentage of combined nitrogen resulted in marked increased in total and non-acid-hydrolyzable nitrogen. The proportion of hydrolyzable nitrogen to the total decreased as the level of non-acid-hydrolyzable nitrogen increased. Relative changes

FIGURE 2

Relationship between total and nitrate nitrogen in Oshtemo sand 40 weeks after incorporation of various organic residues.

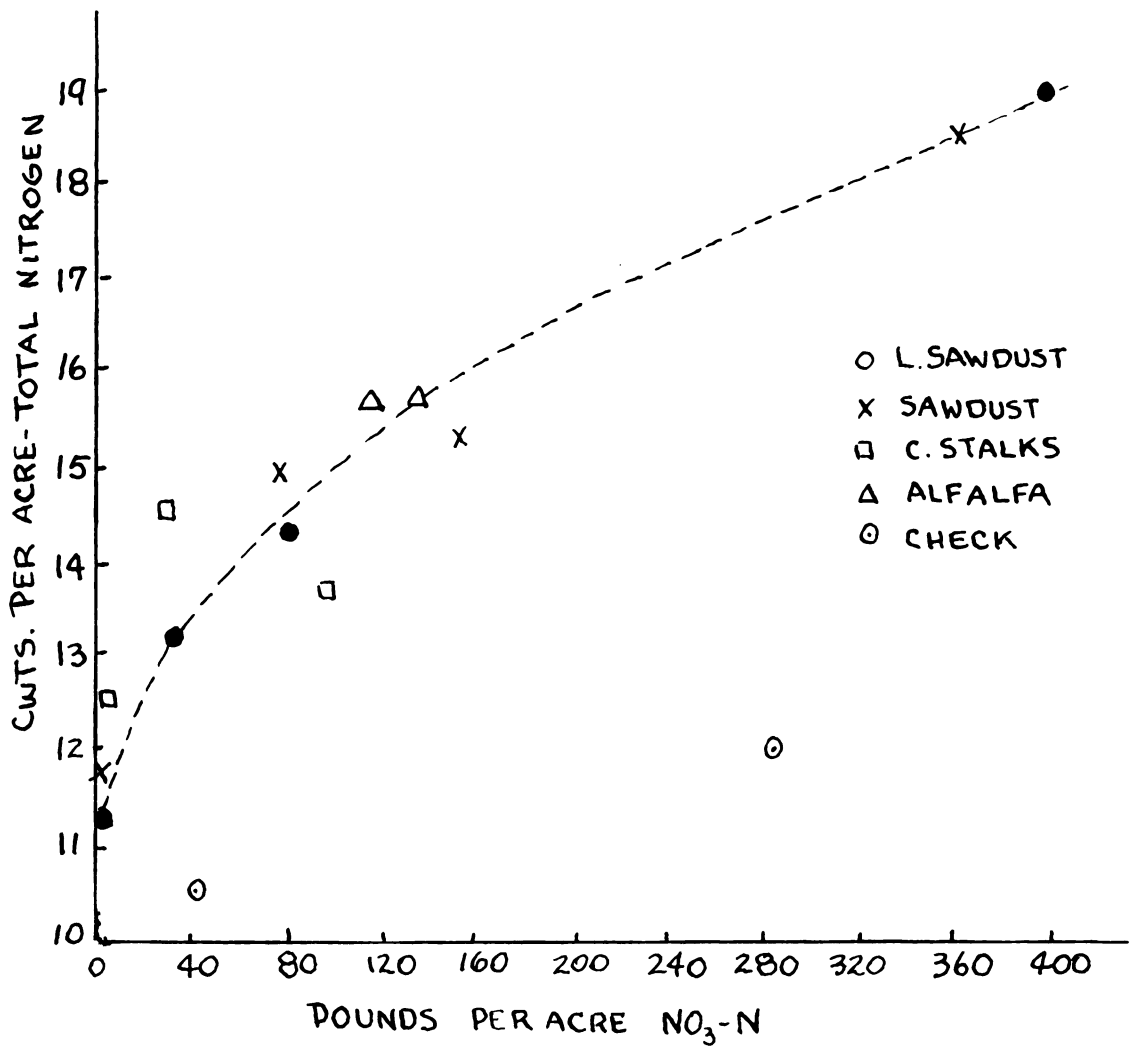

CWTS. PER ACRE-TOTAL NITROGEN
19
18
17
16
15
14
13
12
11
10
0
40
80
120
160
200
240
280
320
360
400
POUNDS PER ACRE NO_3-N
L. SAWDUST
SAWDUST
C. STALKS
ALFALFA
CHECK

Table 10. - Acid-hydrolyzable nitrogen fractions in an Oshtemo sand 40 weeks after incorporation of various residues.*

Treatment	Total N % of soil **	Non-acid hydrolyzable N % of soil ***	Acid hydrolyzable nitrogen fractions in per cent of total soil nitrogen	
			Total acid hydrolyzable N ****	NH_3-N
LS-W-N_o	.058	.015	74.98	15.46
LS-W-N_2	.065	.018	71.77	14.64
LS-W_o-N_2	.078	.032	58.89	14.60
SD-W-N_o	.057	.013	76.54	16.53
SD-W-N_2	.067	.018	72.59	14.07
SD-W_o-N_2	.074	.026	65.50	12.15
CS-W-N_2	.070	.018	74.70	13.93
ALF-W-N_o	.083	.025	69.68	14.17
CK-W-N_o	.057	.014	75.39	16.15
Virgin Houghton muck	3.090	.074	76.10	12.23

* Determinations on samples sieved through 40-mesh screen. 40 grams of soil hydrolysed with 6N HCl for 12-14 hours under a reflux column.

** Total Kjeldahl nitrogen as per cent of sieved samples. (Nitrates were previously removed by leaching with water.)

*** Non-acid hydrolyzable nitrogen as per cent of sieved sample, by difference.

**** Nitrogen in acid hydrolyzate as per cent of total Kjeldahl N. (Nitrates were removed prior to hydrolysis by leaching with water.)

Table 10. - (Continued)

Acid hydrolyzable nitrogen fractions in percent of total soil nitrogen			Ratio of humin N to non-acid hydrolyzable N	Treatment
Amino N	Basic N	Acid hydr. humin N		
35.29	12.03	9.88	.098	LS-W-N_o
30.08	7.92	8.24	.083	LS-W-N_2
30.60	7.77	8.49	.085	LS-W_o-N_2
36.31	8.72	9.58	.096	SD-W-N_o
33.70	8.70	10.70	.107	SD-W-N_2
28.41	7.91	8.88	.089	SD-W_o-N_2
41.70	8.70	8.69	.087	CS-W-N_2
31.50	8.54	9.26	.093	ALF-W-N_o
33.87	10.71	12.11	.121	CK-W-N_o
46.03	7.77	6.31	.063	Virgin Houghton muck

among the various hydrolyzable nitrogen fractions were due principally to changes in the absolute levels of amino nitrogen and non-hydrolyzable nitrogen, as may be seen in Figure 3.

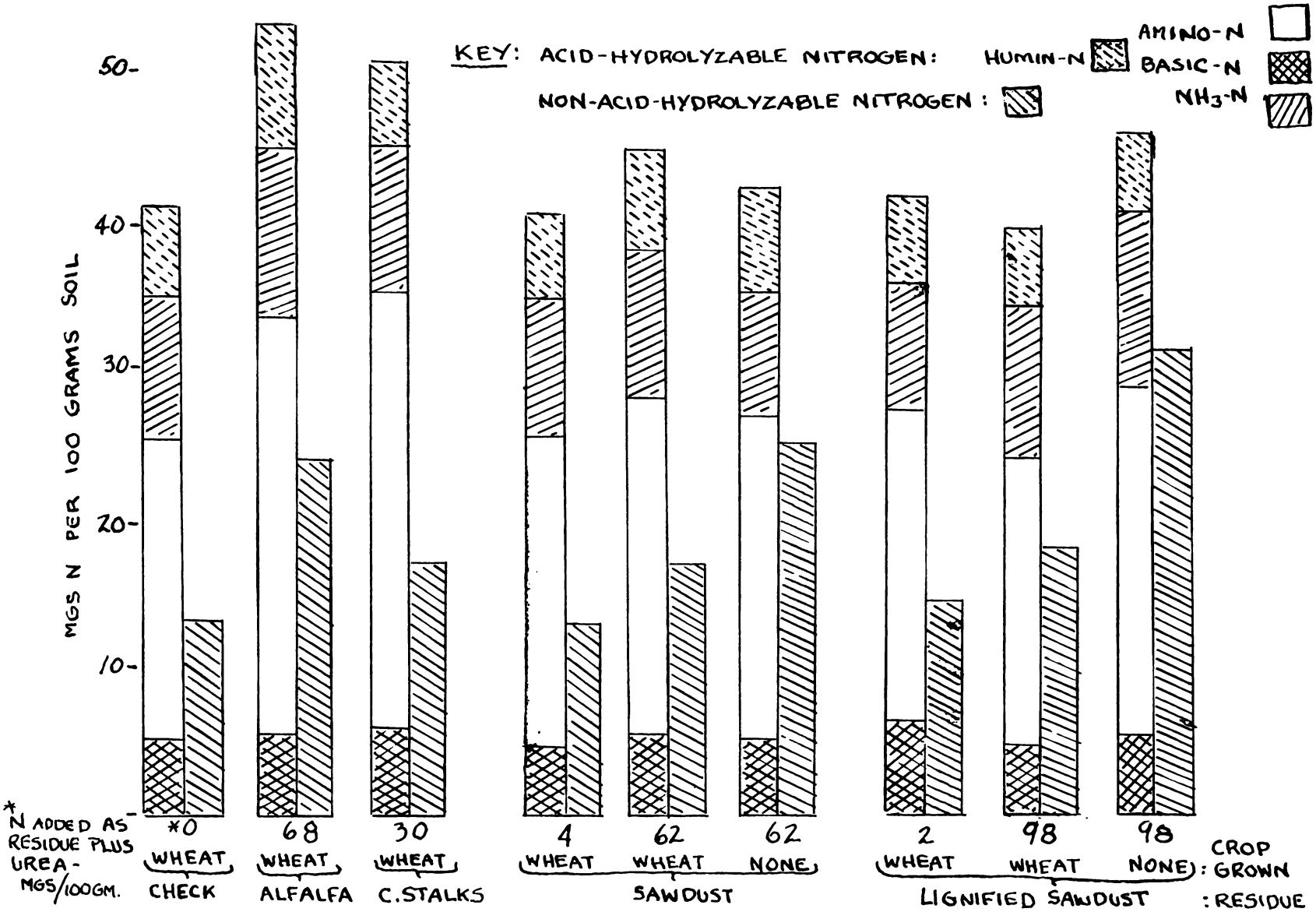
The absolute level of amino nitrogen in Figure 3 was much higher following alfalfa and cornstalks treatments than in the check. It was higher for these two treatments than for any of the other residue treatments for which data was obtained. Microbial numbers (see page 75) were three to ten times larger throughout the decomposition period with alfalfa and corn stalks than with any of the other treatments represented in Figure 3. This supports the interpretation that the acid-hydrolyzable amino nitrogen fraction represents principally materials which have been rather recently synthesized by soil micro-organisms and are present either in the form of microbial cells or as products of microbial metabolism.

The amino nitrogen fraction varied erratically with the treatments which involved sawdust or lignified sawdust. With these two materials, however, the non-acid-hydrolyzable nitrogen fraction increased consistently when supplemental nitrogen was used. This increase was much greater in the uncropped soils. As has been pointed out, cropping reduced the level of mineral nitrogen in the decomposition medium. That this may have been a factor in the accumulation of nitrogen in the acid-resistant fraction would appear to follow from the data plotted in Figure 4.

The increase in non-acid-hydrolyzable nitrogen with increasing nitrate level in Figure 4 was essentially linear for sawdust and lignified sawdust over the range of values encountered. It is known

FIGURE 3

Fractional distribution of nitrogen in Oshtemo sand 40 weeks after treatment with various residues applied at the rate of 2.5 grams per 100 grams of soil.


KEY: ACID-HYDROLYZABLE NITROGEN: HUMIN-N
NON-ACID-HYDROLYZABLE NITROGEN:
AMINO-N
BASIC-N
NH3-N
MGS N PER 100 GRAMS SOIL
50
40
30
20
10
*N ADDED AS RESIDUE PLUS UREA - MGS/100GM.
*0
68
30
4
62
62
2
98
98
WHEAT
WHEAT
WHEAT
WHEAT
WHEAT
NONE
WHEAT
WHEAT
NONE
CHECK
ALFALFA
C.STALKS
SAWDUST
LIGNIFIED SAWDUST
: CROP GROWN
: RESIDUE

that complexing reactions between lignaceous constituents of plant residues or soil humic materials and ammonia or proteinaceous nitrogen compounds give rise to chemically resistant substances. In this connection, it is pointed out that maximum levels of non-hydrolyzable nitrogen were found here in materials known to contain relatively large amounts of lignin (alfalfa, sawdust and lignified sawdust).

The curve for total Kjeldahl nitrogen in the upper half of Figure 4 shows a distinct tendency to level off at high levels of soil nitrate. This would suggest that incorporation of nitrogen into resistant fractions may become increasingly important as rates of nitrogen fertilization are increased or as the ratio of carbon to nitrogen in the soil system decreases. Such a relationship was found in the soils studied here and is depicted in Figure 5.

If the points for lignified sawdust treated samples in Figure 5 are considered by themselves, non-acid-hydrolyzable nitrogen was found to decrease with increasing soil C:N ratio in a curvilinear pattern, approaching the level in the check soil at the widest C:N ratio. The points for the other materials and the check sample clustered along a similar curve which was displaced in the direction of lower C:N ratio, or a higher nitrogen content.

Thus it would seem that an increasing level of nitrogen in the soil promoted an increasingly intense fixation or immobilization of nitrogen in chemically resistant combinations. The quantity fixed was a function, not only of nitrogen level, but also of the quantity of carbonaceous compounds present that were capable of fixing nitrogen in this form. For example, the displacement to the left of the curve

FIGURE 4

Total soil nitrogen and non-acid-hydrolyzable nitrogen in Oshtemo sand as related to nitrate nitrogen after 40 weeks.

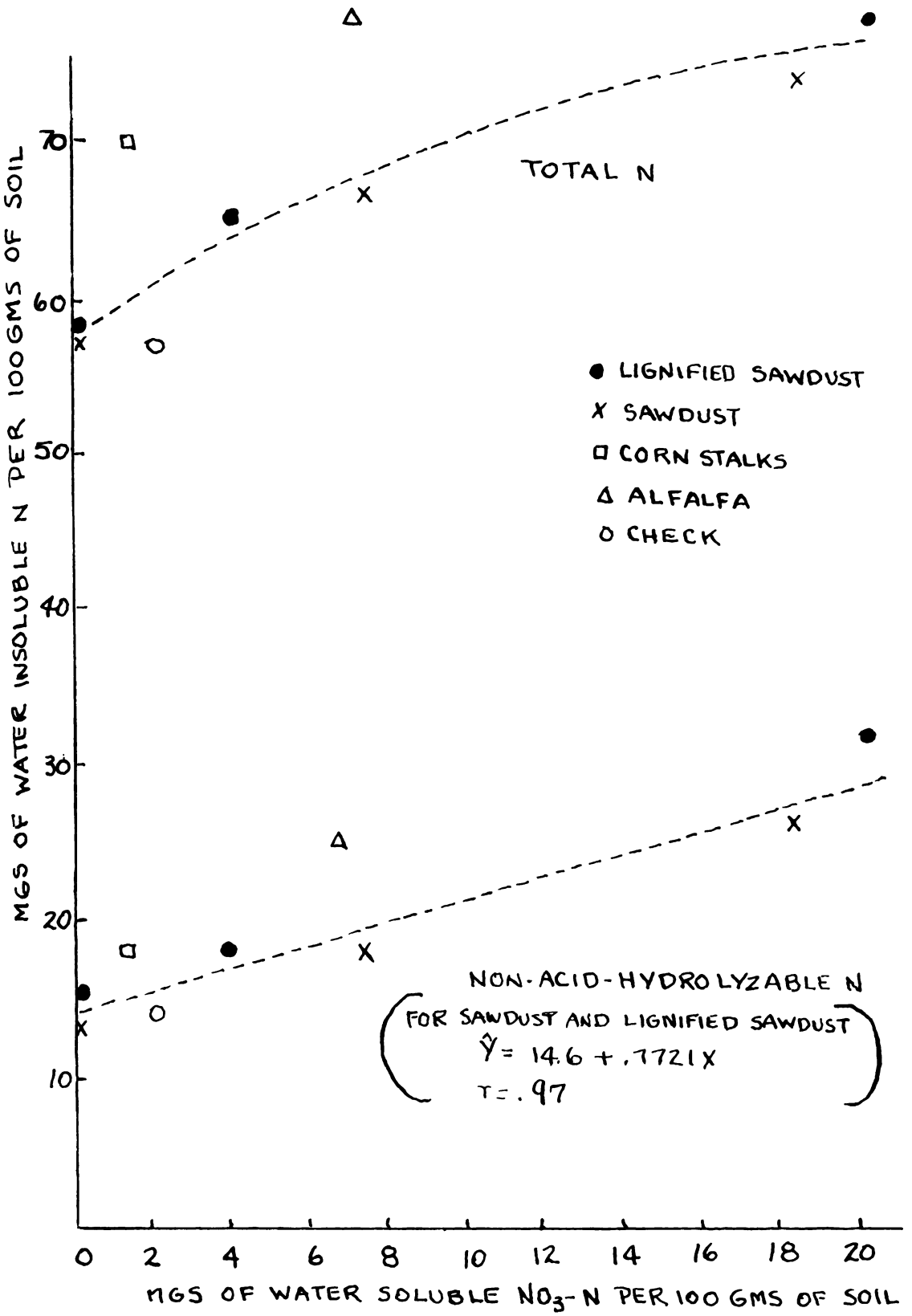
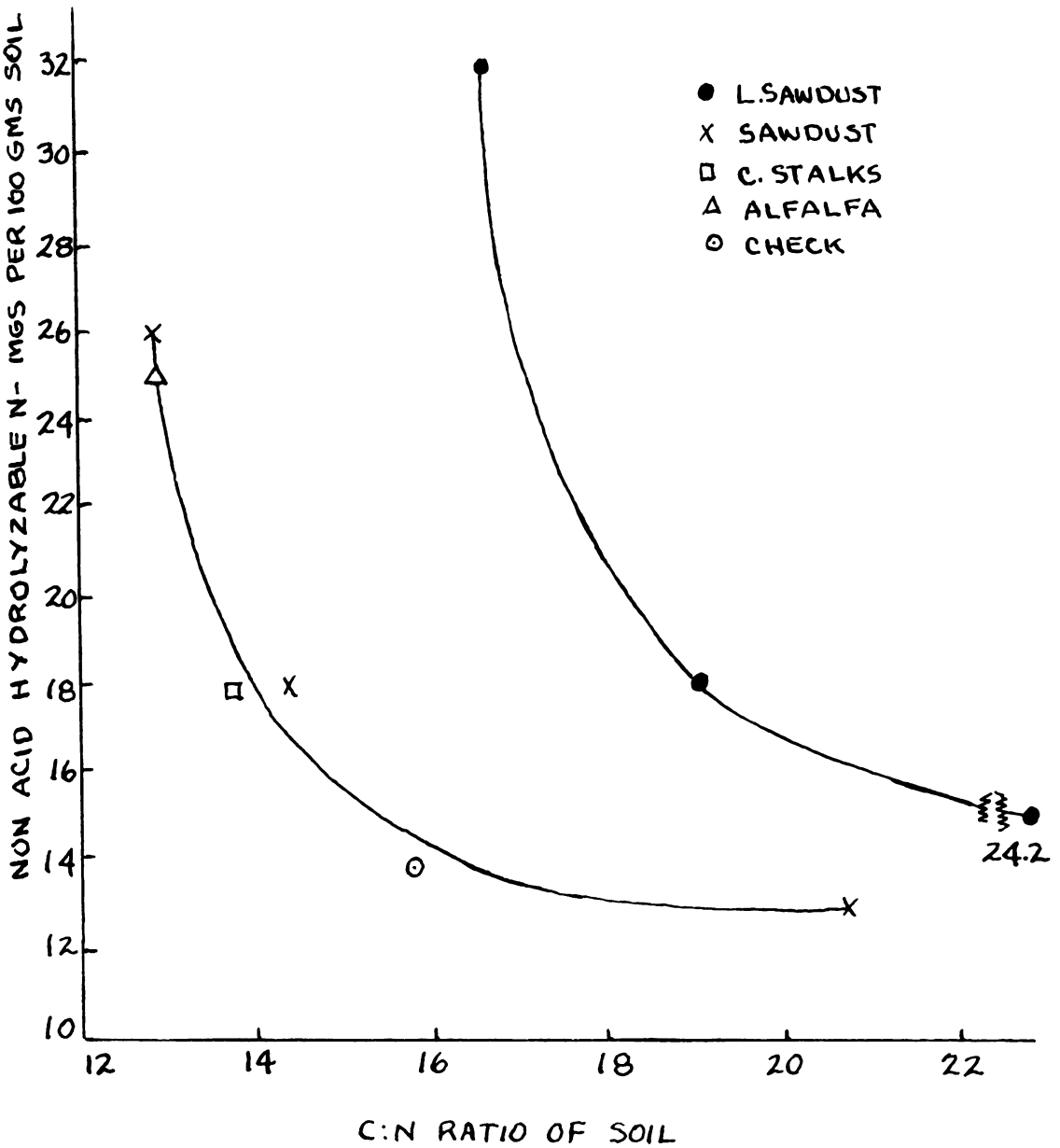

MGS OF WATER INSOLUBLE N PER 100 GMS OF SOIL
70
60
50
40
30
20
10
TOTAL N
LIGNIFIED SAWDUST
SAWDUST
CORN STALKS
ALFALFA
CHECK
NON-ACID-HYDROLYZABLE N
FOR SAWDUST AND LIGNIFIED SAWDUST
$\hat{Y} = 14.6 + .7721X$
$r = .97$
0 2 4 6 8 10 12 14 16 18 20
MGS OF WATER SOLUBLE NO_3-N PER 100 GMS OF SOIL

FIGURE 5

Relation of non-acid-hydrolyzable nitrogen to C:N ratio of the soil in Oshtemo sand 40 weeks after treatment.


NON ACID HYDROLYZABLE N- MGS PER 100 GMS SOIL
32
30
28
26
24
22
20
18
16
14
12
10
12
14
16
18
20
22
C:N RATIO OF SOIL
L.SAWDUST
SAWDUST
C. STALKS
ALFALFA
CHECK
24.2

for sawdust, alfalfa and corn stalks would appear to have been due to a greater respiration loss of carbon resulting from preferential microbial attack on the readily available carbohydrates and proteins with which the lignin (or lignin-like) constituents of these materials were diluted.

The fixation of ammonia by lignin in the laboratory is influenced by pH as well as ammonia concentration. Appropriately high pH's and ammonia concentrations did exist during the early stages of decomposition in the soils to which large amounts of nitrogen were added either as urea or in the form of alfalfa hay. Whether active complex formation occurred primarily during this early period or more or less continuously throughout the 40 weeks is not clear. The performance of successive wheat crops grown with lignified sawdust suggests that initial fixation of ammonia may have been by relatively weak exchange mechanisms, and that ammonia so fixed became progressively more resistant to release as decomposition progressed. These results will be discussed later (page 85).

In these Oshtemo samples there was no evidence of an initial attack on this resistant nitrogenous fraction in the presence of fresh plant materials such as was found in the Sims clay loam samples one year after a field application of sawdust. No simple explanation appears at this point to account for this result. Differences in mineral nitrogen supply, differences in quantity and degree of oxidation of humic materials originally in the soil, and differences in aeration related to soil structure and conditions of moisture and temperature in the field and in the greenhouse may have been involved.

On the other hand, two distinct and essentially independent mechanisms of nitrogen accumulation were seen to be operative in both soils. One was the accumulation of amino nitrogen associated with a large and active microbial population in the presence of a ready supply of energy materials. The second mechanism was the chemical fixation of nitrogen by lignaceous constituents of plant residues and soil humic materials. The latter became increasingly important as the ratio of carbon to nitrogen in the soil decreased.

The ratio of humin N to non-acid-hydrolyzable N in these samples (Table 10) was much lower and more variable than in the case of the Sims samples (Table 5). This tends to undermine the hypothesis that acid-hydrolyzable humin constituents represent equilibrium products of hydrolyzable materials represent the resistant core (cf. page 33). However, it would be expected that such an equilibrium would be influenced by the state of oxidation of the parent substances, as well as by the extent to which clay minerals might be integrated with the parent structures. The relationships which exist between these two fractions would appear to deserve further study.

Alkali-extractable nitrogen

Table 11 shows the amount of nitrogen extracted in alkali and its distribution between the humic acid and fulvic acid fractions for four selected treatments of the Oshtemo sand. Nitrogen not hydrolyzed by acid (from Table 10) is also presented for comparison.

Approximately 55 to 60 percent more nitrogen was extracted by alkali from the three residue-treated soils than from the check.

Table 11. - Effects of various residues on the humic and fulvic fraction of the Oshtemo samd after 40 weeks' decomposition.*

Nitrogen fraction	treatment **						
	Lignified sawdust		Sawdust		Check		Corn stalks
	Rep. I	Rep. II	Rep. I	Rep. II	Rep. I	Rep. II	Rep. I
Total soil nitrogen mgs per 100 gms	72.9	72.0	73.5	79.0	55.9	51.8	72.8
Total soil nitrogen extracted in alkali mgs per 100 gms	29.9	29.2	30.5	31.0	19.4	18.4	28.5
Percent of total soil nitrogen extracted in alkali	41.0	40.5	41.5	39.2	34.7	35.5	40.4
Humic acid nitrogen mgs per 100 gms	16.5	16.0	23.5	23.5	9.9	8.9	16.0
Percent of total	22.6	22.2	32.0	30.0	17.7	17.2	22.0
Fulvic acid nitrogen mgs per 100 gms	13.4	13.2	7.0	7.5	9.5	9.5	13.5
Percent of total	18.3	18.3	9.5	9.4	16.9	18.3	18.5
Non acid hydrolyzable *** nitrogen-mgs per 100 gms	18.0		18.0		14.0		18.0
Percent of total nitrogen	27.7		26.8		24.8		25.7

* Same procedure used as described for Sims clay loam. (Table 6)

** All treatments cropped to wheat at N_2 levels, except the check which received no nitrogen. Rate of residue: 2.5 grams per 100 gms of soil.

*** Non-acid-hydrolyzable nitrogen from Table 10.

Most of this increase occurred in the humic acid fraction. The increases in alkali-soluble nitrogen corresponded to a 28 percent increase in non-acid-hydrolyzable nitrogen in residue-treated soils relative to the check.

In the Sims clay loam, both alkali-soluble nitrogen and non-acid hydrolyzable nitrogen were reduced initially by sawdust treatment (Table 6). This difference between the two soils was most likely due to the higher levels of supplemental nitrogen used with the organic amendments applied to the Oshtemo soil. A high level of nitrogen in the decomposition medium promoted the incorporation of nitrogen into the non-acid-hydrolyzable fraction, as has been shown (Figures 4 and 5). The fact that non-acid-hydrolyzable and alkali-extractable nitrogen fractions showed a tendency in both soils to vary together in their relative and absolute proportions supports the contention that certain organic constituents were common to both fractions.

In the Sims samples, the decline in alkali-extractable nitrogen continued over a longer period of time than did the decline in non-acid-hydrolyzable nitrogen. In the Oshtemo samples, the increase in alkali-extractable nitrogen was twice as great as the increase in the acid-resistant fraction. Both of these results support the view that the alkali-extractable materials include a significant portion of compounds which represent intermediate stages of transformation. As such, they would be expected to accumulate during the early stages of decomposition of fresh plant materials.

Where mineral nitrogen in the soil was low, increases in alkali-soluble nitrogen might be noted principally in the fulvic acid fraction,

as was observed the first year after addition of sawdust to the Sims clay loam (Table 6). In the presence of high levels of nitrogen, processes of complex formation involving lignin-like materials would be enhanced and their products would be expected to appear in the humic acid fraction, as was the case in the Oshtemo samples (Table 11). At later stages of decomposition, this humic acid nitrogen might appear in either the more highly oxidized materials resistant to acid hydrolysis or in the hydrolyzable amino nitrogen fraction, depending on the degree of oxidation or the supply of ready energy materials to promote microbial assimilation.

In the Sims soil, increases in both the hydrolyzable amino fraction and the acid-resistant fraction of nitrogen concurred with a decline in alkali-soluble nitrogen in the third and fifth years (compare Fig. 1 with Table 6). The Oshtemo data represents only the early stages of decomposition and provides no basis for postulating later trends.

Mineralization of carbon and nitrogen

Nitrogen and carbon immobilized in organic compounds is released, or mineralized, primarily by microbial decomposition of these organic compounds. In a given soil, the net mineralization of organic nitrogen will depend on the total quantity present, its availability to microbial attack, and the proportion of carbon to nitrogen in the medium to meet the assimilative requirements of the microorganisms.

It was of interest to know to what extent the observed differences in quantity and distribution of carbon and nitrogen in the Sims and

Oshtemo samples might be correlated with differences in the rates of mineralization of carbon and nitrogen during incubation under controlled conditions. In Table 12 are assembled pertinent data for the Oshtemo samples.

Carbon mineralized was taken as a measure of the availability or decomposability of organic materials left in the soil after the 40-week decomposition period. As seen in Table 12, there was no correlation between carbon mineralized and the total carbon in the soil. With all materials, however, except alfalfa, carbon released as CO_2 decreased sharply as the level of nitrate in the soil increased.

It was pointed out earlier (Table 8) that the quantity of unaltered or water-floatable materials which were recovered at the end of 40-weeks decreased generally with increasing nitrate level. This suggests that carbon mineralized during incubation might have been related to the amount of water-floatable materials, since these might have represented the most readily available energy materials in the incubating samples. The upper half of Figure 6 shows that a generally direct relationship did exist in the case of all treatments except sawdust.

A much closer inverse relationship was found between CO_2 evolution and total nitrogen as is seen in the lower half of Figure 6.

The same inverse relationship between nitrogen content and decomposability of soil organic materials was observed in the sawdust-treated Sims clay loam samples (Table 4), where a one-third increase in nitrogen content from the first to the third year was accompanied by a two-thirds reduction in CO_2 production.

As shown in Figure 4, increases in total nitrogen in Oshtemo

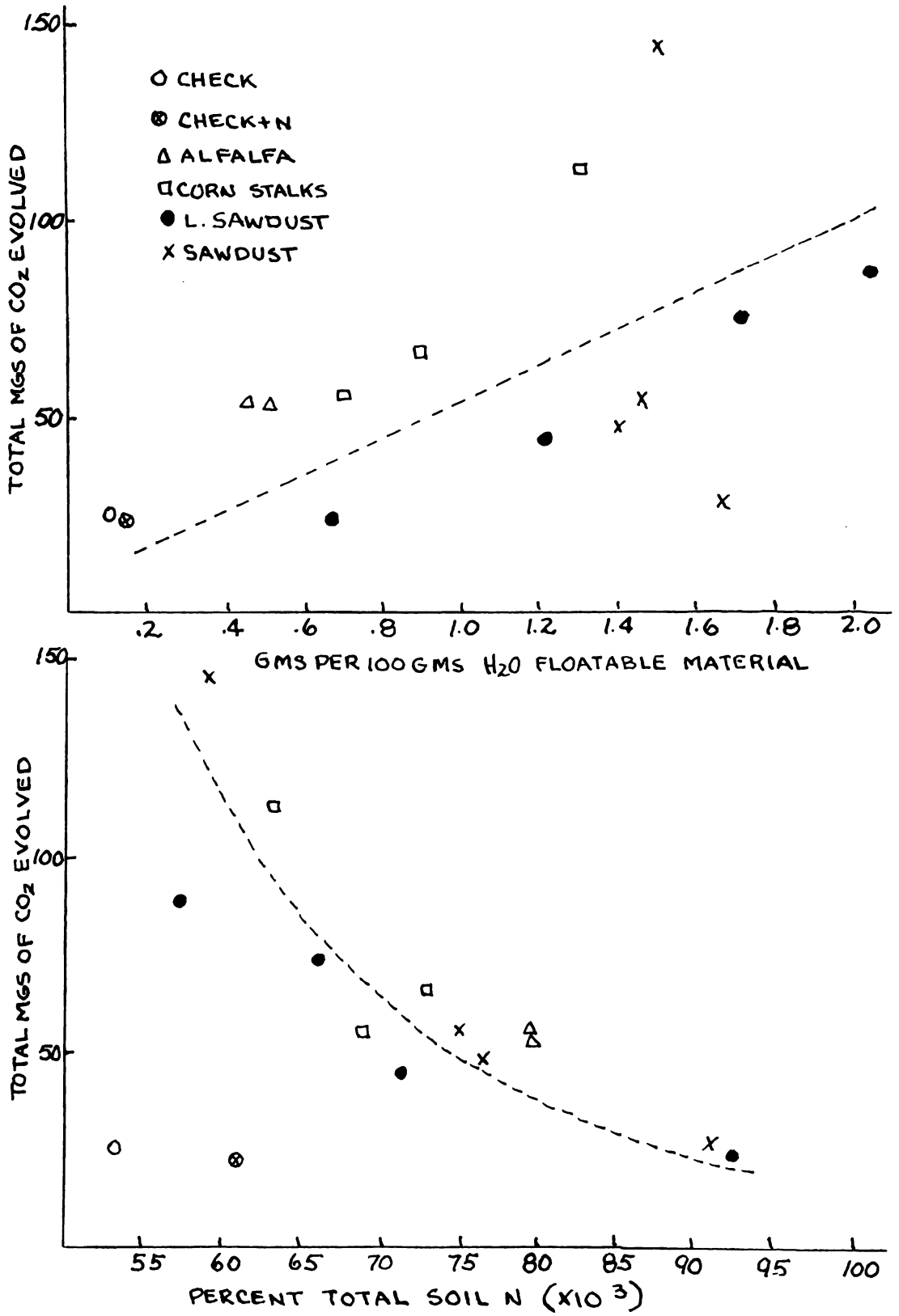
Table 12. - Total carbon and nitrogen and nitrate nitrogen in Oshtemo sand 40 weeks after incorporation of residues and their relation to the mineralization of carbon and nitrogen during incubation.

Treatment	Total C and N			Pounds per acre			
	C %	N %	C:N	Nitrate N	Nitrifiable N	Carbon mineralized	C:N of mineralization
				(1)	(2)	(3)	(4)
LS-W-N_0	1.38	.057	24.2	1	0	481	∞
LS-W-N_1	1.46	.066	22.2	32	35	409	14.3
LS-W-N_2	1.38	.072	19.0	79	37	242	6.5
LS-W_0-N_2	1.53	.093	16.5	510	19	131	6.9
SD-W-N_0	1.22	.059	20.7	0	0	790	∞
SD-W-N_1	1.11	.075	14.8	77	38	311	8.2
SD-W-N_2	1.11	.077	14.4	150	32	258	8.1
SD-W_0-N_2	1.18	.092	12.8	372	40	159	4.0
CS-W-N_0	1.02	.063	16.2	2	43	615	14.3
CS-W-N_2	1.00	.073	13.7	28	45	368	8.1
CS-W_0-N_2	1.03	.069	14.9	98	49	302	6.2
ALF-W-N_0	1.01	.079	12.8	136	58	310	5.3
ALF-W_0-N_0	1.00	.078	12.7	110	55	304	5.5
CK-W-N_0	.84	.053	15.8	42	27	142	5.3
CK-W-N	.78	.061	12.8	284	17	128	7.5

(1) Nitrate nitrogen in initial water extract of air dried soil.
(2) Nitrifiable nitrogen by incubation at 35°C for 14 days.
(3) Total carbon evolved as CO_2 in 10 days at 35°C.
(4) Ratio of carbon mineralized to nitrifiable nitrogen.

FIGURE 6

Comparison of carbon dioxide evolution for various soil residue treatments with water-floatable material and total nitrogen after 40 weeks' decomposition.


150
100
50
TOTAL MGS OF CO_2 EVOLVED
O CHECK
⊗ CHECK+N
Δ ALFALFA
□ CORN STALKS
● L. SAWDUST
X SAWDUST
.2
.4
.6
.8
1.0
1.2
1.4
1.6
1.8
2.0
GMS PER 100 GMS H_2O FLOATABLE MATERIAL
150
100
50
TOTAL MGS OF CO_2 EVOLVED
55
60
65
70
75
80
85
90
95
100
PERCENT TOTAL SOIL N ($X10^3$)

samples were accompanied by increases in non-acid-hydrolyzable nitrogen, although the rates of increase were not parallel. In the upper half of Figure 7, carbon mineralized during incubation is plotted against non-acid-hydrolyzable nitrogen. The values for lignified sawdust and sawdust fall together on a smooth curve. Those for corn stalks and alfalfa fall above this curve, an observation which appears to be related to the higher content of hydrolyzable amino nitrogen which was found in these samples (Fig. 3) and the greater availability of associated energy materials which may be inferred from the nature of the orininal materials themselves.

The significance to nitrogen mineralization of the close relationship observed for sawdust and lignified sawdust treatments between non-acid-hydrolyzable nitrogen and decomposability is suggested by the data plotted in the lower half of Figure 7. The mineralization of nitrogen during incubation was suppressed at low levels of non-acid-hydrolyzable hydrogen. This suppression was due to microbial immobilization in the presence of excess energy materials, as may be inferred from the fact that no supplemental nitrogen was applied with the corresponding sawdust and lignified sawdust treatments. Intense microbial immobilization of nitrogen may also be inferred from the high rate of CO_2 evolution at corresponding nitrogen levels in the upper graph.

Maximum mineralization of nitrogen in Figure 7 coincided with moderate levels of microbial activity and intermediate levels of acid-resistant nitrogen. Reduced mineralization of nitrogen at high levels of non-acid-hydrolyzable nitrogen was associated with a high degree of resistance to microbial attack, as reflected by the low rate of

CO_2 production in the upper graph.

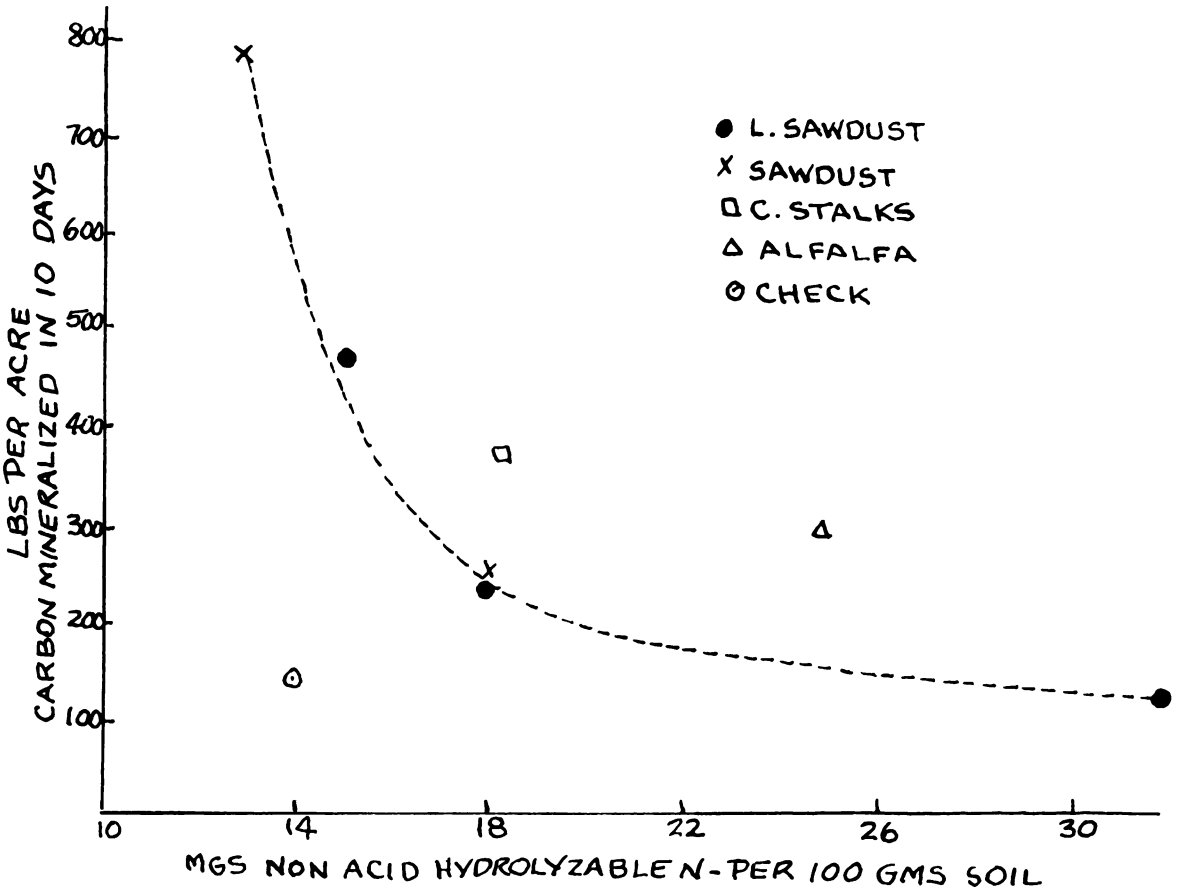
The curves in Figure 7 have been drawn to emphasize the ideal relationships which appear to be reflected by the data for sawdust and lignified sawdust treatments. The chemically resistant materials in soil do not appear to be inert. It is generally recognized that they represent products of complex formation, and that they may themselves represent active complexing agents. The data for the two sawdust materials in Figure 7 suggest that the complexing potential of materials resistant to acid hydrolysis may exert a strong controlling influence on the transformations of carbon and nitrogen in the soil.

The degree of control expressed by this fraction will vary with the quantity and nature of associated organic materials. This is shown by the fact that values in Figure 7 for the check soil and for soils treated with corn stalks and alfalfa do not fall on the same curve as those for the sawdust and lignified sawdust. The effects of associated materials, however, appear to resolve themselves primarily into differences in their availability as energy substrates for the soil microbial population. This point is brought out more clearly in Figure 8, where the carbon and nitrogen mineralization data are plotted against C:N ratio.

In the upper graph in Figure 8 it is seen that the mineralization of carbon was greater at any given C:N ratio for the sawdust treatment than for lignified sawdust. This would be expected since the more readily available energy materials had been removed from the latter by acid extraction. The residual materials from corn stalks and alfalfa were decomposed more rapidly than those from sawdust, reflecting the

FIGURE 7

Relation of non-acid-hydrolyzable nitrogen in the soil to the mineralization of carbon and nitrogen in Oshtemo sand.


LBS PER ACRE
CARBON MINERALIZED IN 10 DAYS
800
700
600
500
400
300
200
100
L. SAWDUST
SAWDUST
C. STALKS
ALFALFA
CHECK
10
14
18
22
26
30
MGS NON ACID HYDROLYZABLE N-PER 100 GMS SOIL

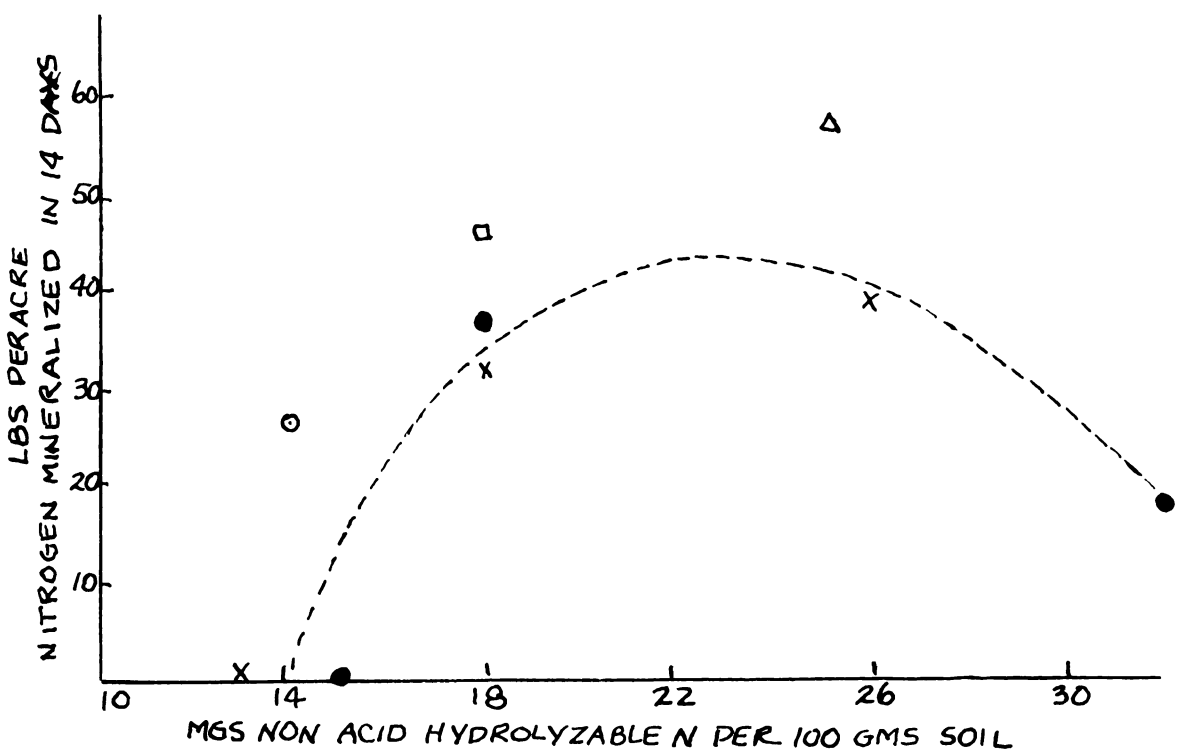
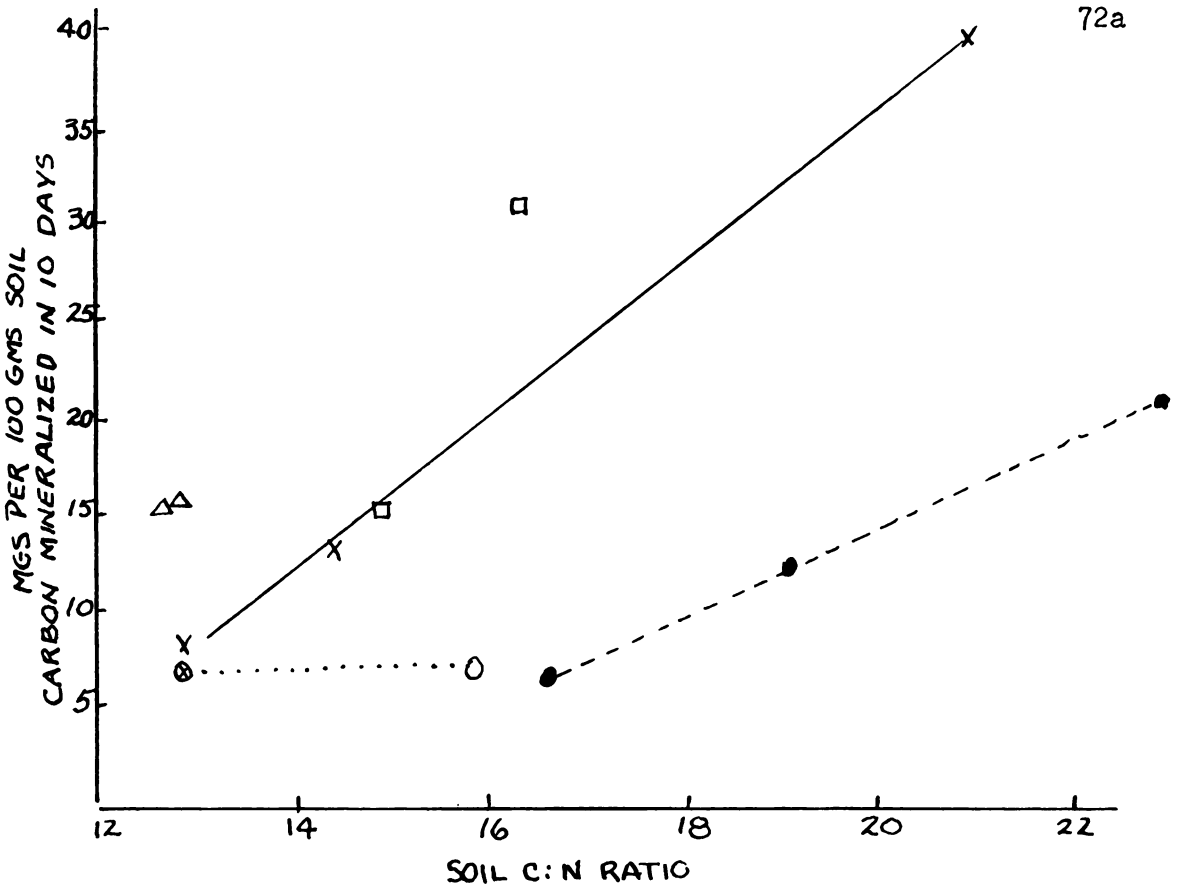
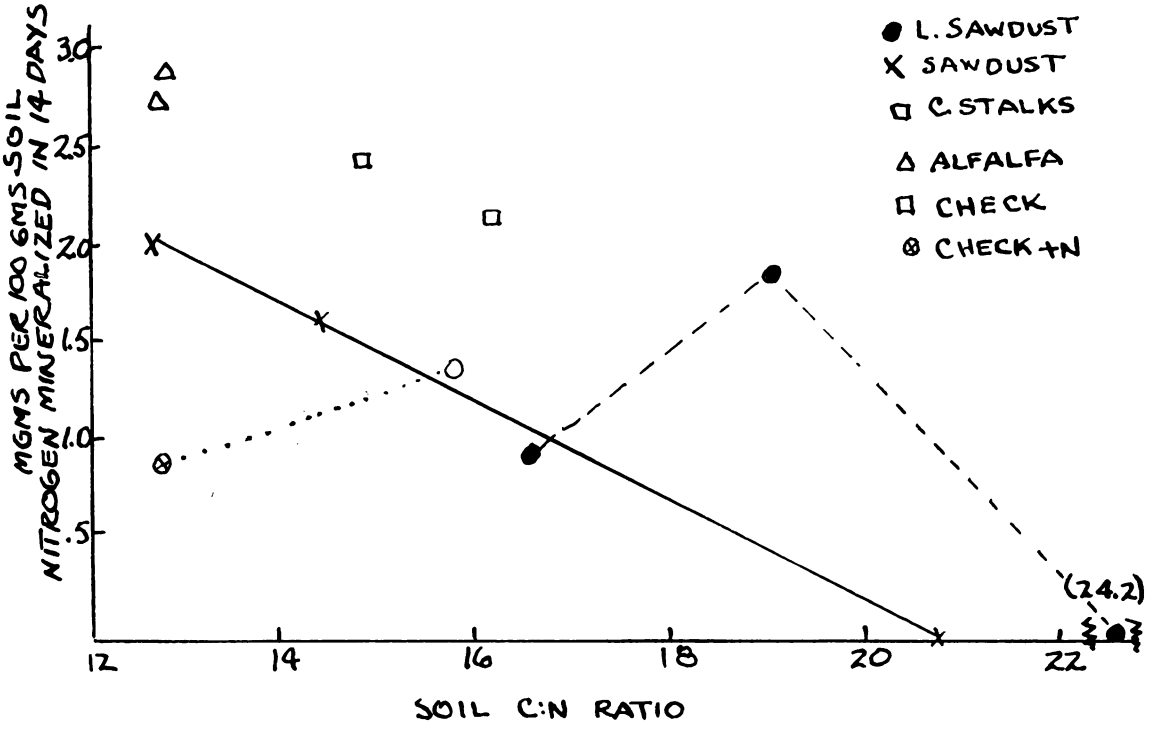

LBS PERACRE
NITROGEN MINERALIZED IN 14 DAYS
60
50
40
30
20
10
10
14
18
22
26
30
MGS NON ACID HYDROLYZABLE N PER 100 GMS SOIL

FIGURE 8

Carbon and nitrogen mineralized during incubation as related to soil C:N ratio and plant residues applied 40 weeks previously to Oshtemo sand.


MGS PER 100 GMS SOIL
CARBON MINERALIZED IN 10 DAYS
40
35
30
25
20
15
10
5
12
14
16
18
20
22
SOIL C:N RATIO


MGMS PER 100 GMS SOIL
NITROGEN MINERALIZED IN 14 DAYS
3.0
2.5
2.0
1.5
1.0
.5
L. SAWDUST
SAWDUST
C. STALKS
ALFALFA
CHECK
CHECK+N
(24.2)
12
14
16
18
20
22
SOIL C:N RATIO

normally greater decomposability of these plant materials, as well as the much higher hydrolyzable amino nitrogen content found in these soil samples.

The mineralization of nitrogen from sawdust treated soils (lower graph, Fig. 8) was inversely related to C:N ratio. This was true also for the alfalfa-and cornstalk-treated soils considered as a group, although the release of nitrogen was more rapid at a given C:N ratio for these than for the sawdust series. The release of nitrogen in these samples was inversely related to CO_2 evolution, or microbial activity. In other words, increasing rate of mineralization of nitrogen was due in part to decreasing intensity of microbial immobilization which was associated with decreasing C:N ratio.

The three lignified sawdust treatments and the checks showed a different behavior. Maximum mineralization occured in the lignified sawdust sample which had an intermediate C:N ratio and fell off sharply at lower and higher ratios. A heavy application (962 lbs per acre) of nitrogen to the check soil, which resulted in a narrower C:N ratio, also resulted in a reduced rate of nitrogen mineralization.

From the carbon mineralization data in the upper graph in Figure 8, it is seen that both the check and lignified sawdust treated soils contained smaller amounts of readily available energy materials at any given C:N ratio than the soils to which other organic amendments had been added. The decline in nitrogen mineralization rate following high levels of nitrogen amendment in these soils of low energy content is presumed to have been due to the formation of resistant chemical complexes of nitrogen with lignin or soil humic materials. As was

seen in Figure 5, the formation of such complexes was greatly increased in soils of low C:N ratio.

Numbers of bacteria and fungi

Periodically during the 40 week incubation period, plate counts for bacteria and fungi were taken to determine the effect of the various treatments on the soil microbial population. The results of these counts are tabulated in Table 22 of the Appendix. Data for representative treatments are presented graphically in Figure 9.

The counts shown in Figure 9 for the second week of decomposition were made on samples taken after the first crop of wheat was up in the pots in which wheat had been planted. Those for 15 and 25 weeks were taken after the harvest of the first and second wheat crops respectively. Consequently, the first three counts were influenced by the presence or absence of living roots and root residues from an associated wheat crop. All pots were uncropped during the period from 25 to 40 weeks, so the counts at 40 weeks reflect the residual effects of the initial treatments plus modifying residual effects of previous cropping as carried over a 15-week fallow period.

In Figure 9-A, it is seen that, where no nitrogen was applied, bacterial populations were consistently higher with lignified sawdust and sawdust than in the checks. Comparison with Figure 9-B shows that bacteria were three to ten times more numerous where alfalfa hay was incorporated than with the sawdust materials. These results are in keeping with recognized differences in decomposability, or energy-availability, of similar plant materials. These results have already

Figure 9. Effects of various organic amendments and nitrogen on numbers of bacteria and fungi over a 40 week period with and without cropping.*

A. Effects of lignified sawdust and sawdust without supplemental nitrogen on bacterial numbers compared with the unamended check with and without nitrogen.

B. Effects of alfalfa hay on bacterial numbers.

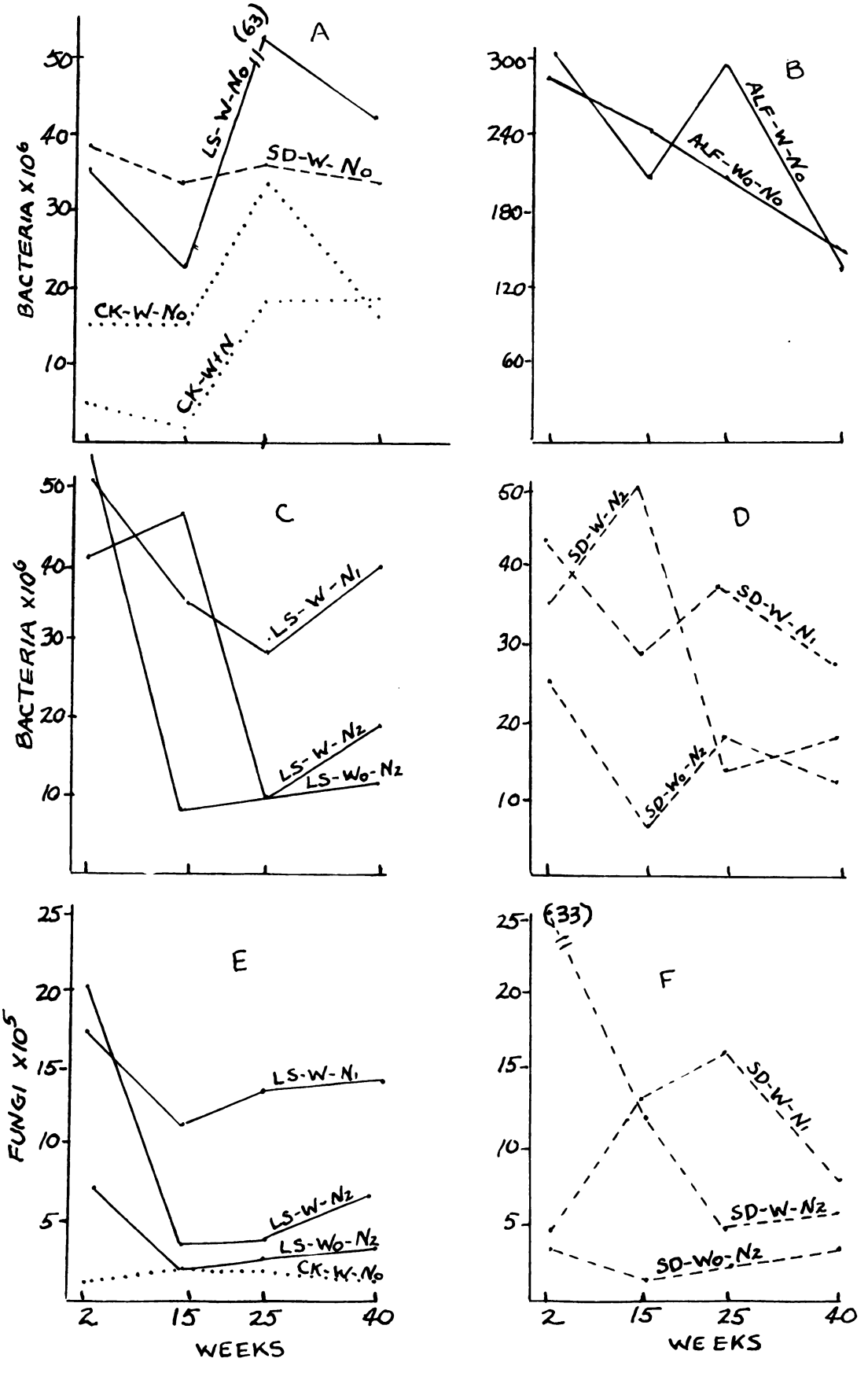
C. Effect of nitrogen level and crop on bacterial numbers in soil treated with lignified sawdust.

D. Effect of nitrogen level and crop on bacterial numbers in soil treated with sawdust.

E. Effects of nitrogen level and crop on fungal numbers in soil treated with lignified sawdust.

F. Effects of nitrogen level and crop on fungal numbers in soil treated with sawdust.

* All amendments were added at the rate of 2.5 grams per pot, or 25 tons per acre.


A
(63)
LS-W-No
SD-W-No
CK-W-No
CK-W-N
BACTERIA X10⁶
50
40
30
20
10
B
ALF-W-No
ALF-Wo-No
300
240
180
120
60
C
LS-W-N1
LS-W-N2
LS-Wo-N2
BACTERIA X10⁶
50
40
30
20
10
D
SD-W-N2
SD-W-N1
SD-Wo-N2
60
40
30
20
10
E
LS-W-N1
LS-W-N2
LS-Wo-N2
CK-W-No
FUNGI X10⁵
25
20
15
10
5
2
15
25
40
WEEKS
F
(33)
SD-W-N1
SD-W-N2
SD-Wo-N2
25
20
15
10
5
2
15
25
40
WEEKS

been alluded to in support of the conclusion that the acid-hydrolyzable amino nitrogen found in these soils after 40 weeks represented materials recently synthesized by soil microorganisms (page 56).

When a large amount (equivalent to 962 pounds per acre) of urea nitrogen was added to the check soil in Figure 9-A, bacterial numbers were depressed relative to the no-nitrogen check through the first three samplings. When nitrogen was added with lignified sawdust and sawdust (Figs. 9-C and 9-D), bacterial numbers in the first two samplings were generally larger than with the same materials at the N_o level of nitrogen treatment (Fig. 9-A). However, in the last two samplings, bacterial numbers were sharply depressed by the N_2 level of nitrogen treatment.

The same depressive effect of high nitrogen levels was expressed on the fungal population, as is shown in Figures 9-E and 9-F.

The low numbers of microorganisms which were found after 40 weeks in the lignified sawdust and sawdust treated soils following extremely high levels of nitrogen treatment correspond to the low rates of CO_2 evolution which were obtained during subsequent incubation of the same soils (Fig. 6). Thus it would appear that both microbial numbers and CO_2 evolution reflected the decreasing availability of energy materials which was associated with increasing levels of non-acid-hydrolyzable nitrogen (Fig. 7).

It may be argued that microbial numbers and activites in these samples were influenced by pH rather than by intrinsic differences in decomposability of residual energy substrates. It is true that pH levels in the soils which had received N_2 levels of nitrogen treatment

were low (Table 8). However, no smoothly regular relationship was found between CO_2 evolution and pH such as was found between CO_2 evolution and non-acid-hydrolyzable nitrogen with sawdust and lignified sawdust.

Levels of nitrate in the soil after 40 weeks were obviously important in determining the final pH of the variously treated soils. However, the pH at any given nitrate level was also influenced by plant origin of the organic amendments (see discussion on page 47). It must be assumed that nitrates were in equilibrium with a dynamic soil system in every case. Since nitrates in soil are the end products of a system of oxidative processes, their absolute level in the absence of cropping or leaching losses must reflect the degree of oxidation attained in the total system. A linear relationship was found between nitrates and non-acid-hydrolyzable nitrogen in these Oshtemo samples (Fig. 4). This resistant nitrogen fraction appears to have been the product of processes of complex formation involving lignin and ammonia, which are known to be oxidative in nature.

Thus, increasing levels of nitrate and non-acid hydrolyzable nitrogen both appear to have been the result of the increasing degree of oxidation of organic residues in these soils. Increasing levels of initially added nitrogen hastened the dissipation of masking high-energy materials and the subsequent oxidation of resistant lignaceous constituents. The associated decrease in availability of energy appears from this data to have been at least as significant a factor as pH in the decline in microbial numbers with progressive decomposition, as well as in the observed relationship between CO_2 evolution and

acid-resistant nitrogen.

Striking effects of cropping on microbial numbers at N_2 levels of nitrogen were observed (Fig. 9). Bacterial numbers were higher in the first sampling in the pots where wheat was grown in the presence of lignified sawdust at the N_2 level of nitrogen treatment than where no wheat was planted (Fig. 9-C). The same thing was true in the first two samplings from the corresponding sawdust treatments (Fig. 9-D). The behavior of fungi was essentially the same as for bacteria with both materials (Fig. 9-E and 9-F), except that numbers were increased by cropping to an even greater extent in the first sampling than with bacteria.

Three distinctly different effects of cropping may be inferred from the data at hand. As has been pointed out (P.48), a principle effect of the two wheat crops, in the case of sawdust and lignified sawdust treatments, was to reduce the level of mineral nitrogen in the soil. A part of this reduction would have resulted from uptake and removal of nitrogen in the harvested portions of the wheat. A part would have resulted from microbial immobilization in the presence of energy materials contributed to the soil in the form of root residues and, in the case of the growing crop, in the form of root secretions or abraded fragments.

The removal of mineral nitrogen by these two processes associated with the crop would have moderated the effects described earlier of nitrogen level on oxidative processes. A third effect of the crop is indicated by the magnitude of the stimulus to microbial numbers two weeks after planting of the first wheat crop. At this stage of

development the wheat could not have contributed greatly to the supply of energy materials in the soil. The effect here appears to have been of catalytic proportions.

Numerous heterotrophic organisms have been shown to require small amounts of certain amino acids, organic acids or vitamins as essential growth factors in their external environment (58). The supply of such growth factors would be extremely low in such materials as sawdust, and even lower after acid extraction as in lignified sawdust. On the other hand, it has been shown that a number of such compounds are secreted by living plant roots (29). The marked stimulus to microbial numbers of the first wheat crop two weeks after planting would appear to have been due to rhizosphere effects of such secretions.

Following sawdust treatments, this apparent rhizosphere effect was still in evidence prior to planting of the second wheat crop 13 weeks later (Figs. 9-D and 9-F). With lignified sawdust, however, it had completely disappeared by the time of this second sampling (Figs. 9-C and 9-E). Since most of the more readily available energy materials had been removed from the latter material by acid extraction, only a very transient benefit from catalytic root secretions would have been expected.

A final point of interest in Figure 9 is the fact that microbial numbers were better maintained to the end of the decomposition period where no nitrogen was used with sawdust and lignified sawdust than where high levels were employed. The high levels of energy materials indicated by bacterial numbers in these no-nitrogen samples was re-

flected again by their high rate of CO_2 evolution during the subsequent respiration experiment.

The distribution of nitrogen among hydrolytic fractions was essentially unaltered from that in the check by lignified sawdust or sawdust when no supplemental nitrogen was used (Fig. 3). This would imply that the larger numbers of bacteria found were the result of more rapid recycling of nitrogen contained in microbial cells, rather than by net increases in microbial proteins, since the latter would have resulted in increased amounts of amino nitrogen. Large changes in nitrogen distribution occured only when supplemental nitrogen was used. The data for microbial numbers and CO_2 evolution both point to rapid depletion of readily available energy materials and extensive oxidation of resistant lignin constituents as factors responsible for the increase in acid-resistant nitrogen when supplemental nitrogen was used with sawdust and lignified sawdust.

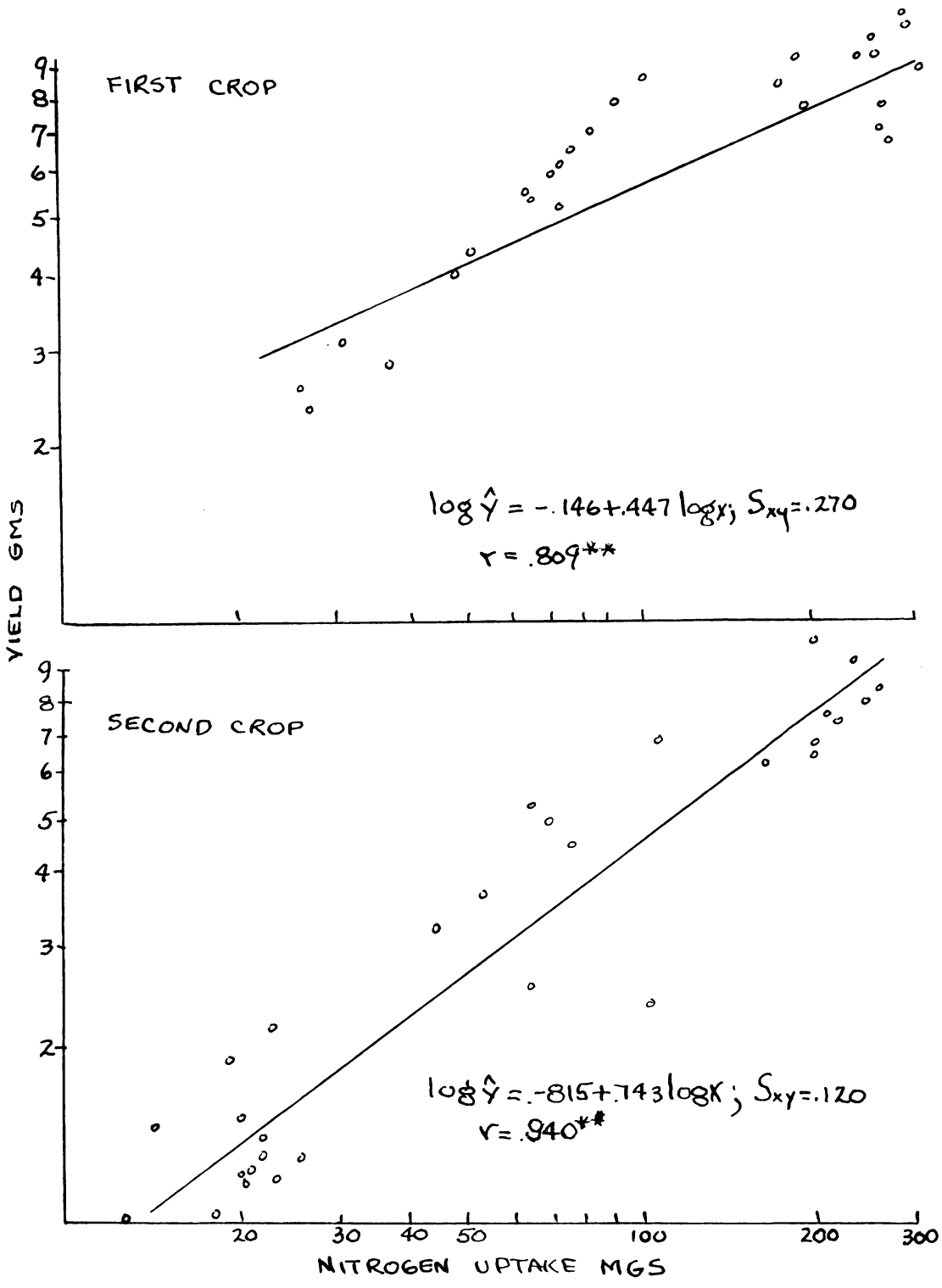
Nitrogen uptake and crop yields

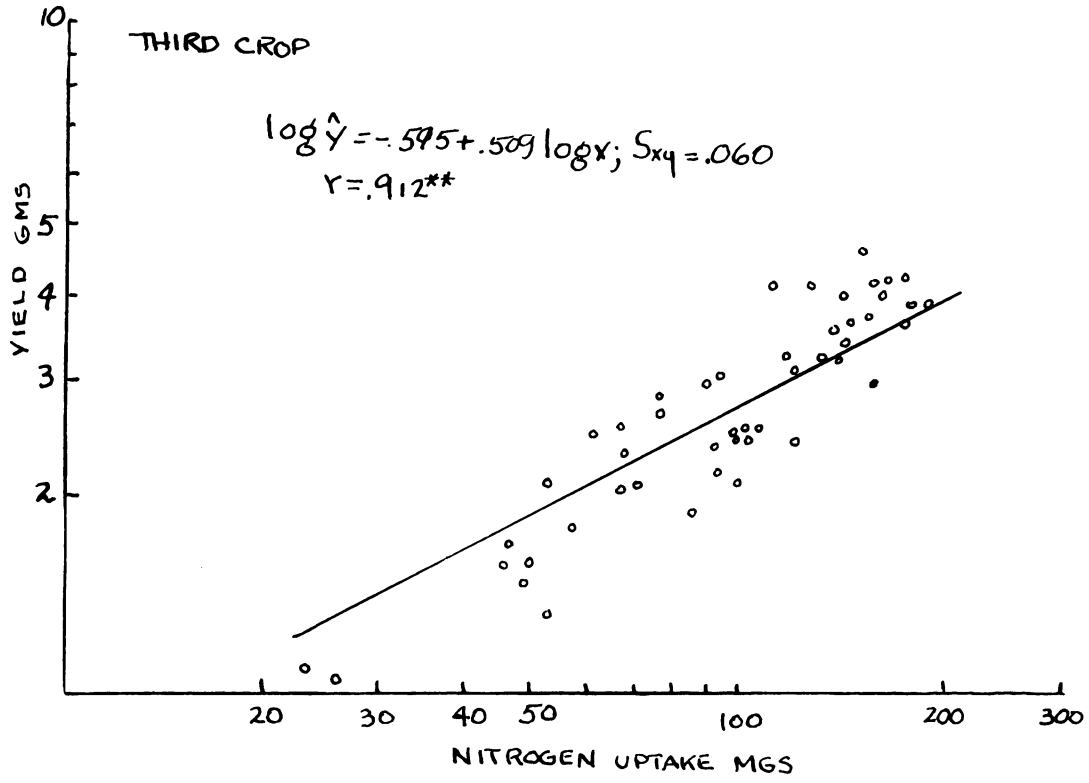
Yields and nitrogen uptake by three successive wheat crops are tabulated by greenhouse pot numbers in Table 18 of the Appendix. The treatments which correspond to each pot are described in Table 17.

The overall relationship between yields of wheat and nitrogen uptake is shown in Figure 10. The high correlations obtained for each crop indicate that nitrogen availability was a controlling factor in determining wheat yield in most pots. A number of divergent points plotted for each crop, however, suggest that other factors may have been operating also. This is further substantiated by the extremely

FIGURE 10

Correlation between yield and nitrogen uptake of three crops of wheat grown on Oshtemo sand in the greenhouse.


FIRST CROP
9
8
7
6
5
4
3
2
$\log \hat{Y} = -.146 + .447 \log x$; $S_{xy} = .270$
$r = .809^{**}$
SECOND CROP
$\log \hat{Y} = .815 + .743 \log x$; $S_{xy} = .120$
$r = .940^{**}$
YIELD GMS
20
30
40
50
100
200
300
NITROGEN UPTAKE MGS


THIRD CROP
$\log \hat{Y} = -.595 + .509 \log X$; $S_{xy} = .060$
$r = .912^{**}$
YIELD GMS
10
5
4
3
2
20
30
40
50
100
200
300
NITROGEN UPTAKE MGS

high nitrogen content (4.0 per cent or more) of first crop wheat plants grown at the high level of nitrogen in check pots and with lignified sawdust and sawdust, as well as of third crop wheat plants following all four materials at the high nitrogen levels, (Table 18, Appendix). Uniformly low to moderate N contents in the second crop plants indicate that available soil nitrogen was the factor principally controlling nitrogen uptake and yields at this stage of decomposition for all materials at both rates of addition and at all three nitrogen levels. The highest correlation coeficient ($r = .940$) was also obtained with this second crop.

Nitrogen taken up by the three wheat crops is plotted against level of nitrogen treatment for four organic amendments in Figure 11. The actual amounts of urea nitrogen used and a key to the various treatments is presented in Table 13.

Three points which are relevant to discussions in previous chapters are made with respect to the data plotted in Figure 11:

1. When no supplemental nitrogen was used, lignified sawdust and sawdust depressed nitrogen uptake to very low levels in all three crops. Straw and corn stalks depressed nitrogen uptake in the first two crops, but in the third crop there was a significant release of nitrogen to the wheat. The data on microbial numbers and CO_2 evolution have shown that suppressed nitrogen availability where no nitrogen was used was due to microbial immobilization. The longer duration of suppression with sawdust and lignified sawdust was due to their lower nitrogen content and the lower decomposability of their cellulosic and lignaceous constituents. Energy materials were dissipated less

Table 13. - Key to organic amendments and nitrogen treatments for which nitrogen uptake is plotted in Figure 11.

Organic Amendment	Pounds N per acre (applied as urea)			
	L*		H*	
	N_1 **	N_2 **	N_1 **	N_2 **
Lignified sawdust (LS)	321	962	641	1,923
Sawdust (SD)	191	589	393	1,178
Corn stalks (CS)	63	190	126	379
Wheat straw (ST)	32	110	73	219
Check (CK)	None			
Check + N (CK+N)	962			

* L = Organic amendment applied at $12\frac{1}{2}$ tons per acre.
H = Organic amendment applied at 25 tons per acre.

** N_1 = Intermediate C:N ratio (different for each material).

N_2 = Lowest C:N ratio (different for each material).

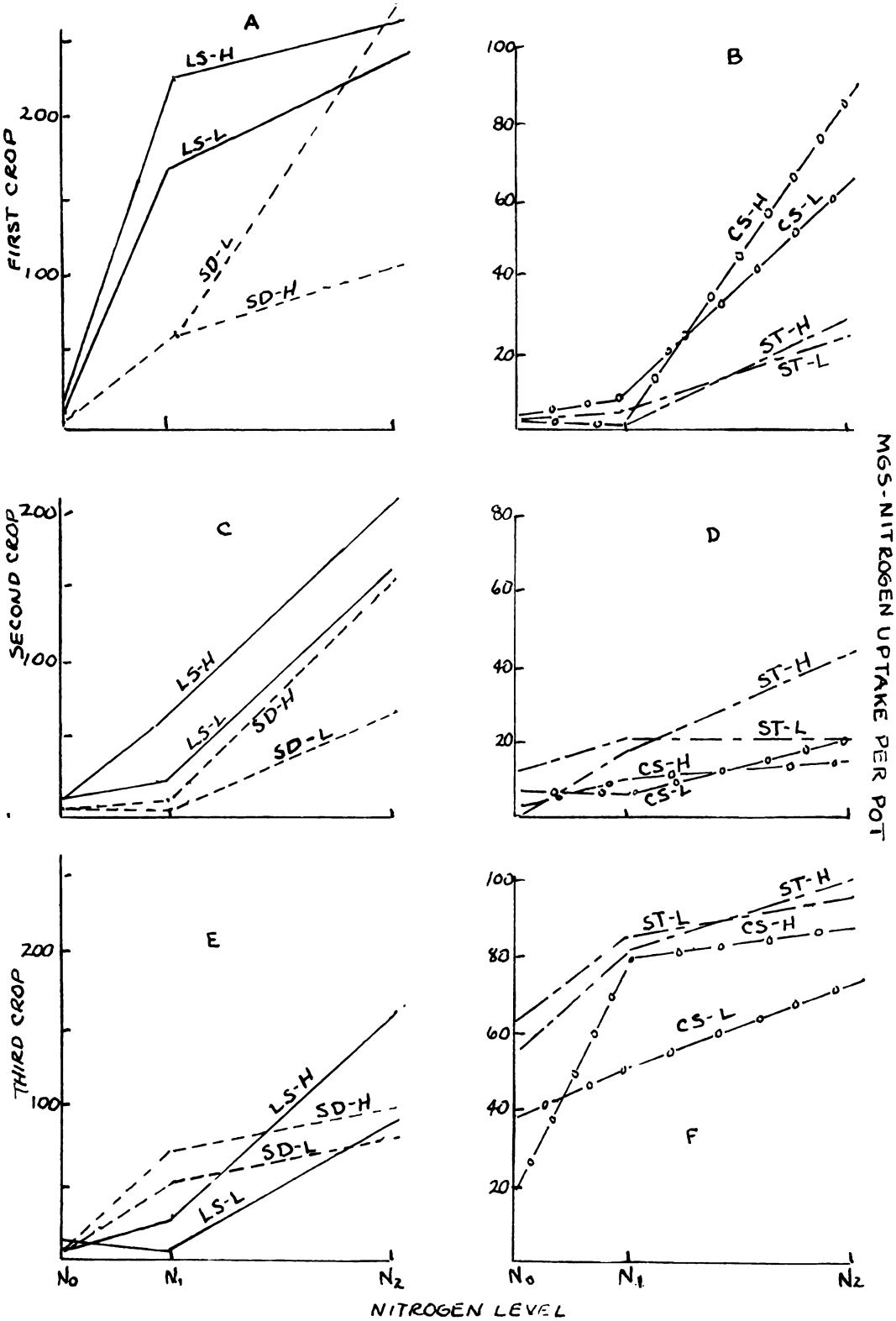
N_o = No supplemental nitrogen used.

FIGURE 11

Total nitrogen taken up by three crops of wheat following treatment of an Oshtemo sand with organic amendments and nitrogen according to the schedule presented in Table 13.*

* Note: Nitrogen uptake for check pots with and without nitrogen were as follows:

	1st crop mgs	2nd crop mgs	3rd crop mgs
CK	83.5	17.5	38.0
CK+N	22.5	246.5	117.5


A
LS-H
LS-L
SD-L
SD-H
FIRST CROP
200
100
B
100
80
60
40
20
CS-H
CS-L
ST-H
ST-L
C
SECOND CROP
200
100
LS-H
LS-L
SD-H
SD-L
D
80
60
40
20
ST-H
ST-L
CS-H
CS-L
E
THIRD CROP
200
100
LS-H
SD-H
SD-L
LS-L
F
100
80
60
40
20
ST-H
ST-L
CS-H
CS-L
N0
N1
N2
NITROGEN LEVEL
MGS-NITROGEN UPTAKE PER POT

rapidly and microbial numbers were better maintained throughout the decomposition period than in the case of the other materials.

2. When high rates of nitrogen were used with these four materials, nitrogen availability reached a minimum in the second crop with corn stalks and straw, but continued to decline in availability through the third crop with lignified sawdust and sawdust. The data on microgial numbers and CO_2 evolution showed that available energy substrates in sawdust and lignified sawdust at the high rate of nitrogen treatment had been largely dissipated by the time the third wheat crop was planted. Nitrogen immobilization at this time was principally chemical rather than microbial, as shown by the associated increases in non-acid-hydrolyzable nitrogen.

3. Indirect evidence for the great capacity of lignin to take up ammonia chemically is to be found in the immediate effect of lignified sawdust on nitrogen uptake in the first crop of wheat. Nitrogen added to the check soil at a rate equivalent to 962 pounds per acre was extremely toxic to the wheat plants, - yield of dry matter was reduced from 7.3 grams per pot to 0.6 grams, and nitrogen uptake was reduced from 83.5 to 22.5 mgms. When the same amount of nitrogen was added with 12.5 tons per acre of lignigied sawdust, nitrogen uptake was increased threefold to 247.5 mgms, although dry matter yield was still slightly reduced to 6.6 grams per pot. This protective action was equally effective when the added amounts of both lignified sawdust and urea nitrogen were doubled to 25 tons and 1,923 pounds per acre, respectively.

The high buffering capacity of lignin with respect to ammonia

was also reflected in pH levels two weeks after treatments were applied (Table 8). The high degree of availability of the sorbed ammonia to the first wheat crop indicates that initial fixation of ammonia by lignin occurred by action of relatively weak physical forces. The early drop in microbial numbers (Fig. 9), and the progressive decline in nitrogen availability where lignified sawdust was added with large amounts of nitrogen (Fig. 11) both point to the probability that nitrogen sorbed by lignin is rather quickly fixed by chemical forces which arise by oxidation.

The third crop of wheat was planted primarily as a biological assay agent to evaluate the effects of the various treatments on nitrogen availability at the end of the 40-week decomposition period. Attempts to relate nitrogen uptake in this third crop directly with original treatment showed a generally direct relationship between uptake and the total of nitrogen added in residues plus urea. However, the response to increasing rates of individual organic amendments was highly erratic and showed no consistent similarity in slope to the general response curve.

Accordingly, a detailed study was made of representative treatments for which complete chemical data was available from soil samples taken just before planting of the third wheat crop. When nitrogen uptake was plotted against soil C:N ratio, distinctly separate curves were described by the values for cropped samples of several organic treatments, as is shown in Figure 12. The points for uncropped (W_o) samples failed to conform to the functions described by the values for cropped (W) soils. If the data plotted in this figure are compared

with the amounts of nitrogen originally added (Table 13), it will be seen that different plant materials differentially altered the residual availability of fertilizer nitrogen and that these effects were further modified by cropping. The fact that equivalent levels of nitrogen availability occured at widely different soil C:N ratios following different organic amendments provides an explanation for the failure of numerous reported attempts to relate the nitrogen supplying power of soils to their C:N ratio.

Incubation mineralization rates for nitrogen showed wide differences at a given C:N ratio for different materials in these same soil samples (Fig. 8). However, no correlation at all was found between the incubation tests for nitrifiable nitrogen and uptake of nitrogen by the third crop of wheat. This was not surprising, since most soils contained moderately to extremely large amounts of water soluble nitrates at this time. Figure 13 shows that nitrogen uptake was closely related to water-soluble nitrates in all but four of the soils in this group of treatments.

When nitrogen uptake was plotted against the combined total of water-soluble nitrates and nitrifiable nitrogen, all but two of the cropped (W) soils gave values which fell along a smooth curve, shown in Figure 14. There was an inflexion in the curve about the value for the no-nitrogen check. The four soils below the check on this curve represent treatments in which extensive microbial immobilization may be inferred from the data on microbial numbers (Fig. 9) from CO_2 evolution data (Table 12) and from crop yield data (Fig. 11). Thus, the shape of this lower portion of the curve appears to reflect the

FIGURE 12

Nitrogen uptake by wheat as related to soil C:N ratio and various organic amendments, supplemental nitrogen treatment and previous cropping. (Oshtemo sand)

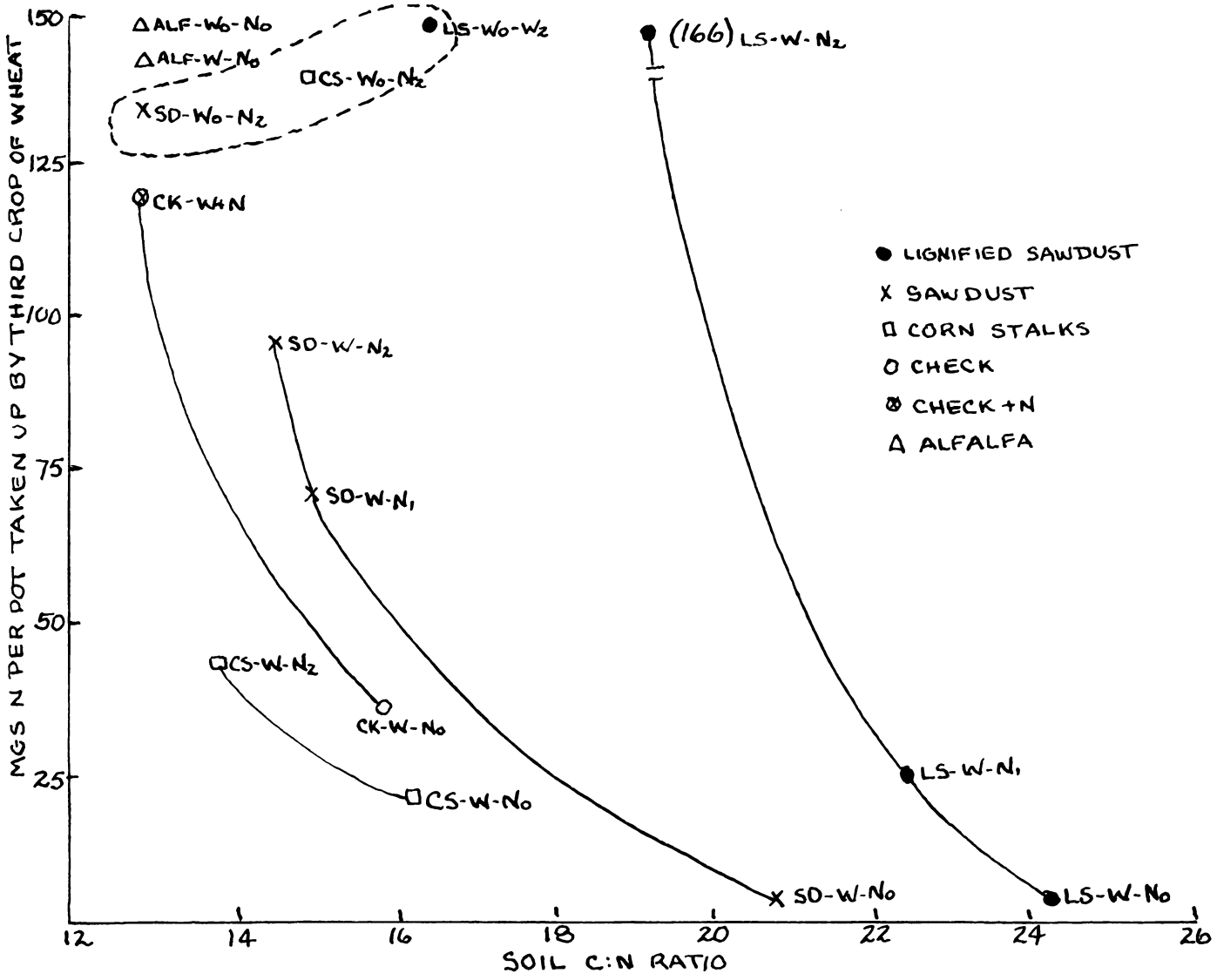

150
125
100
75
50
25
MGS N PER POT TAKEN UP BY THIRD CROP OF WHEAT
ΔALF-W0-N0
LS-W0-W2
ΔALF-W-N0
CS-W0-N2
XSD-W0-N2
(166) LS-W-N2
CK-W+N
XSD-W-N2
XSD-W-N1
CS-W-N2
CK-W-N0
CS-W-N0
XSD-W-N0
LS-W-N1
LS-W-N0
LIGNIFIED SAWDUST
X SAWDUST
CORN STALKS
O CHECK
CHECK+N
Δ ALFALFA
12
14
16
18
20
22
24
26
SOIL C:N RATIO

FIGURE 13

Nitrogen uptake of wheat as related to water-soluble nitrate in the soil at planting time. (Oshtemo sand)

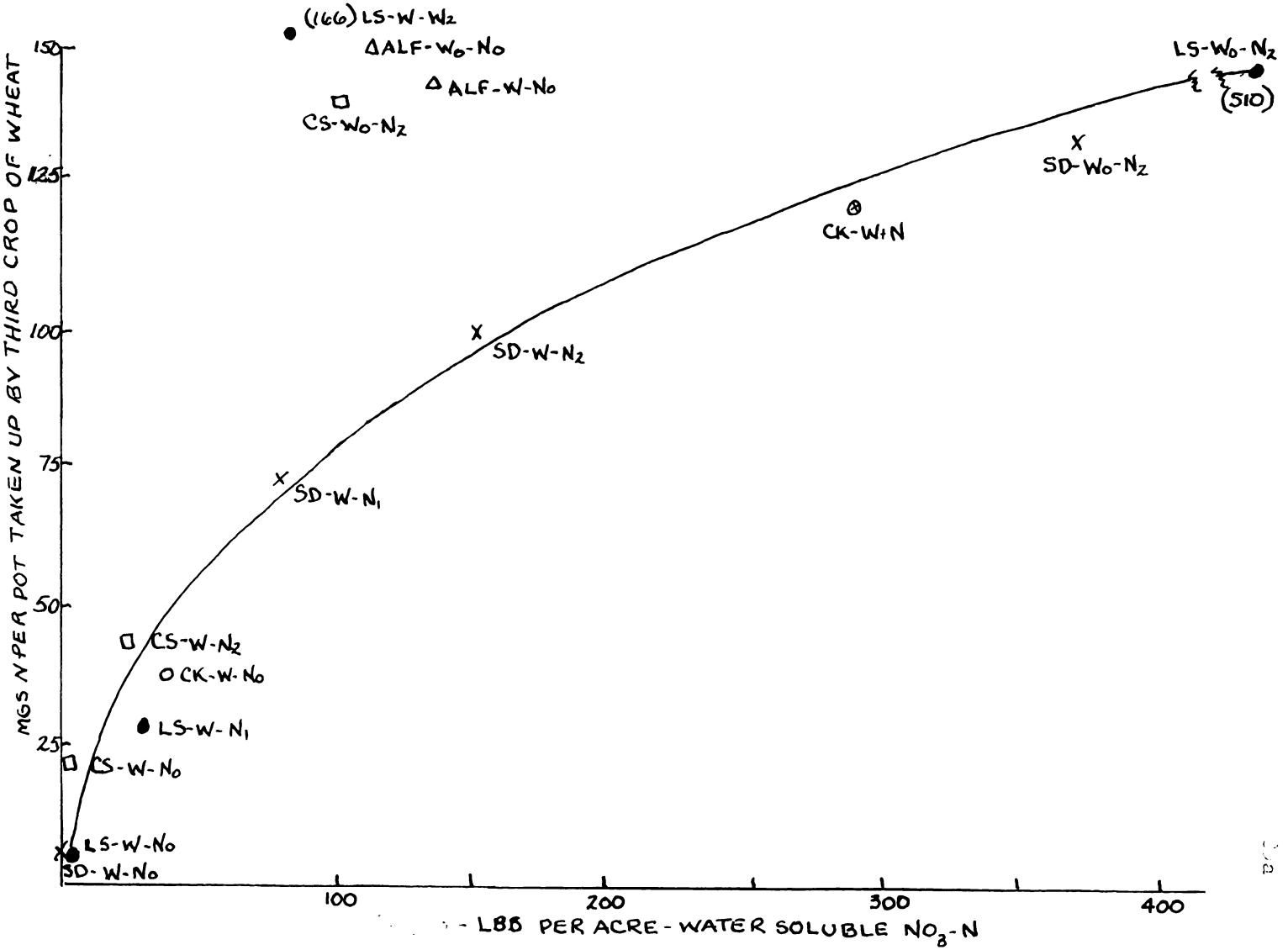
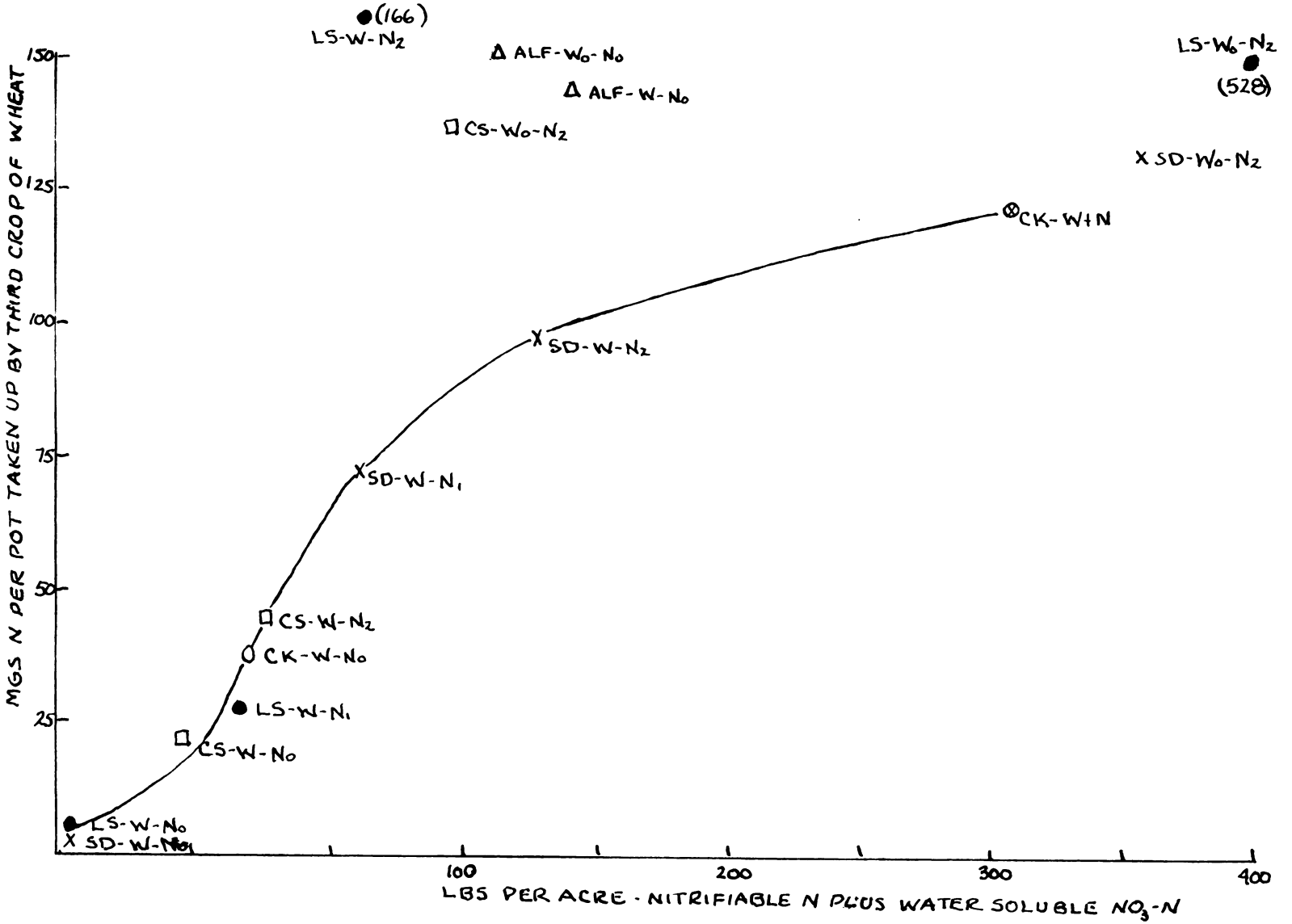

(166) LS-W-W2
ΔALF-W0-N0
Δ ALF-W-N0
CS-W0-N2
LS-W0-N2
(510)
X
SD-W0-N2
⊗
CK-W1N
X
SD-W-N2
X
SD-W-N1
CS-W-N2
O CK-W-N0
LS-W-N1
CS-W-N0
LS-W-N0
SD-W-N0
MGS N PER POT TAKEN UP BY THIRD CROP OF WHEAT
150
125
100
75
50
25
100
200
300
400
- LBS PER ACRE - WATER SOLUBLE NO3-N

FIGURE 14

Nitrogen uptake by wheat as related to the sum of water soluble nitrate in the soil at planting time plus nitrifiable nitrogen released as nitrate during 14 days' incubation at 35°C.


●(166)
LS-W-N2
Δ ALF-W0-N0
Δ ALF-W-N0
LS-W0-N2
●
(528)
□ CS-W0-N2
X SD-W0-N2
⊗CK-W+N
X SD-W-N2
X SD-W-N1
□ CS-W-N2
○ CK-W-N0
● LS-W-N1
□ CS-W-N0
● LS-W-N0
X SD-W-N0
150
125
100
75
50
25
100
200
300
400
MGS N PER POT TAKEN UP BY THIRD CROP OF WHEAT
LBS PER ACRE - NITRIFIABLE N PLUS WATER SOLUBLE NO3-N

declining immobilization potential of energy substrates remaining in these soils in excess of the check, 40 weeks after treatment. The portion of the curve above the check appears to be essentially a normal response curve to increasing increments of nitrate nitrogen.

In six of the soils in Figure 14, nitrogen was taken up in excess of the rate function described by the majority of cropped soils. Included in this six were the uncropped soils treated with each of the four materials at the highest level of nitrogen treatment. From the data on microbial numbers and CO_2 evolution it was seen that microbial immobilization of nitrogen was less extensive where energy supplies were not augmented by root residues during the decomposition period. This may have been a factor in the abnormally high rate of nitrogen uptake from previously uncropped soils.

However, very low levels of microbial activity were associated with reduced rates of nitrogen mineralization during incubation. This reduction in mineralization appeared to be due to the high degree of resistance to microbial attack of nitrogen in non-acid-hydrolyzable forms. Four of the aberrant soils in Figure 14 were found to be unusually high in non-acid-hydrolyzable nitrogen (Fig. 3). Low availability to microorganisms of nitrogen in resistant forms was associated with an abnormally high rate of release of nitrate to wheat in the same soils. Apparently the availability of this resistant nitrogen was enhanced by a rhizosphere effect associated with the growing wheat crop.

In field application, soil samples are usually taken in the fall or winter and it is assumed that nitrates already present will be re-

moved by leaching before the next crop, for which fertilizer recommendations are to be made, is planted. Nonetheless, it does appear that nitrifiable nitrogen determined by incubation procedures is subject to numerous unpredictable factors related to previous treatment and cropping which seriously prejudice its use as a sole basis for predicting nitrogen fertilizer needs. The irregular behavior of alfalfa treatments in Figure 14 is of interest in this regard. In Iowa it has been found that predictable relationships between the incubation test for nitrate and corn yields can only be obtained when corn follows a nonlegume in the rotation. The test does not reveal the high nitrogen supplying potential of an immediately preceding legume (34).

Residual effects of alfalfa and wheat on nitrogen taken up by a succeeding wheat crop

To test the effect of sawdust and lignified sawdust treatments on growth and nitrogen uptake of a legume, alfalfa was grown in soil treated with these materials. Oshtemo sand was used with the additional nutrients previously described. The rates of addition of sawdust materials and nitrogen are shown in Table 14. Also shown are the total yield and nitrogen uptake for three cuttings of alfalfa taken during the first 40 weeks of decomposition and for two crops of wheat grown in a parallel series of pots during the same period. The residual effects of treatment and previous crop on yield, nitrogen uptake and percentage nitrogen content of dry matter of wheat planted 40 weeks after initial treatment are shown in the last three columns of Table 14.

Table 14. - Yield and nitrogen uptake of two crops of wheat and three cuttings of alfalfa grown during the first 40 weeks following treatment of Oshtemo sand with sawdust and lignified sawdust, and the residual effects on yield and nitrogen uptake of a succeeding wheat crop.

Treatment			First 40 weeks			Residual response of wheat planted after 40 weeks		
Organic amendment	Rate	Nitrogen added	Crop grown	Yield	Nitrogen uptake	Yield	Nitrogen uptake	% N in tissue
	gm*	gm*		gm**	mgm	gm***	mgm***	
Control		0	Wheat	8.90	102	1.52	38	2.45
"		1.923	"	8.95	270	2.71	118	4.36
Sawdust	50	0	Wheat	.65	6	.69	15	2.18
"	50	.392	"	5.83	73	1.88	59	3.12
"	50	0	Alfalfa	8.21	216	2.03	49	2.42
"	50	.392	"	8.30	246	2.96	95	3.21
Sawdust	100	0	Wheat	.33	3	.19	4	1.81
"	100	.785	"	5.14	71	2.36	73	3.03
"	100	0	Alfalfa	7.73	201	.97	23	2.38
"	100	.785	"	5.99	172	2.99	88	2.94
Lignified Sawdust	50	0	Wheat	.79	11	1.08	20	1.87
"	50	.641	"	11.24	203	.77	19	2.48
"	50	0	Alfalfa	8.96	278	2.29	58	2.53
"	50	1.281 #	"	11.82	378	3.37	96	2.85
Lignified Sawdust	100	0	Wheat	1.24	11	.22	4	1.57
"	100	1.281	"	14.80	311	.86	27	2.91
"	100	0	Alfalfa	8.40	237	2.11	54	2.56
"	100	.641 #	"	10.98	321	3.11	107	3.44

* Grams per 4,000 grams of soil.

** Total dry matter from 2 crops for wheat and 3 cuttings for alfalfa.

*** Dry matter produced and nitrogen taken up in 8 weeks.

\# Rates of nitrogen were inadvertently reversed for these two treatments at the time the soils were treated.

Where no nitrogen was used, total yield and nitrogen uptake of the two crops of wheat grown during the first 40 weeks were severely curtailed by sawdust and lignified sawdust treatments. By contrast, the dry matter produced and the nitrogen taken up by three cuttings of alfalfa during the same period in the presence of these materials without added nitrogen compared favorably with dry matter yield and nitrogen taken up by wheat on the control soil to which supplemental nitrogen had been applied.

The addition of nitrogen with lignified sawdust increased yields and nitrogen uptake of alfalfa. With the higher level of raw sawdust the addition of nitrogen depressed alfalfa yield and nitrogen uptake. Presumably this reflects the competitive microbial tie-up in the presence of sawdust of some other nutrient, probably phosphorus, since the results with and without nitrogen at the low level of sawdust addition indicate that the ability of alfalfa to fix atmospheric nitrogen was not greatly impared by the presence in the soil of rapidily decomposing sawdust.

It may be assumed that nitrogen taken up by wheat during the first 40 weeks represented the actual availability of nitrogen from soil sources during this period, particularly where no nitrogen was used in conjunction with the organic amendments. Nitrogen taken up by alfalfa in excess of this may be ascribed to symbiotic fixation from the atmosphere. On this basis, alfalfa fixed 204 mgs of nitrogen as an average for the two levels of sawdust treatment without supplemental nitrogen and 247 mgs as an average for the two levels of lignified sawdust. On an acre basis, these would have been equivalent to

102 and 123 pounds per acre of nitrogen fixed during this 40-week period. This compares favorably with reported annual rates of nitrogen fixation by alfalfa in field and greenhouse studies (88). The higher figure for the lignified sawdust reflects the less intense microbial competition for nitrogen and other nutrients in the presence of this relatively refractory lignaceous material, as well as the greater availability of ammonia nitrogen which was presumably held on the lignaceous complexes by base exchange forces during the early stages of decomposition. These results were paralled by those for wheat, since the highest yields and nitrogen uptake for any treatment during this period were achieved where wheat was grown with supplemental nitrogen in pots treated with lignified sawdust.

The residual availability after 40 weeks of applied nitrogen in the control soils was high. This is shown in Table 14 by the fact that 118 mgs of nitrogen was taken up from the nitrogen treated control by the third wheat crop to produce tissue containing 4.36 percent nitrogen. These values were the highest for any treatment.

Where this third crop of wheat followed wheat grown in pots treated with sawdust or lignified sawdust without nitrogen, the availability of nitrogen was about as low as it had been in the case of the first two crops. Where supplemental nitrogen had been added with these materials before the first two wheat crops, the residual availability of nitrogen to the third crop was greatly increased in the case of sawdust but not in the case of lignified sawdust. It is evident that rapid dissipation of energy materials in the sawdust in the presence of supplemental nitrogen had shortened the period of intense microbial immobilization and that nitrogen from dead cells

left by a declining microbial population was being mineralized and was contributing to the available supply after 40 weeks. The low residual availability of nitrogen with lignified sawdust appears to be related to the fact that maximum yields of dry matter had been produced in the two proceeding crops of wheat with this treatment. Maximum quantities of root residues would have been added to the soil and microbial immobilization at the time of the third crop would have been intensified relative to the comparable sawdust treatments.

Where no nitrogen was used with the two sawdust materials and alfalfa was grown during the first ten months, a large residual benefit to the following wheat crop from alfalfa was observed in all cases except the high rate of sawdust application. That nitrogen was still limiting with this treatment is indicated by the fact that only a moderate level of nitrogen was found in the harvested wheat tissue (2.38 percent). That the growing alfalfa had contributed to some extent to the more rapid decomposition of the sawdust is shown by the fact that nitrogen uptake by the third wheat crop was greater than where wheat had been grown during the first 40 weeks without nitrogen and in sawdust treated pots. However, at the higher rate of application, nitrogen from alfalfa was inadequate to promote maximum decomposition of energy materials. As a result, microbial immobilization was still a dominant factor in nitrogen availability after 40 weeks.

Where nitrogen had been applied in the beginning, the maximum residual benefit from this applied nitrogen in terms of wheat yields was found where alfalfa was grown during the first ten months in pots treated with lignified sawdust. The residual benefit was less

where sawdust had been used, but it was still greater after alfalfa than after wheat.

Although wheat yields were greater after alfalfa with nitrogen treated organic amendments than after wheat in the nitrogen treated control, nitrogen uptake and nitrogen content of wheat tissue was less. The extremely high nitrogen content of wheat from the nitrogen treated control suggests that the nitrogen level in the soil was excessively high and that a toxic effect may have limited yields with this treatment. Certainly an excessive amount of nitrogen was used (962 pounds per acre), and a distinctly toxic effect was observed on the first crop of wheat (Fig. 11). The highly beneficial residual effects of lignified sawdust observed in Table 14 where nitrogen was applied in the beginning at rates of .641 and 1.281 gms per pot (320 and 640 pounds per acre) may be interpreted in part as a protection against excessive nitrogen concentrations afforded by the buffering action of the lignin.

The results with sawdust show that the injurious effects of massive additions of such low nitrogen materials can be materially reduced if sufficient nitrogen is used. They show, further, that such corrective fertilizer treatment may be much more effective if combined with the planting of a strong legume such as alfalfa. The broadcasting of carbonaceous materials such as sawdust on established stands of alfalfa suggests itself as a practical means for handling such materials. This would minimize the economic losses which occur due to microbial immobilization of nitrogen when non-legumes are planted after incorporation of residues low in nitrogen. If organic materials high

in lignaceous constituents are used, the favorable effects of lignin which were observed here in the case of the acid-extracted sawdust may enhance the residual benefits from their use.

SUMMARY AND CONCLUSIONS: PART ONE

Mechanisms of Nitrogen Immobilization

Two distinct mechanisms were observed whereby nitrogen was immobilized in organic combinations in soils to which plant materials were added:

A. During the early stages of decomposition, immobilization by microbial assimilation was a dominant process. In field and greenhouse experiments the intensity of microbial immobilization was reflected in crop yields, microbial numbers, mineralization of carbon and nitrogen in incubated soils, or in the level of amino nitrogen in acid hydrolysates.

B. At advanced stages of decomposition, moderate to large increases in the soil nitrogen fraction which was not hydrolyzed by acid were observed in both soils. At the end of a 40-week decomposition period in the greenhouse experiment, the level of non-acid-hydrolyzable nitrogen increased as a continuous geometric function of decreasing soil C:N ratio. The curves for soils with different organic amendments were different. For any given soil C:N ratio, much larger quantities of acid-resistant nitrogen were found following the addition of sawdust or acid-extracted ("lignified") sawdust than with the more readily decomposed materials.

The curves for sawdust and lignified sawdust were congruent, which indicated that the acid-resistant nitrogen found was associated with a constituent common to both materials and presumed to be lignin. The lignin content of hardwood sawdust is known to be higher than in

corn stalks. These and other considerations lead to the conclusion that increases in non-acid-hydrolyzable nitrogen observed in the greenhouse samples were due to chemical fixation of ammonia and proteinaceous nitrogen compounds by lignin or lignaceous constituents of the organic amendments.

Similar increases in non-acid-hydrolyzable nitrogen were observed in soil samples taken three and five years after addition of sawdust in the field experiment and were similarly ascribed to the formation of chemical complexes with lignin.

Factors Affecting Microbial Immobilization

The intensity of microbial immobilization was directly related to the quantity and availability of energy materials contained in the various organic amendments or in their residues at various stages of decomposition. The intensity of microbial immobilization was also increased by the addition of nitrogen in high nitrogen materials or as supplemental nitrogen with materials low in contained nitrogen. This latter effect was reflected in increased microbial numbers or increased quantities of amino nitrogen recovered in acid hydrolysates, even though it was rarely reflected in reduced crop yields at the abnormally high rates of supplemental nitrogen which were used.

The length of time over which intense microbial immobilization occurred following the addition of various organic amendments was inversely related to the availability of energy substrates contained in the original materials. Effects of nitrogen on the duration of microbial immobilization were difficult to separate from its effect on

chemical fixation. However, an earlier decline in microbial numbers where nitrogen was added with residues low in contained nitrogen indicated an inverse relation between level of nitrogen treatment and the duration of intense microbial immobilization.

The presence of a young, growing wheat crop stimulated microbial assimilation of nitrogen, as reflected in greatly increased microbial numbers two weeks after the start of the greenhouse experiment. This rhizosphere effect was of catalytic proportions. Microbial numbers were maintained at higher levels in cropped than in uncropped soils all through the decomposition period by reason of energy materials contributed by root residues from two successive wheat crops. The enhanced microbial immobilization of nitrogen attributable to cropping was reflected at the end of the 40-week decomposition period by increased CO_2 evolution and decreased mineralization of nitrogen during incubation, as well as by reduced uptake of nitrogen by the third crop of wheat.

Factors Affecting Chemical Immobilization

The chemical fixation of ammonia by lignin is known to occur at pH's around neutrality or above. It is also known that the amount fixed in chemically resistant form increases with ammonia concentration and the degree of oxidation of the lignin.

In the greenhouse soils to which supplemental nitrogen was added as urea, appropriately high pH's and ammonia concentrations for ammonia fixation were attained during the first two weeks. Crop response and pH data showed that ammonia was quickly absorbed by the

lignified sawdust and somewhat less rapidly by sawdust. The sorbed ammonia nitrogen was readily available to the first wheat crop but declined rapidly in availability to the next two crops. The decline in availability of nitrogen to the wheat was accompanied by sharply reduced numbers of bacteria and fungi, from which it was inferred that masking energy materials were rapidly dissipated, exposing the more resistant lignin to oxidation.

The level of acid-resistant nitrogen found after 40 weeks was a function of the degree of oxidation of energy materials in these soils. This was shown by the fact that CO_2 evolved during a 10-day incubation declined geometrically with increasing non-acid-hydrolyzable nitrogen. Carbon dioxide evolved from soils treated with a given organic amendment decreased linearly with soil C:N ratio. At any given C:N ratio, the CO_2 evolution for different materials was of the order expected from the known decomposability of the original materials.

Thus, the chemical immobilization of nitrogen in the greenhouse experiment appears to have been related directly to level of nitrogen treatment, the quantity of lignaceous materials added in the various organic amendments, and the degree of decomposition or dissipation of associated energy materials. The effect of cropping was to reduce the level of acid-resistant nitrogen, presumably by reducing nitrogen level through crop removal and by adding to the energy supply in the form of root residues from the wheat.

Factors Affecting Release of Microbially Immobilized Nitrogen

Comparison of nitrogen taken up by successive crops of wheat with microbial numbers showed that nitrogen initially immobilized by microbial assimilation was released again as the more readily available energy materials were dissipated and the numbers and activity of soil microorganisms declined. This was also shown after 40 weeks by the inverse relationships that were found between nitrogen released and microbial activity when the carbon and nitrogen mineralized during incubation were both plotted against soil C:N ratio.

Factors Affecting Release of Chemically Fixed Nitrogen

Bremner and Shaw (23) have shown that ammonia or protein nitrogen in chemical complexes with lignin is very resistant to mineralization by soil microorganisms. In the present study, the decomposability of organic residues in soils 40 weeks after treatment declined according to a geometric function of increasing content of non-acid-hydrolyzable nitrogen, as shown by CO_2 evolution from incubated soil samples. Mineralization of nitrogen during incubation also appeared to bear a continuous relationship to acid-resistant nitrogen, but the shape of the curve for sawdust and lignified sawdust reflected the action of two independent variables, namely decreasing microbial immobilization and increasing resistance to mineralization of chemically fixed nitrogen.

The data were fragmentary, but the values for sawdust and

lignified sawdust showed a distinct tendency for nitrogen mineralized to increase with non-acid-hydrolyzable nitrogen over the lower range where decreasing intensity of microbial immobilization was indicated by rapidly decreasing CO_2 evolution. In the higher range of non-acid-hydrolyzable nitrogen where microbial activity had been reduced to very low levels, nitrogen mineralized during incubation was sharply reduced. In these more highly oxidized soils, the resistance to microbial mineralization of chemically fixed nitrogen was clearly seen.

One year after application of sawdust to the Sims clay loam in the field experiment, the proportion of non-acid-hydrolyzable nitrogen was less than in the check soil and the proportion of amino nitrogen was greater. This result suggested that chemically fixed nitrogen may have been attacked and utilized by microorganisms in the presence of fresh energy materials.

In the greenhouse, non-acid-hydrolyzable nitrogen after ten months was not reduced below the level of the check by any treatment. However, from the shapes of the curves relating acid-resistant nitrogen to soil C:N ratio, it was apparent that decreases in this fraction following addition of carbonaceous energy materials would be much easier to detect in soils of narrow C:N ratio. The C:N ratio of the Oshtemo sand used in the greenhouse experiment was 16.0:1, that of the Sims clay loam in the field experiment was 11.4:1. These differences in soil C:N ratio help to explain the fact that decreases in non-acid hydrolyzable nitrogen were detected in the field after sawdust application and not in the greenhouse.

The addition of root residues from two crops of wheat in the

greenhouse resulted in wider C:N ratios and lower levels of non-acid-hydrolyzable nitrogen than in the corresponding uncropped soils. Thus, non-acid-hydrolyzable nitrogen appeared to be an equilibrium product of oxidative processes in the soil. As such, its level in the soil would be expected to decrease when fresh organic materials containing reduced carbon compounds of high energy content are added to the soil. This process would be analogous to the "priming" action of fresh residues on decomposition of soil humic materials which has been reported in tracer studies. The conflicting evidence that has been reported regarding this "priming" phenomenon may well have arisen by reason of differences in oxidative status and associated levels of chemically fixed nitrogen in the soils used by different workers.

Significance to Crop Response

It is generally assumed that nitrate is the principal form in which nitrogen is taken up by most plants. Nitrogen taken up by the third crop of wheat planted after 40 weeks in the greenhouse experiment was found to be very closely related to water-soluble nitrate in the soil at the time the wheat was planted. No discernible relationship existed between nitrogen uptake and incubation tests for nitrifiable nitrogen. However, when nitrate released during incubation was added to nitrate already in the soil at planting time, the apparent functional relationship between nitrogen uptake and nitrate nitrogen was improved for a majority of soils.

A direct linear relationship was found between nitrate nitrogen and non-acid-hydrolyzable nitrogen in these soils at the time this

third wheat crop was planted. This indicated that both forms of nitrogen were equilibrium products of the oxidation of organic materials in the soil. In confirmation of this interpretation, it was found that nitrogen availability to the third wheat crop was an inverse function of soil C:N ratio for soils with any given organic amendment. The residual availability of nitrogen in previously cropped soils appeared to have been determined largely by the nature of the original organic amendment and the soil C:N ratio after 40 weeks, with only a general and erratic relation to the total quantity of nitrogen originally added.

The failure of the incubation tests for nitrifiable nitrogen by themselves to reflect levels of nitrogen availability was due partly to the fact that large amounts of nitrate had accumulated in a number of these soils prior to planting of the wheat. However, with uncropped soils and soils treated with alfalfa hay, unusually large amounts of nitrogen were taken up by wheat at given levels of nitrate or nitrate-plus-nitrifiable nitrogen. Several of these soils were unusually high in non-acid-hydrolyzable nitrogen. Apparently, rhizosphere activities associated with the growing wheat crop greatly enhanced the availability of chemically fixed nitrogen to the crop.

Of these irregular soils, those treated with sawdust or lignified sawdust at high levels of nitrogen without previous cropping were unusually high in total nitrogen, so that a capacity factor, as well as a rate factor, was involved in the net release of nitrogen to the wheat. With all uncropped soils, microbial immobilization was less intense (shown by reduced CO_2 evolution) than in the corresponding

cropped soils because of the absence of energy materials contributed from root residues from preceding wheat crops.

The abnormally high uptake of nitrogen with the previously cropped alfalfa treatment was consistent with experience in other areas where it has been found that the incubation test for nitrifiable nitrogen fails to reflect the high nitrogen supplying potential of a legume preceding corn. The complexity of factors that were found to influence the tie-up and release of nitrogen during incubation in the greenhouse and in the laboratory and the additional interactions of these factors with the succeeding crop indicate that no single chemical or incubation test can be relied on to predict the availability of nitrogen to that crop.

Practical Implications

From the results that have been reported here, a number of practical implications may be drawn:

1. The quantity of humified soil organic matter can be materially increased by massive additions of plant materials. Such increases are probably only temporary. Decomposition is relatively rapid. Materials high in lignin, such as sawdust or extracted wood products will have residual effects detectable for at least five years after application. Effects of rapidly decomposable materials such as corn stalks or cereal straws will be dissipated much more quickly. Alfalfa hay is decomposed more rapidly in the initial stages than cereal straws or corn stalks, but leaves a larger residue of resistant organic matter because of its higher lignin content.

2. The rate of transformation of fresh plant materials to humic substances is hastened by the addition of nitrogen. Nitrogen retained in organic combinations in the soil is increased by nitrogen treatment. Except with very highly lignaceous materials, the quantity of carbon retained in resistant humic materials at advanced stages of decomposition appears to be relatively unaffected over a very wide range of nitrogen treatment.

3. The initial injurious effects on non-leguminous crops of large additions of plant materials low in nitrogen can be corrected by the addition of large quantities of nitrogen (of the order of 30 to 40 pounds per ton of sawdust). With plant materials low in phosphorus, extra fertilizer phosphorus will also be needed (5 pounds per ton for sawdust).

4. A leguminous crop such as alfalfa is not seriously reduced in yield by the presence of large quantities of sawdust. The addition of extra phosphate may be all that is needed to eliminate any interference with alfalfa production. The broadcasting of sawdust on established stands of alfalfa would appear to be an economical method for using sawdust for soil building purposes.

5. Lignin materials extracted from sawdust have a large capacity for absorbing ammonia. The sorbed ammonia was found to be highly available to the first crop of wheat in concentrations which were highly toxic to wheat grown without lignin treatment. The lignin-ammonia complex quickly became resistant to microbial attack and yet retained a surprisingly high level of availability to subsequent wheat crops. These properties suggest the need for investigating the use of

lignin-ammonia complexes in fertilizer formulations, particularly for localized placement of heavy rates of application and for controlled release effects.

PART TWO

PHYSICO-CHEMICAL PROPERTIES OF SOME SOIL HUMIC MATERIALS AND SYNTHETHIC MODELS

INTRODUCTION TO PART TWO

A large number of organic constituents have been isolated and identified in hydrolysates of soil humic materials. These include hexose and pentose sugars and their uronic acids and amino derivatives, numerous amino acids characteristic of plant and microbial proteins, nucleic acids and inositol phosphates, resins and waxes, and a complex of phenolic compounds combined with nitrogen. In their natural state, the humic materials are largely insoluble in water, which indicates a fairly high degree of polymerization of the simple organic constituents. The behavior of gross fractions of soil organic matter extracted from soils by various extracting procedures does not correspond to that of polysaccharides, proteins or other natural polymers isolated from living plant, animal or microbial tissues. Thus, many of the larger organic groupings in soil organic matter appear to arise by processes of chemical and physical complex formation rather than by well defined patterns of biological condensation and polymerization.

It must be assumed that the actual processes whereby simple organic building blocks are combined into complex structures in the soil will be controlled or modified by the interplay of chemical, physical and biotic forces. Qualitative and quantitative differences in the composition and structure of humic materials will occur from soil to soil, reflecting differences in parent materials, topography, age, climate, vegetation and cultural practices.

On the other hand, certain patterns of recombination will recur in all soils. The qualitative composition of plant materials which

are the principal source of organic matter in soils varies but little with the species of plant. When various plant residues are attacked by decay organisms, an essentially similar assortment or spectrum of active organic groupings are exposed. These include carbonyl groups, ketones, carboxyls and hydroxyls, amino, imino, amido and imido nitrogen groups, sulfhydryl groups and various phosphate esters, to mention only a few. The chemical properties of these active groupings will determine the nature of the reactions in which they can participate in the chemico-physical environment represented by the soil solution and the interfaces between the gaseous, liquid and solid phases of the soil. For these reasons, a certain skeletal similarity in molecular structure of soil humic materials would be expected regardless of soil type or climatic situation.

A primary goal of fundamental soil organic matter studies has long been to define this "humus skeleton". A major stumbling block to realizing this goal has been the difficulty of separating organic materials in unaltered form from the mineral constituents of soils. The results of chemical studies on extracted fractions cannot be directly projected to soil organic matter in situ.

One approach to this difficulty has been to synthesize hypothetical model substances and draw inferences from observed similarities in chemical or physical behavior between these models and organic matter in natural soil systems. This was the approach taken by Waksman and others in developing the concept of a ligno-protein complex (107-110). It is again being vigorously pursued by Flaig and his co-workers in

Germany in their studies of synthetic polymers of quinones and hydroquinones (35-37). This remains an essentially undeveloped area of experimentation.

Further limitations in fundamental organic matter studies are the tedious, time-consuming nature of chemical procedures available for characterizing the organic constituents of soils and the low specificity of many of them. Chromatographic and electrophoretic procedures have come into the picture to greatly facilitate such studies (94, 96, 99). However, these are not entirely satisfactory for extensive surveys of large groups of soils. Furthermore, they provide no direct evidence as to the way in which the simpler components are combined into larger groupings in the soil.

The absorption of infrared and ultraviolet light by appropriately prepared solutions or films of matter has in recent years been developed into tools for the elucidation of molecular structure. The criteria presently available for the interpretation of absorption spectra in these extra-visible wavelengths are still rather limited, but rapid advances are being made. The techniques involved are rapid and should provide a means for quickly surveying the chemical structure of the organic constituents of large groups of soils, and for determining the nature of their association with soil minerals. The theoretical basis for interpretation of the spectra derived with natural soil preparations can be developed by corollary studies with synthetic models prepared to test hypothetical modes of chemical combination or complex formation.

Preliminary studies were conducted employing this approach.

Infrared spectra were developed using organic fractions extracted from three soils with alkali or with neutral salt solutions. Synthetic models used for comparison included Wyoming bentonite and a mixture of Wyoming bentonite and alpha humus extracted from muck, and complexes of casein with varying proportions of lignin prepared from sawdust by acid extraction and by alkali extraction. Ultraviolet absorption studies were conducted on two mobile components separated from the alpha humus of Sims clay loam by paper electrophoresis.

MATERIALS AND METHODS

Preparation of lignins

Acid lignin was prepared by removing non-lignaceous constituents from hardwood sawdust by hydrolysis with cold 72 percent sulfuric acid followed by hydrolysis with hot dilute sulfuric acid according to the method described by Norman (73).

Alkali lignin was extracted from hardwood sawdust, using 4 percent sodium hydroxide under pressure, as described by Waksman (108).

Both materials were freed from chlorides and sulfates by dialysis.

Preparation of lignin-casein complexes

Casein and lignin were mixed in the dry, powdered form. Separate mixtures were made with each type of lignin in two proportions, one-half gram of casein being added to 1½ and 3 grams of each lignin material. The mixtures were dissolved in 2 percent NaOH and shaken intermittently for eight hours. The complexed materials were then precipitated by addition of HCl, washed free of chlorides, dried at 45-50 degrees C and ground to a fine powder.

Table 15 shows the carbon and nitrogen contents of the lignins and the changes in nitrogen content where casein was complexed with six parts of lignin.

Preparation of alpha humus

A sample of Oshtemo sand which had been treated with lignin in the greenhouse experiment reported in Part One and samples of muck and Sims clay loam were extracted with 2 percent NaOH overnight at

room temperature and were then centrifuged. The black supernatant liquid was recovered by decantation and acidified until a precipitate was formed. This precipitate was centrifuged out, washed free of chlorides by dialysis, dried at 45-50 degrees C and then ground to a fine powder.

Table 15. - Carbon and nitrogen found in lignin-casein complexes used for infrared spectrum analysis.

Material	C Percent	N Percent	Theoretical N Percent
Acid lignin	57.3**	0.3	
Alkali lignin	53.5	0.2	
Casein	-----	13.6	
Alkali lignin-casein complex*	-----	1.9	2.1
Acid lignin-casein complex*	-----	2.2	2.2

* Both analysis from complex of one part casein to six parts of lignin.

** Carbon by dry combustion.

Soil organic matter extracted with neutral salt solution

A sample of Sims clay loam was extracted overnight at room temperature with .05M sodium pyrophosphate. The dissolved organic matter was separated from the soil by centrifugation and then precipitated, freed of chlorides, dried and ground in the same manner as the alpha humus.

Infrared spectra

Materials for infrared spectrum analysis were mounted in a Nujol mull in a Perkin-Elmer recording infrared spectrophotmometer. Absorbancy curves were developed over a range of wavelengths from 2 to 14.5 microns.

Ultraviolet spectra

Ultraviolet spectra were developed on a Beckman DK-2 recording spectrophotometer. Materials were dissolved in dilute sodium hydroxide, and introduced into the light path in quartz absorption cells. Absorbancy curves were developed over a range of wavelengths from 220 to 340 microns.

Paper electrophoresis

Electrophoretic separations from the alpha humus fraction of Sims clay loam were made using a Spinco Model R paper electrophoresis cell. The transporting medium was a barbiturate buffer at pH9.0 with an ionic strength of .1 gamma per ml. Runs were made for four-hour periods with a current of 10 millamperes. Migrant spots were located visually under white or ultraviolet light, then cut from the paper and redissolved in .001 M NaOH in ethyl alcohol for ultraviolet analysis.

EXPERIMENTAL RESULTS

Infrared Spectra

Infrared spectra for acid lignin, alkali lignin and casein are shown in Figures 15, 16, and 17. The three major absorption peaks which appear at wavelengths of 3.45, 6.85 and 7.25 microns are characteristic of the Nujol dispersing medium and are to be observed in all infrared spectra reported here. The sharp break at 9.2 microns is a mechanical irregularity which results when the instrument is switched over from a lower to a higher range of wavelengths.

In the spectra for alkali lignin and casein an absorption band appears at 3.00 microns which is not to be seen in the spectrum for acid lignin. This is a region where characteristic absorbance by N-H or O-H bonds has been shown. At 8.4 to 8.5 microns, an absorbance band for casein and acid lignin coincides with an abrupt damping of absorbance in the spectrum of alkali lignin. Again at 8.9 and 9.8 microns, there are absorbance bands in the alkali lignin spectrum which are not found in the spectra of acid lignin or casein. All three spectra show a peak of absorbance at 14 microns.

The significance in terms of molecular structure of these similarities and dissimilarities in infrared absorption by these three materials is not known, but they do reflect the fact that these materials differ in their chemical constitution. More exact interpretation of such spectra is possible through comparison with absorption spectra that have been reported for numerous reference compounds. Such data were not readily available at the time this report was

FIGURE 15

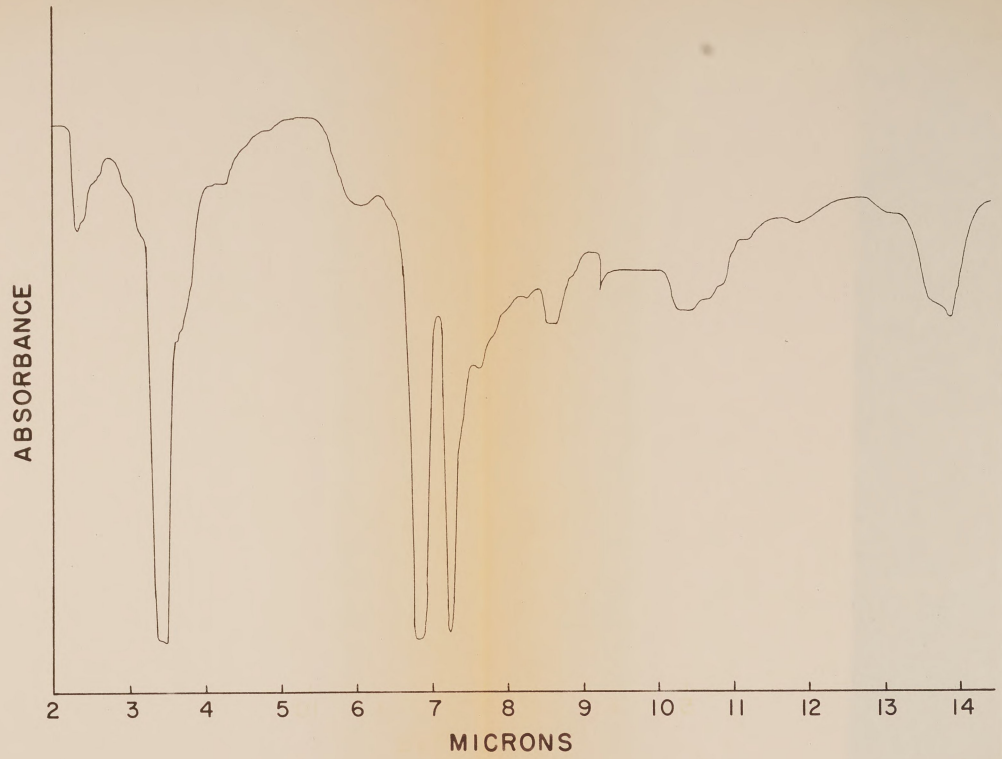


FIGURE 16

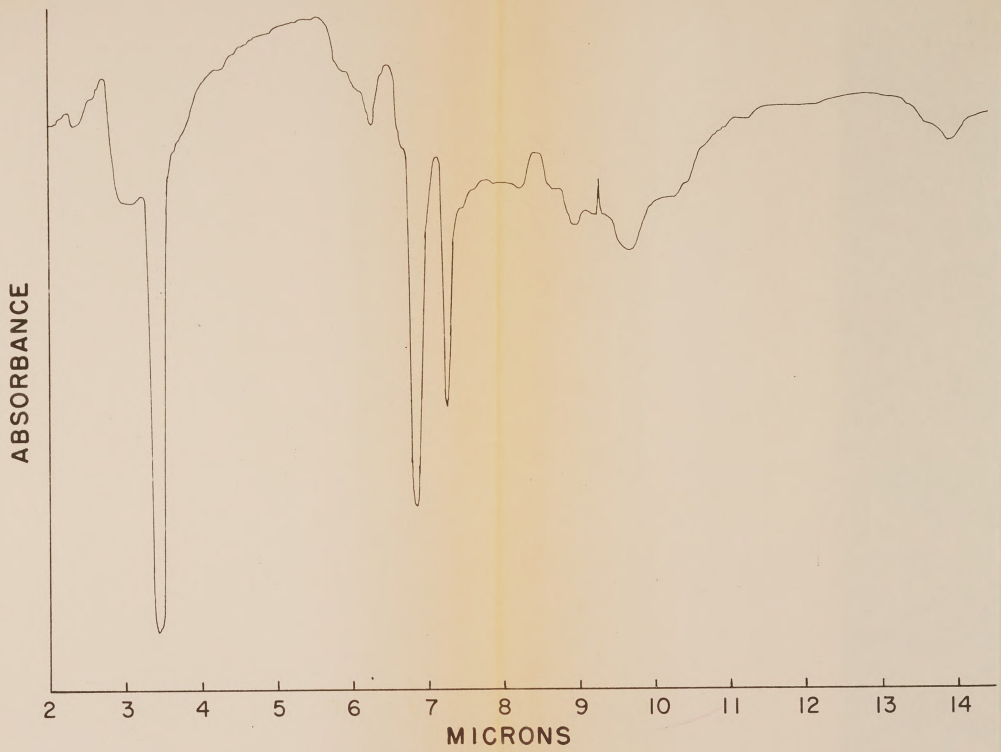
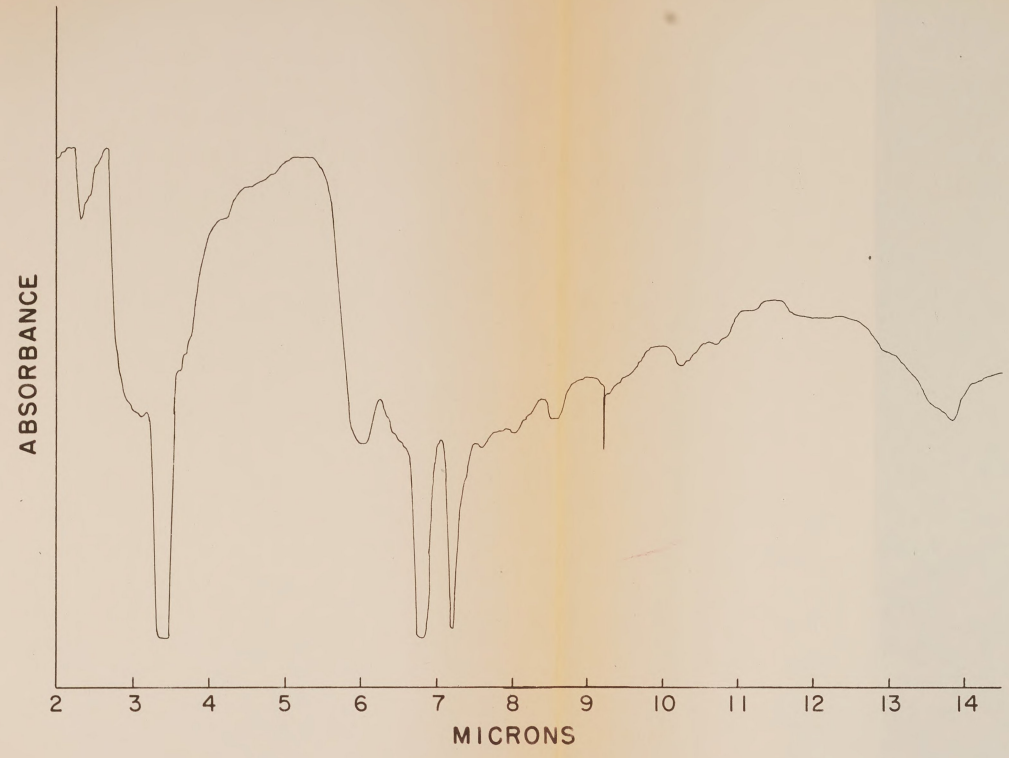


FIGURE 17



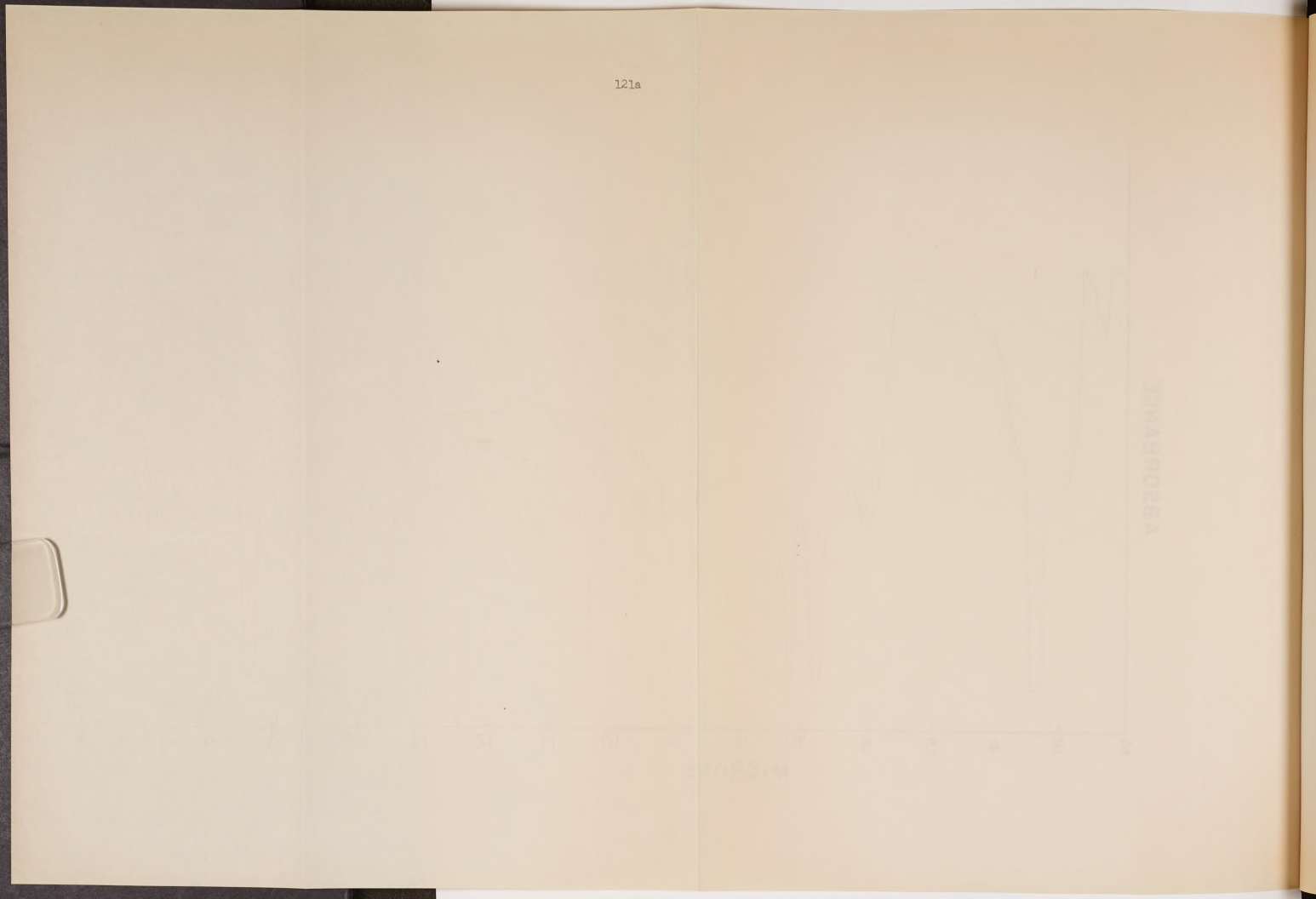


FIGURE 18

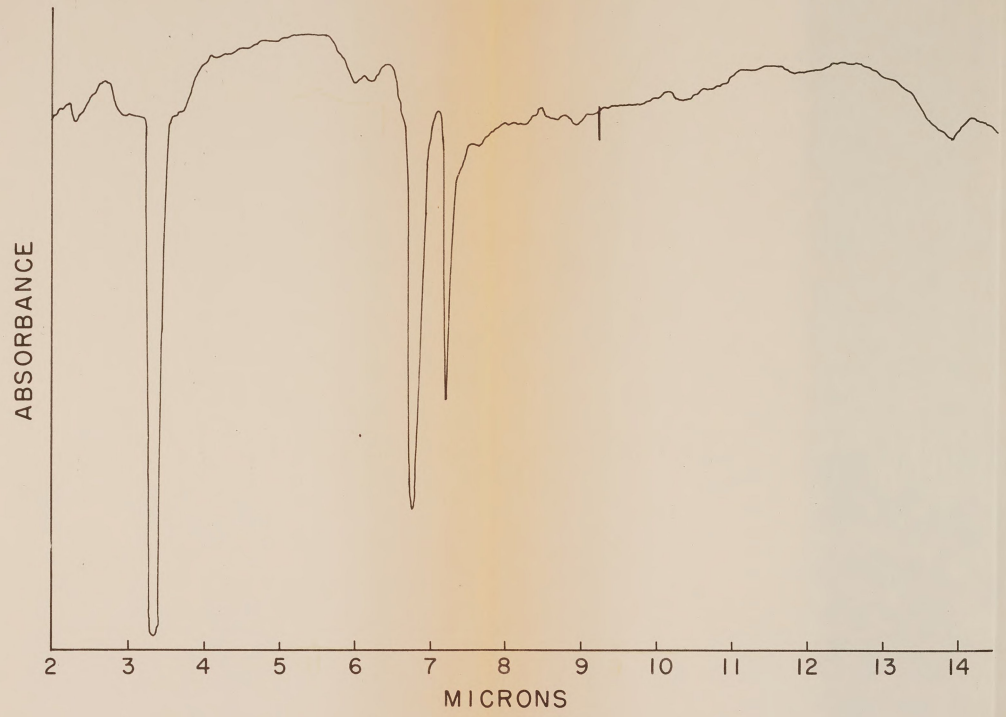


FIGURE 19

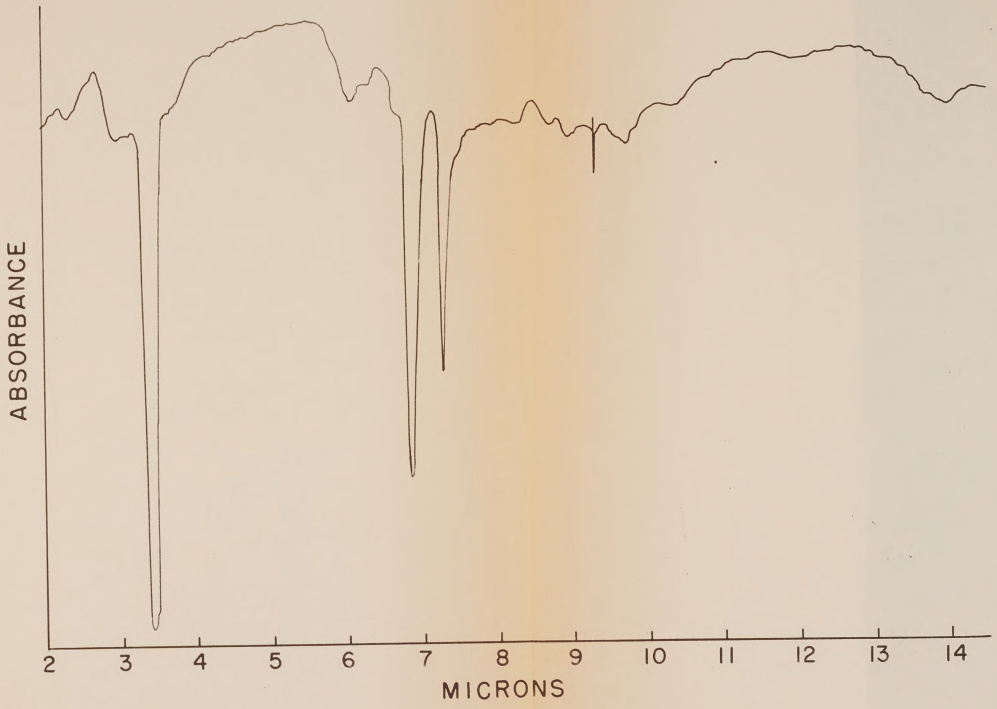
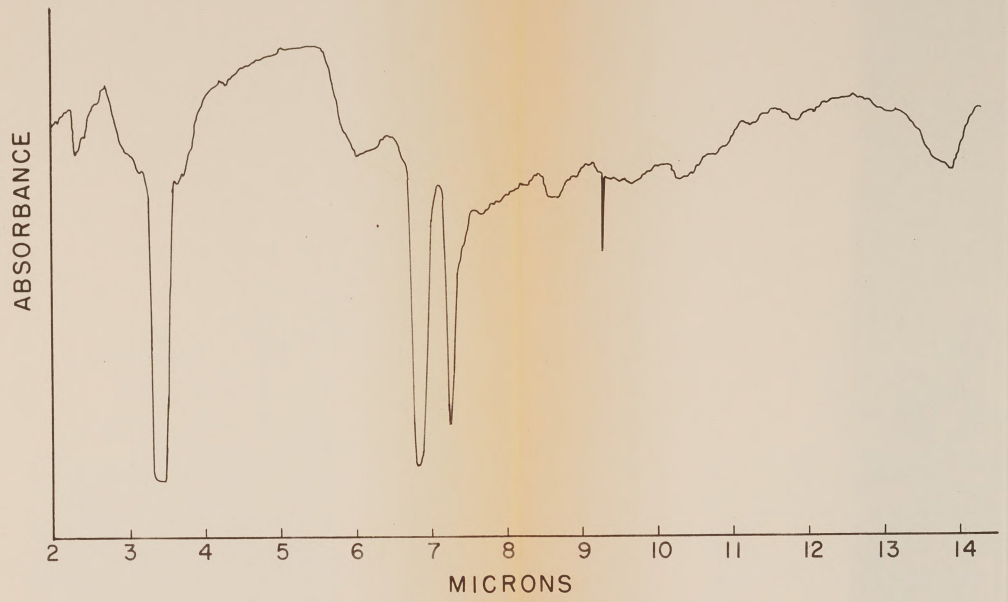


FIGURE 20



prepared.

In Figures 18 and 19 are shown the spectra for the synthetic complexes of casein with acid lignin and with alkali lignin. The shape of the two absorption curves was essentially similar, even to the exact correspondence of numerous absorption maxima and minima at the same wavelengths for both materials. Similar curves were obtained whether three or six parts of lignin were mixed with one of casein.

When Figures 18 and 19 are compared with Figures 15, 16 and 17, it will be seen that numerous characteristic absorption peaks in the parent materials have been sharply reduced in the complexes. This is particularly evident in the region from 7.5 to 12 microns. Here the damping effect of the mixture might reflect merely the interference of opposing regions of maximal and minimal absorbance contributed by elements of the two parent materials in a mechanical mixture.

However, in the case of the alkali lignin-casein comples (Fig. 19), if a purely mechanical mixture were involved, it would be expected that there should be reinforcement of the absorption peak exhibited at 3.00 microns by both alkali lignin (Fig. 16) and casein (Fig. 17). Instead, this peak was less pronounced than in either parent material. Apparently a chemical combination had taken place which resulted in the disappearance of N-H group or O-H group or both.

In the case of the acid lignin-casein complex (Fig. 18) there was a similar damping of absorption at 2.3 microns where reinforcement would have been expected, since peaks occurred here in acid lignin (Fig. 15) and casein (Fig. 17). Again a chemical transformation is indicated. No postulation is offered as to active groups or bonds which may have been involved.

In Figure 20 is shown the infrared absorption diagram for alpha humus extracted from muck. There is a striking similarity between this spectrum and that for acid lignin (Fig. 15). Both have a sharply defined absorption peak at 2.3 microns. Neither shows the peak at 3.0 microns that was shown by alkali lignin and casein and weakly by the two complexes. In the region above 7.5 microns numerous maxima and minima coincide exactly, although they are less sharply defined in the alpha humus from muck. This close similarity in absorbance behavior between humic material from muck and lignin isolated from sawdust by virtue of its resistance to hydrolysis by strong acid is consistent with the generally accepted concept that resistant lignaceous residues from plant decomposition are a principle constituent of soil humus, particularly in poorly drained situations where aeration is poor.

An alpha humus was also extracted from one of the Oshtemo sand samples which had been treated with acid lignin and incubated in the greenhouse for 30 weeks in the experiment reported in Part One. The infrared spectrum of this preparation is shown in Figure 21. There are sharp absorption peaks at 3 and 6 microns which correspond to those shown by casein (Fig. 17). The intensity of these peaks compared with those obtained where casein was complexed with lignin at ratios of 1 to 3 (Figs. 18 and 19) provides indirect evidence that proteins were extensively stabilized by the lignin treatment in this soil. This had been inferred from the large increases in acid-hydrolyzable amino nitrogen and non-hydrolyzable nitrogen reported in Part One.

The fact that the peaks were more pronounced for the alkali

extract from the incubated soil (Fig. 21) than for the complex formed in alkaline solution (Figs. 18 and 19) may reflect quantitative differences in the proportion of proteinaceous materials present. On the other hand, the mechanisms of complex formation or protein stabilization in the incubated soil may have been different than under the conditions existing in the laboratory synthesis.

In Figure 22 the spectrum for alpha humus extracted from Sims clay loam with neutral sodium pyrophosphate is presented. Rather weak but distinct absorption peaks at 3 and 6 microns appear. No attempt at comparison with previous spectra can be made since a different extractant was used as well as a different soil. However, the point is made that characteristic peaks are recognizable regardless of extractant. This suggests that successive fractional extraction using different extractants may provide one means for identifying chemical groups responsible for specific peaks in infrared spectra of soils.

The pattern of absorbance in Figures 21 and 22 for alpha humus extracted from mineral soils showed no similarity above 7.5 microns to any of the previous spectra. A broad absorption band around 9.5 microns has been reported to be characteristic of clay and other silicate minerals. Accordingly a spectrum was developed for hydrogen-saturated Wyoming bentonite and for a mixture of Wyoming bentonite and alpha humus extracted from muck. These are presented in Figures 23 and 24. Major peaks occur in the bentonite spectrum at 2.3, 6.1, 9.4 to 9.8, and at 14 microns. These are also the major peaks in the spectrum of the mixture.

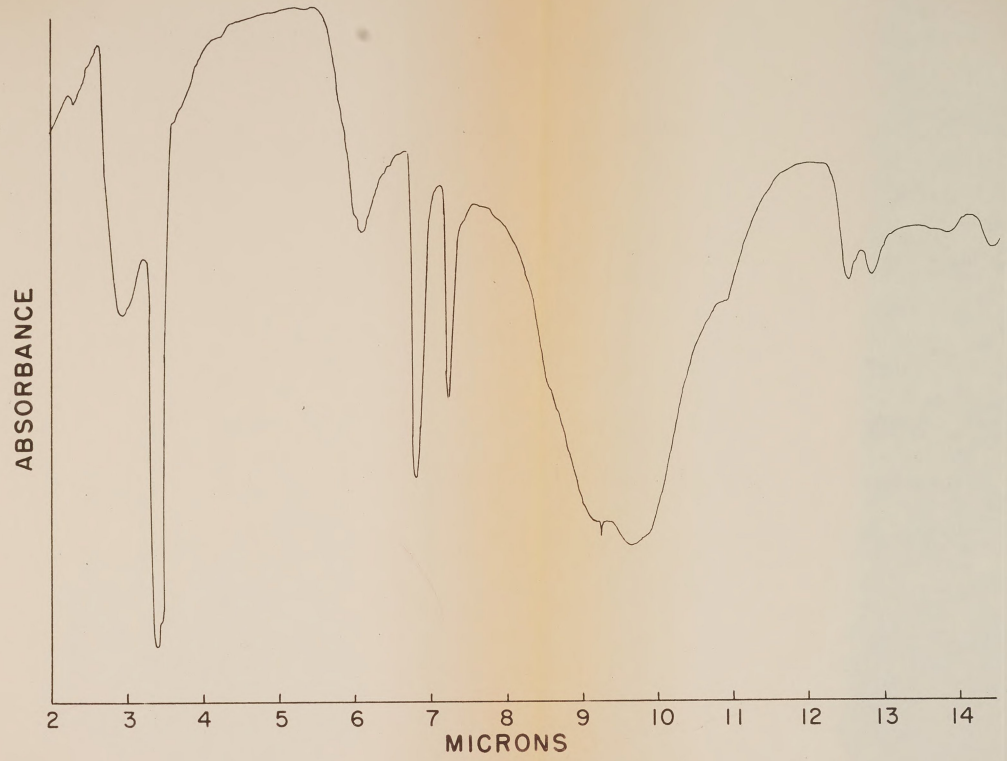
Figure 21. - Infrared absorption spectrum of alpha humus extracted with NaOH from an Oshtemo sand. The soil had been incubated for 30 weeks following an application of acid lignin.

Figure 22. - Infrared absorption spectrum of alpha humus extracted from Sims clay loam with sodium pyrophosphate. Check treatment.

Figure 23. - Infrared absorption spectrum of hydrogen saturated Wyoming bentonite.

Figure 24. - Infrared absorption spectrum of alpha humus prepared from four parts muck complexed with one part bentonite.

FIGURE 21



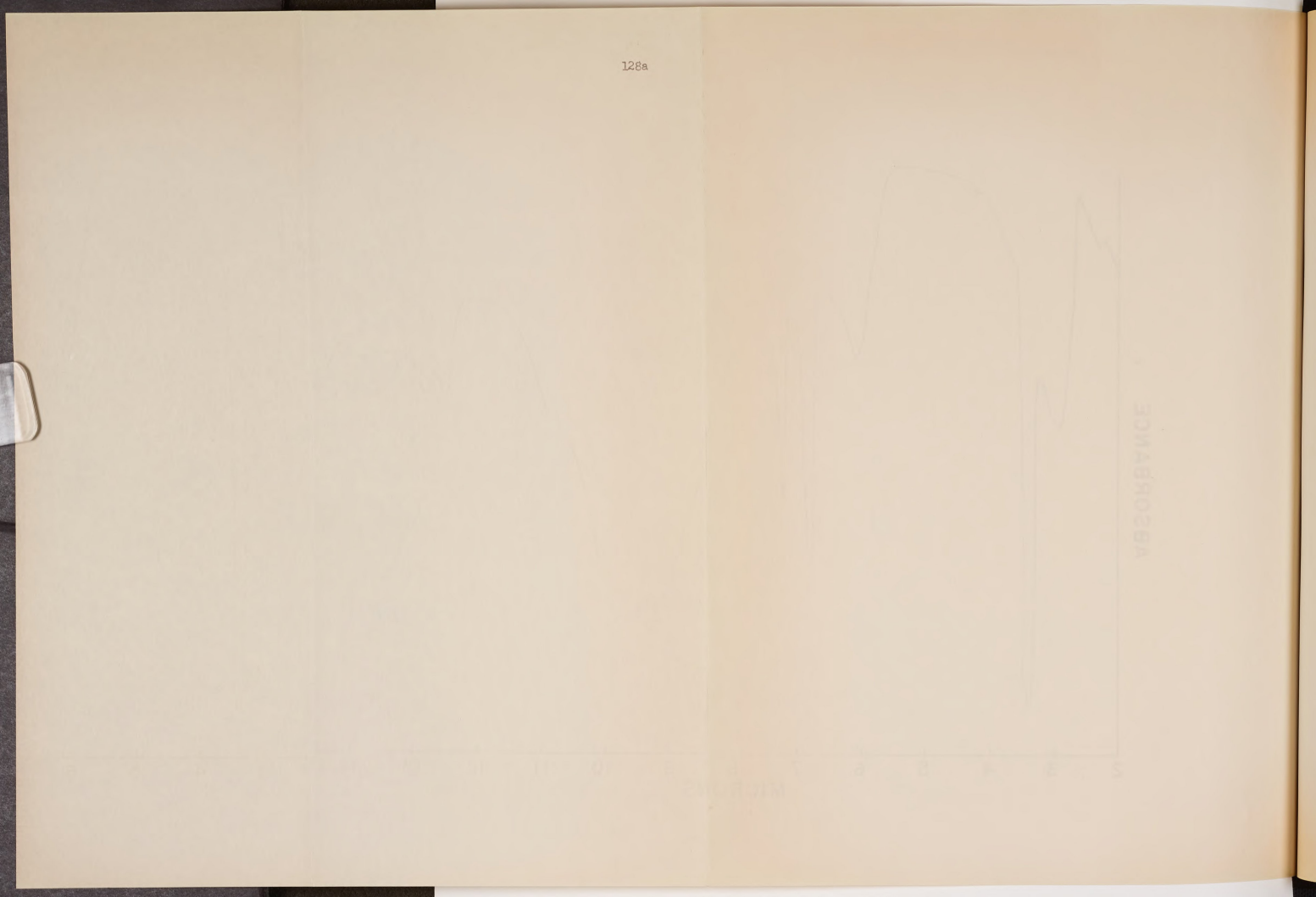


FIGURE 22

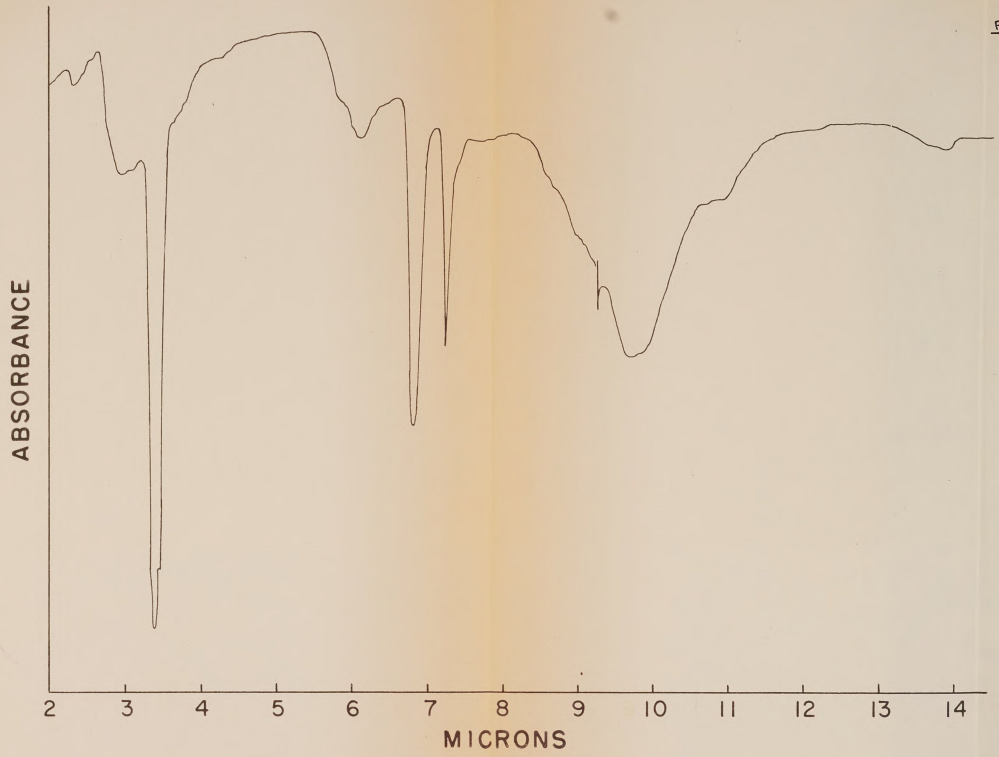


FIGURE 23

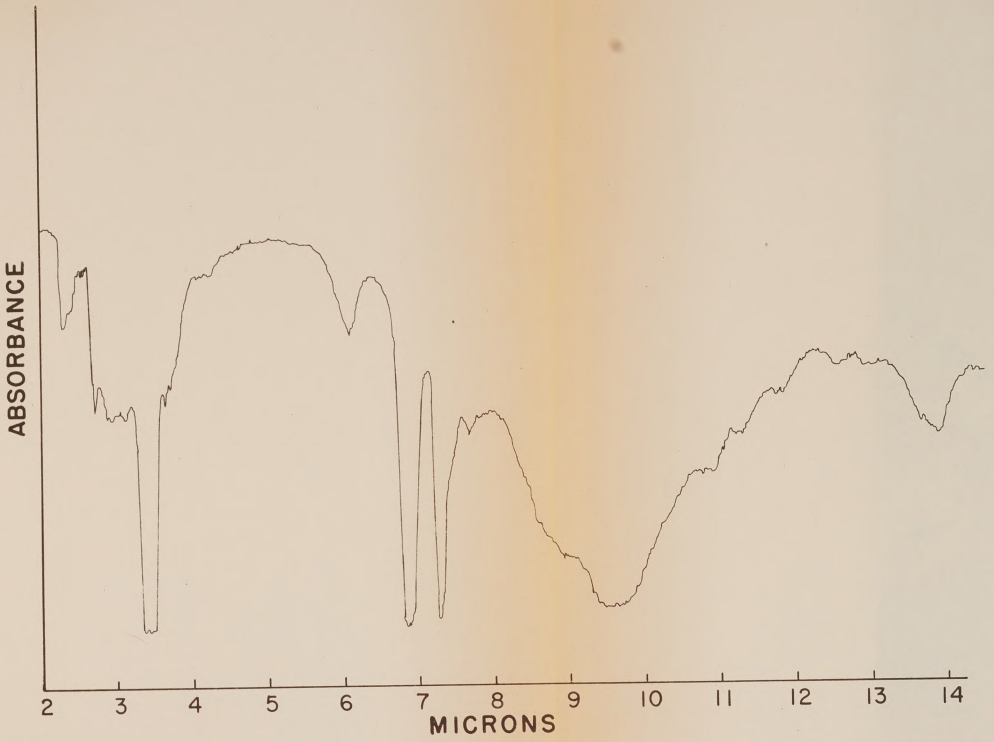
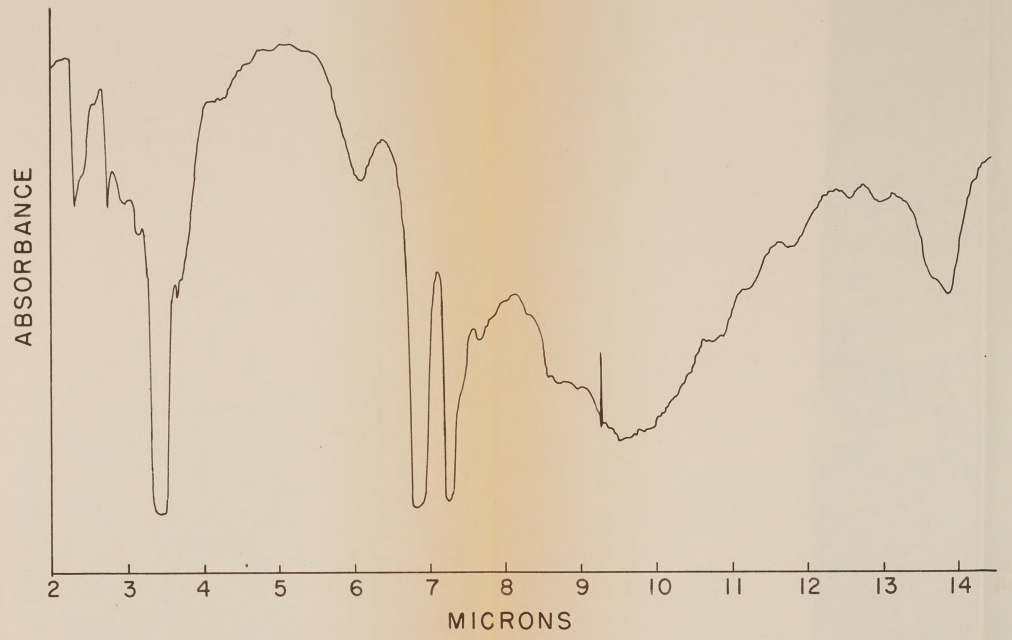


FIGURE 24



This masking of the organic fraction by mineral impurities represents a major technical difficulty in the application of infrared spectrum analysis to studies of the organic fraction of mineral soils. Further pitfalls in the sort of interpretation which has been developed tentatively thus far are represented by the fact that O-H bonds in minerals such bentonite can account for the absorption peak at 3 microns and that water absorbed on organic or mineral colloids may be responsible for the peak around 6 microns. However, different materials do leave characteristic tracings in the infrared spectrum. By systematic model systems it should be possible to interpret these tracings with increasing assurance.

Ultraviolet Spectra

Attempts were made to develop ultraviolet spectra with solutions of alpha humus extracted from soils.

The upper curve in Figure 25 is typical of the results obtained. The absence of any maximum or minimum transmittance peaks indicates that this gross fraction is chemically too complex for study with ultraviolet techniques.

In an effort to separate a fraction that might be less heterogeneous, a sample of the same soil was first hydrolyzed in boiling 6 N HCl and washed free of chlorides before extracting an alpha humus fraction with NaOH. The second curve in Figure 25 shows that this fraction was no more specific in its behavior towards ultraviolet light than the first.

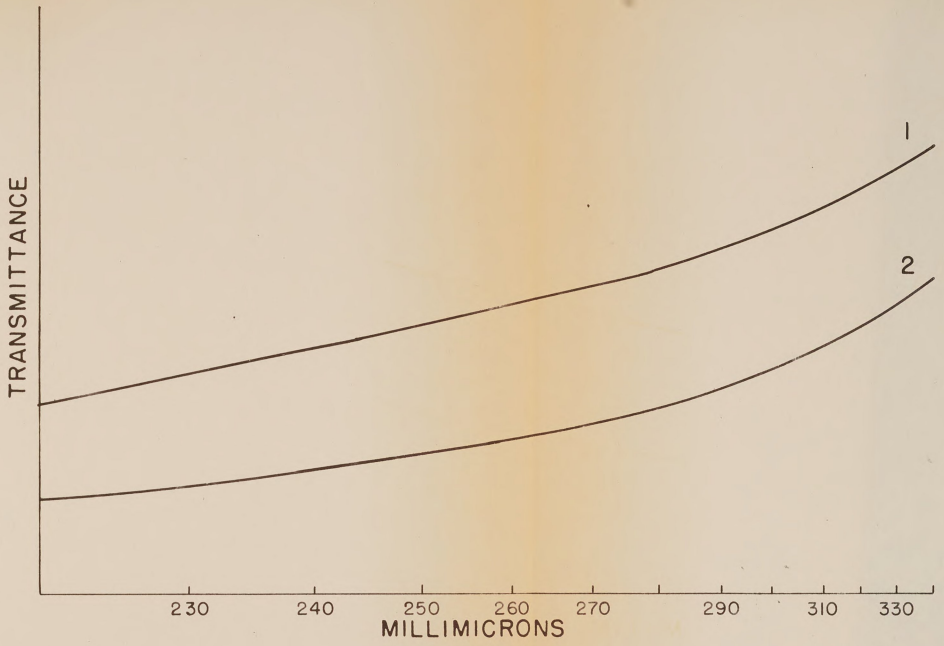
Stevenson (99) had reported the separation of a single mobile

FIGURE 25

Ultraviolet spectrum of alpha humus from a Sims clay loam.

1. Alpha humus dissolved in .001 M NAOH

2. Alpha humus dissolved in .001 M NAOH after soil had been hydrolyzed with 6 NHCL.


TRANSMITTANCE
1
2
230
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250
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270
290
310
330
MILLIMICRONS

component from alpha humus, using a Tiselius-Klett electrophoresis cell. This appeared to be a means for separating a humic fraction that might be better defined chemically and which might give an ultraviolet spectrum with better resolution. Electrophoresis apparatus of the type used by Stevenson was not available, so separations were made using paper electrophoresis equipment. Alpha humus fractions extracted before and after acid hydrolysis of Sims clay loam were used.

Photographs in daylight and under ultraviolet light of a typical electrophoresis paper pattern for alpha humus are presented in Figure 26. Under ordinary light a single band of dark colored material was found to have migrated out of the alpha humus in the direction of the cathode. Under ultraviolet light it was found that the dark colored material was preceded by a band of fluorescence. The bands of dark colored and fluorescent materials were not sharply separated, but the observed phenomena indicate that at least two distinct mobile components were present.

The area of the bands was cut from the paper and extracted with .001 M NaOH in 95 percent ethanol. Ultraviolet spectra of the fluorescent component are presented in Figure 27. At the higher dilution, it appeared that a great improvement in resolution had been achieved over that obtained with the original alpha humus fractions in Figure 25. A similar spectrum was obtained with the material extracted from the dark colored band so that it must be assumed that the separation of the colored and fluorescent components was incomplete. With additional purification, the fluorescent component would be expected

FIGURE 26

Paper electrophoresis separation of alpha humus fraction from a Sims clay loam

A. Ordinary separation observed under daylight.

B. Separation observed under ultraviolet light.

O is the origin.

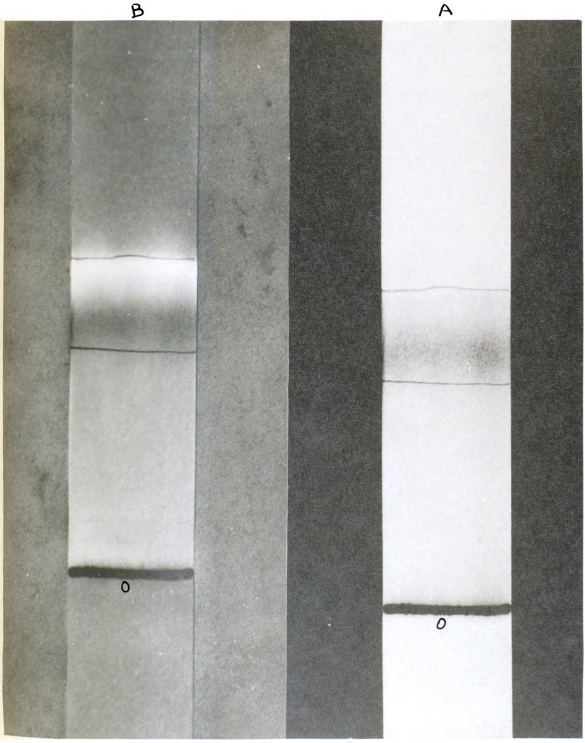
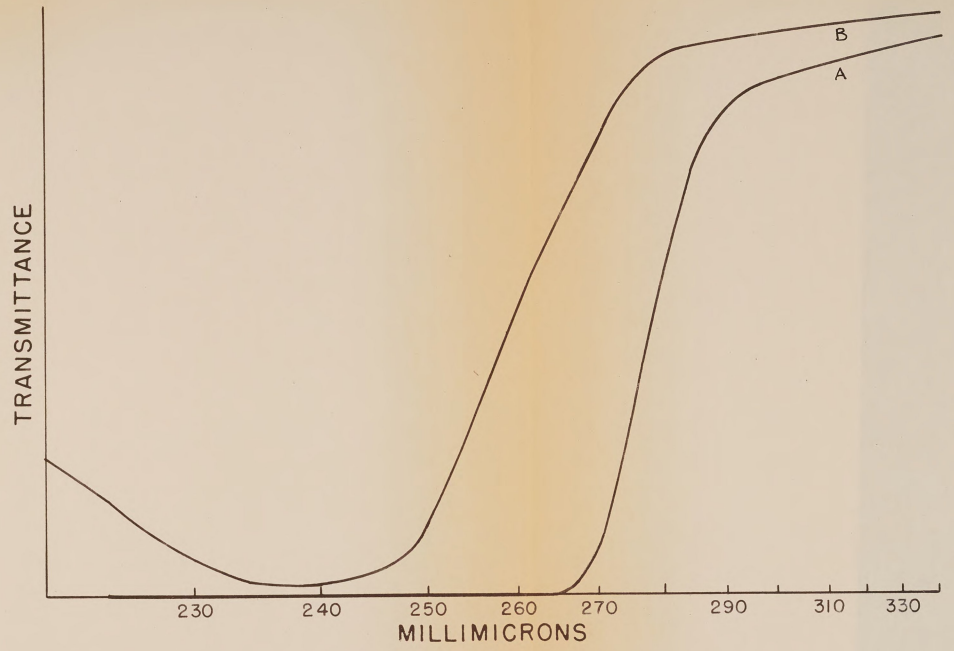

B
A
O
O

FIGURE 27

Ultraviolet spectrum of fluorescent component separated by paper electrophoresis

A. Original component dissolved in .001 M NaOH after being cut from the paper.

B. Same Component dilute app. 200 times.


TRANSMITTANCE
B
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270
290
310
330
MILLIMICRONS

to give a much sharper absorbance spectrum in the ultraviolet range. This would provide a clue to its identity. It would also provide a criterion for establishing the purity of similar soil organic matter separates as a guide in further chemical studies directed towards positive identification.

It is significant that the fluorescent band was obtained in electrophoretic separations from alpha humus fractions extracted both before and after hydrolysis of soil with 6N HCl. While it must be assumed that certain humic constituents are released from soils both by alkali extraction and acid hydrolysis, it would appear that distinct components are specifically characteristic of the alkali-soluble fraction. This had been inferred from the similarities and dissimilarities observed in the trends with time of acid-hydrolyzable and alkali-soluble nitrogen in the soils studied in Part One.

After acid hydrolysis the alpha humus extracted from Sims clay loam still contained 1 percent or more of nitrogen. Several observations support the conclusion that the nitrogen in the alpha humus fractions of this soil was not present as protein. Only the dark colored and the fluorescent materials were observed to migrate under the influence of an electric current. This was true whether the alpha humus was extracted before or after acid hydrolysis. The migration velocities of the mobile components were greater than those of the usual proteins found in blood, and they were not stained by bromphenol blue. If nitrogen was present in the form of amino acids, these amino acids were not in the usual protein combinations, or at least not as free proteins.

It was considered that mineral impurities in the alpha humus preparations might have contributed to the immobility of the dark colored materials which failed to migrate away from the origin (Fig. 12). Accordingly, suspensions of pure clay minerals were applied to paper strips and subjected to the same electrophoretic potential that was used in the humic separations. The blue color formed by the action of benzidine on clay minerals was used to visualize the location of the clay. No movement of the clay was observed after four hours.

Since the infrared spectrum of alpha humus from Sims clay loam (Fig. 22) showed the presence of silicates as impurities, it appears likely that electrophoretic immobility of certain humus components may derive from complex formation between mineral and organic constituents of soils. Electrophoretic procedures provide a tool for their separation and further study.

Conductometric and High Frequency Titrations

In the work with the synthetic casein-lignin complexes, it was of interest to duplicate studies reported in the literature on the cation exchange properties of such complexes, using, however, newer and more rapid procedures for estimating exchange capacity.

Lignin, casein and lignin-casein complexes were saturated with barium by the method described by Mehlich (66). The barium saturated materials were washed free of chlorides and dried. Two methods were used to estimate cation exchange capacity. These included the conductometric titration suggested by Mortland and Mellor (71) and a high frequency titration employing apparatus described by Johnson and Timnik (52).

In Figures 28, 29, 30 are presented curves obtained with the two methods during the titration of suspensions of the barium saturated lignins and casein with 0.1 N $MgSO_4$. Both methods gave sharp end points, but there was little agreement between the values obtained for cation exchange capacity. Since the high frequency method eliminates the use of electrodes which might interfere with the exchange reaction, this method was used for titration of the lignin-casein complexes.

In Figures 31 and 32 are shown the high frequency titration curves obtained with complexes of casein with acid lignin and alkali lignin. A striking result is the appearance of a second end point in these curves. The first end point suggests the presence of new exchange sites characterized by relatively low sorption energy for barium. The second inflection in the curves represents the point where barium has been completely replaced by magnesium on exchange sites and corresponds to the point on the other curves which was taken as an estimate of total cation exchange capacity.

Cation exchange capacities taken from the high frequency titration curves of all materials are tabulated in Table 16.

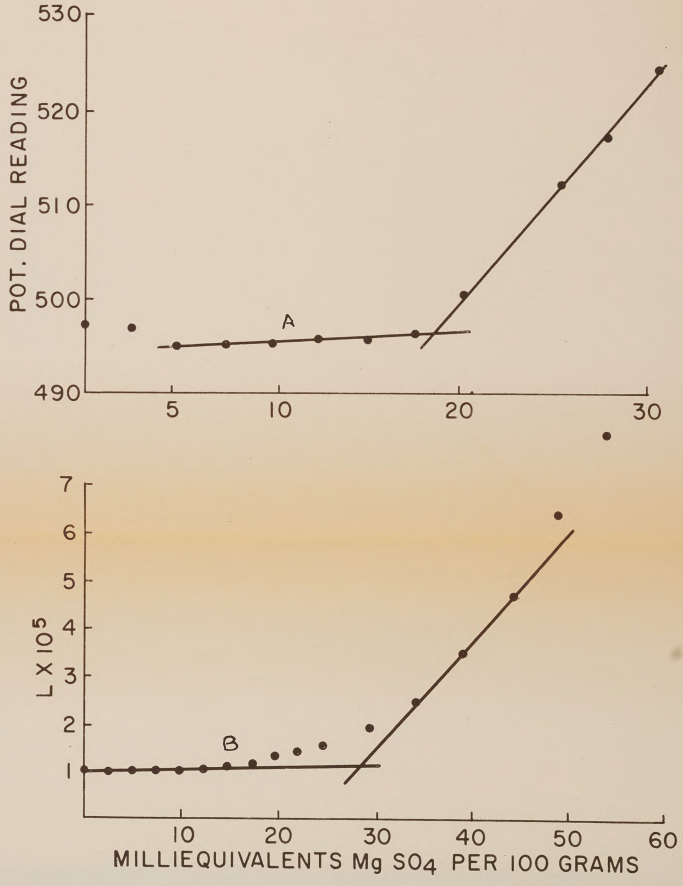
The total cation exchange capacity of acid lignin was increased in the 6:1 complex with lignin to a value intermediate between the capacities of the two materials alone. The increase was equivalent to the capacity of new exchange sites represented by the first end point.

In the 3:1 complex of acid-lignin and casein, total exchange capacity was greater than that of either material alone. An increase

Figure 28. - High frequency (A) and conductometric (B) titration curves for barium saturated acid lignin in 100 ml. of water and 50 ml. of alcohol.

Figure 29. - High frequency (A) and conductometric (B) titration curves for barium saturated alkaline lignin in 100 ml. of water and 50 ml. of alcohol.

Figure 30. - High frequency (A) and conductometric (B) titration curves for barium saturated casein in 100 ml. of water and 50 ml. of alcohol.


530
520
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490
POT. DIAL READING
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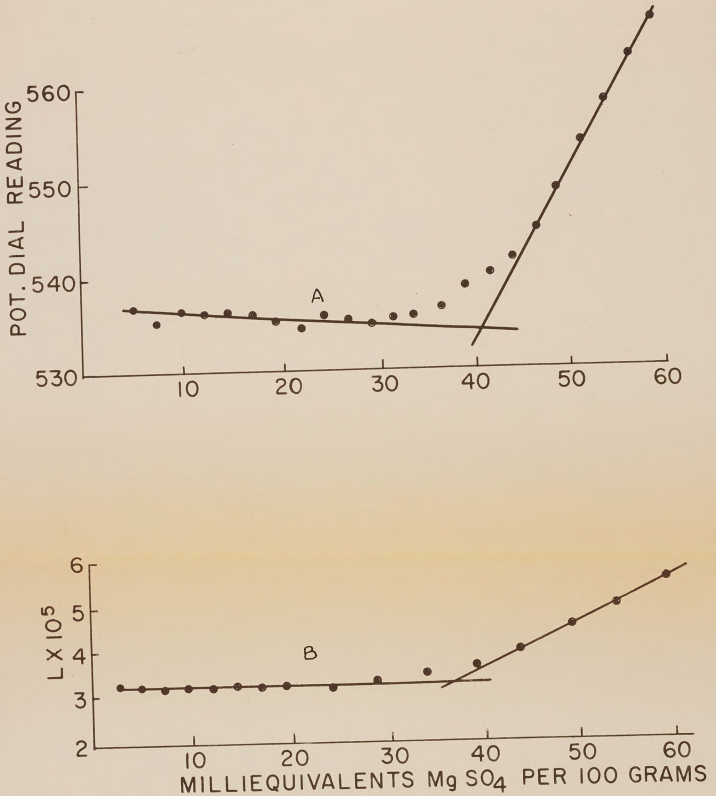
10
20
30
7
6
5
4
3
2
1
L X 10⁵
B
10
20
30
40
50
60
MILLIEQUIVALENTS Mg SO4 PER 100 GRAMS

FIGURE 28


POT. DIAL READING
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530
A
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30
40
50
60
L X 10⁵
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4
3
2
B
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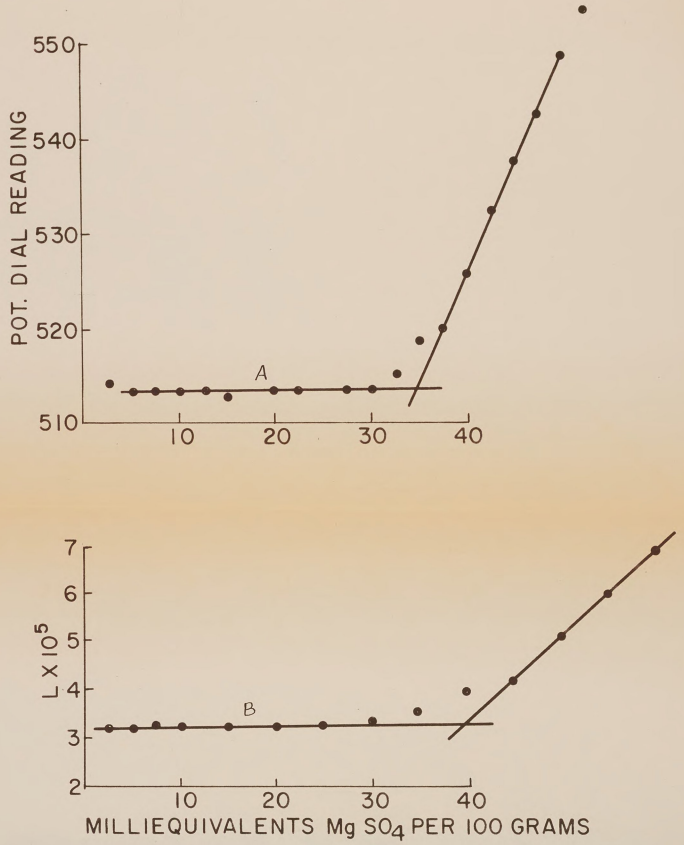
20
30
40
50
60
MILLIEQUIVALENTS Mg SO4 PER 100 GRAMS

FIGURE 29


POT. DIAL READING
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20
30
40
L X 10^5
7
6
5
4
3
2
B
10
20
30
40
MILLIEQUIVALENTS Mg SO4 PER 100 GRAMS

FIGURE 30

FIGURE 31

High frequency titrations curves for barium saturated acid-lignin-casein complex in 100 ml. of water and 50 ml. of alcohol.

A. Complex composed of six parts of lignin to one part of casein.

B. Complex composed of three parts of lignin to one part of casein.

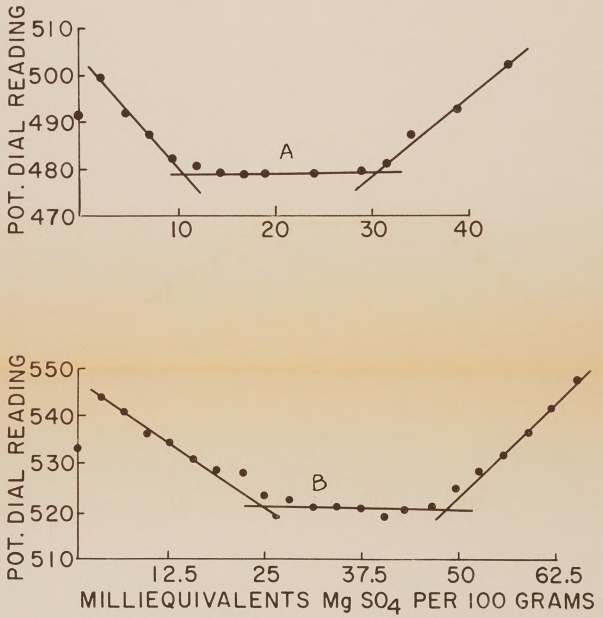
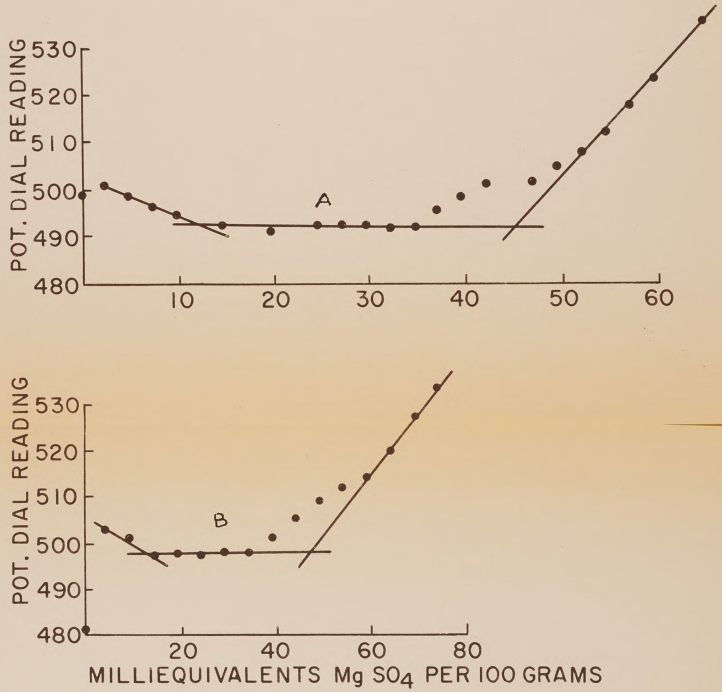

POT. DIAL READING
510
500
490
480
470
A
10
20
30
40
POT. DIAL READING
550
540
530
520
510
B
12.5
25
37.5
50
62.5
MILLIEQUIVALENTS Mg SO4 PER 100 GRAMS

FIGURE 32

High frequency titrations curves for barium saturated alkali lignin-casein complex in 100 ml. of water and 50 ml. of alcohol.

A. Complex composed of six parts of lignin to one part of casein.

B. Complex composed of three parts of lignin to one part of casein.


POT. DIAL READING
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40
50
60
POT. DIAL READING
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510
500
490
480
B
20
40
60
80
MILLIEQUIVALENTS Mg SO4 PER 100 GRAMS

of 29 m.e. in total exchange capacity over that of the acid lignin alone was largely accounted for by the appearance of 25 m.e. of exchange capacity in the titration preceding the first end point.

Table 16. - Cation exchange capacities of lignins, casein and lignin-casein complexes as determined by high frequency titration of Ba-saturated materials with N/10 $MgSO_4$.

Material	Material alone	Cation exchange capacity, m.e. per 100 gms: Complex with casein		
		Ratio	First endpoint	Total
Acid lignin	19	6:1*	11	30
		3:1	25	48
Alkali lignin	41	6:1	11	45
		3:1	11	46
Casein	35	---	--	--

* Ratio of lignin to casein in the complex.

The behavior of the alkali lignin complex was quite different from that of the acid lignin complex. There were no differences in either lower energy or total exchange capacities between the 6:1 and the 3:1 ratios of lignin to casein. With both ratios, the total exchange capacity of the complex was only 4 or 5 m.e. greater than that of the alkali lignin alone. It is interesting to note that the total capacity of the complex was 10 or 11 m.e. greater than that of casein alone, which is equivalent to the capacity of new exchange sites represented by the first endpoint.

The significance of the observed cation exchange phenomena is not known. The different results with acid lignin as compared with alkali lignin help to explain why some investigators have found that the exchange capacity of lignin-protein complexes was greater than that of lignin alone, whereas other investigators have been unable to show such changes. The manner in which lignin is extracted from natural sources greatly influences the chemical properties of the product. This was indicated by the infrared spectra as well as by the differences in cation exchange properties of the materials themselves and of their complexes with casein.

The appearance of new exchange sites in lignin-casein complexes substantiates the inferences made from infrared spectra that actual chemical combinations had occurred between active groups in the two materials when they were mixed in alkaline solution.

SUMMARY AND CONCLUSIONS

The physico-chemical studies reported here represent an attempt to apply several new techniques to the study of the fundamental nature and properties of soil organic matter. It is recognized that the results obtained are fragmentary and that their theoretical significance is not clear. However, the experiences recorded may be a guide to future studies along these lines. Several specific results appear to justify further investigation.

Infrared absorbance spectra were developed for a number of extracted soil organic matter fractions and synthetic models involving casein, acid- and alkali-extracted lignin and clay minerals. The spectra for organic materials were highly characteristic; for example distinctly different absorbance patterns were exhibited by lignins extracted from hardwood sawdust with acid and with alkali. Structural differences revealed by infrared spectra were reflected in differences in cation exchange properties of acid and alkali lignins alone and in synthetic complexes with casein.

The infrared spectrum for alpha humus extracted with alkali from muck was very similar to that for acid extracted lignin, but was distinctly different from that of alkali lignin extracted from sawdust. This result is consistent with the accepted concept that chemically resistant lignaceous constituents of plants accumulate as the principle component of humus in poorly drained situations where biological oxidation is limited.

Alpha humus extracted from mineral soils gave spectra in which the patterns characteristic for organic fractions were obscured

above 7.5 microns by mineral impurities. Comparison of these spectra with those obtained with bentonite alone and in mixture with alpha humus from muck indicated that the mineral impurities were probably silicates.

Future infrared studies with the organic fractions of mineral soils will need to consider means for eliminating these mineral impurities. On the other hand, studies with synthetic clay-organic matter systems in the regions of the infrared spectrum where silicates interfere may reveal clues as to the active groups involved in interactions between clays and organic compounds.

The usefulness of the infrared absorbance diagrams was limited by the lack of an extensive reference list of absorbance data for pure compounds. The absorption of electromagnetic energy by matter is known to be a function of the bonding energy between units of molecular structure. Specific absorption wavelengths have been determined for numerous interatomic combination. A necessary phase of any investigation involving radiation absorbance phenomena should be the tabulation from the literature of available data of this sort.

Infrared analysis appears to have considerable promise as a means for surveying large numbers of soils with a view to characterizing gross organic fractions which can be readily freed from mineral impurities. Ultraviolet absorption, on the other hand, has more limited application as a means of tentative identification of certain fundamental humic components after they have been isolated in rather pure form. Presumably these will include compounds with aromatic ring structure or heterocyclic compounds derived from lignaceous

materials. Ultraviolet absorption spectra may serve as criteria of the purity of isolates to be used for further chemical analysis.

Preliminary experiments with paper electrophoresis suggest that this may provide a method for isolating components of alpha humus with sufficient purity to give characteristic ultraviolet absorbance spectra. Whereas others have reported a single mobile component in alpha humus preparations employing free electrophoresis in a Tiselius cell, in the present work with paper electrophoresis two distinct components were observed. One of these was a colorless material which fluoresced under ultraviolet light. The separation of the two mobile components was not complete, but their ultraviolet absorbance spectra showed much better resolution than those for the original alpha humus.

The extreme complexity of soil organic matter and its intimate association with soil minerials has made it a rather unrewarding object of study in the past. A number of new instruments and techniques, including those used here have opened new possibilities for the fruitful examination of soil organic matter. No one method can be expected to accomplish much by itself. There is considerable promise, however, in an integrated approach involving several methods on both natural soil systems and synthetic models.

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APPENDIX

Table 17. - Treatments used with Oshtemo sand in greenhouse experiment

Pot number (a)	Material added	Rate gms.	C:N ratio of original material	C:N ratio actual	Nitrogen added (d) gms.	Crop grown
1,2	Lignified sawdust	100	577:1	12.7:1	3.846	wheat(b)
3,4	"	"	"	36.5:1	1.281	"
5,6	"	"	"	12.7:1	3.846	---(c)
7,8	"	"	"	36.5:1	1.281	---
9,10	"	50	"	12.7:1	1.923	wheat
11,12	"	"	"	36.5:1	.641	"
13,14	"	"	"	12.7:1	1.923	---
15,16	"	"	"	36.5:1	.641	---
49,50	Sawdust	100	353:1	20:1	2.355	wheat
51,52	"	"	"	60.5:1	.785	"
53,54	"	"	"	20:1	2.355	---
55,56	"	"	"	60.5:1	.785	---

(a) All pots contained 4000 gms of a uniformly sieved Oshtemo sandy loam.

(b) Five plants were grown in each pot, 2 crops were grown during first 25 weeks of decomposition period.

(c) No plants were grown.

(d) Nitrogen added as urea.

Table 17 (Continued)

Pot number	Material added	Rate gms.	C:N ratio of original material	C:N ratio actual	Nitrogen added (d) gms.	Crop grown
57,58	Sawdust	50	353:1	20:1	1.177	wheat
59,60	"	"	"	60.5:1	.392	"
61,62	"	"	"	20:1	1.177	---
63,64	"	"	"	60.5:1	.382	---
17,18	Straw	100	65.7:1	41.6:1	.438	wheat
19,20	"	"	"	55.1:1	.146	"
21,22	"	"	"	41.6:1	.438	---
23,24	"	"	"	55.1:1	.146	---
25,26	"	50	"	41.6:1	.219	wheat
27,28	"	50	"	55.1:1	.073	"
29,30	"	"	"	41.6:1	.219	---
31,32	"	"	"	55.1:1	.073	---
33,34	Corn stalks	100	113.6:1	40.9:1	.757	wheat
35,36	"	"	"	72.2:1	.252	"
37,38	"	"	"	40.9:1	.757	---
39,40	"	"	"	72.2:1	.252	---
41,42	"	50	"	40.9:1	.379	wheat
43,44	"	"	"	72.2:1	.126	"
45,46	"	"	"	40.9:1	.379	---

Table 17 (Continued)

Pot number	Material added	Rate gms.	C:N ratio of original material	C:N ratio actual	Nitrogen added (d) gms.	Crop grown
47,48	Corn stalks	50	113.6:1	72.2:1	.126	---
65,66	none	---	-----	---	1.923	wheat
67,68	Lignified sawdust	50	577:1	---	-----	"
69,70	Sawdust	"	353:1	---	-----	"
71,72	Straw	"	65.7:1	---	-----	"
73,74	Corn stalks	"	113.6:1	---	-----	"
75,76	Lignified sawdust	100	577:1	---	-----	"
77,78	Sawdust	"	353:1	---	-----	"
79,80	Straw	"	65.7:1	---	-----	"
81,82	Corn stalks	"	113.6:1	---	-----	"
83,84	none	---	-----	---	-----	"
*85,86	Alfalfa	100	18.4:1	---	-----	wheat
87,88	"	"	"	---	-----	---
89,90	"	50	"	---	-----	wheat
91,92	"	"	"	---	-----	---
101,102	Sawdust	100	353:1	---	-----	alfalfa
103,104	"	"	"	60.5:1	.785	"
105,106	"	50	"	---	-----	"

* 2.708 gms. of nitrogen added per pot as alfalfa hay.

Table 17 (Continued)

Pot number	Material added	Rate gms.	C:N ratio of original material	C:N ratio actual	Nitrogen added (d) gms.	Crop grown
107,108	Sawdust	50	353:1	60.5:1	.392	alfalfa
109,110	Lignified sawdust	"	577:1	---	------	"
111,112	"	"	"	18.2:1	1.281	"
113,114	"	100	"	---	------	"
115,116	"	"	"	68.7:1	.641	"
117,118	Sawdust	"	353:1	---	------	"
119,120	"	50	"	---	------	"

Table 18a. - Yield and nitrogen uptake of wheat crops in greenhouse experiment

Pot number	1st Crop Yield gms.	%N	Nitrogen uptake mgs.	2nd Crop Yield gms.	%N	Nitrogen uptake mgs.
1	6.805	4.0	272.2	7.535	2.88	217.0
2	7.195	2.72	267.6	6.870	2.84	195.1
3	7.885	2.43	191.6	3.250	1.34	43.5
4	11.640	2.44	284.0	6.830	1.51	103.1
5						
6						
7						
8						
9	10.130	3.44	384.4	6.100	2.70	164.7
10	2.990	3.70	110.6	7.770	2.67	207.4
11	8.670	2.05	177.7	1.950	.99	19.3
12	9.590	1.92	184.1	2.260	1.13	25.5
13						
14						
15						
16						
49	2.850	4.40	125.4	6.840	2.92	199.7
50	1.925	4.60	88.5	2.410	4.53	109.1
51	5.210	1.41	73.4	.440	1.62	7.1
52	4.015	1.20	48.2	.620	2.22	13.7
53						
54						

Table 18a (Continued)

3rd Crop			Totals		Average		
Yield gms.	%N	Nitrogen uptake mgs.	N mgs.	Yield gms.	N mgs.	Yield gms.	Pot number
3.777	4.95	186.7	675.9	18.113			1
					642.5	17.930	
3.683	3.98	146.5	609.2	17.748			2
.223	2.60	5.8	240.9	11.358			3
					338.0	15.655	
1.493	3.22	48.0	435.1	19.953			4
2.493	4.78	119.1					5
					150.3	3.198	
3.903	4.65	181.4					6
3.473	4.13	143.4					7
					134.9	3.823	
4.173	3.03	126.4					8
1.953	4.30	83.9	597.0	18.183			9
					501.7	15.728	
2.513	4.24	106.5	424.5	13.273			10
.473	2.10	9.9	206.9	11.093			11
					221.1	12.003	
1.063	2.42	25.7	235.3	12.913			12
2.883	3.80	109.5					13
					141.2	3.583	
4.283	4.04	173.0					14
3.213	4.10	131.7					15
					147.9	3.633	
4.053	4.05	164.1					16
2.433	3.86	93.9	419.0	12.123			49
					359.8	9.565	
2.573	4.01	103.1	300.7	7.008			50
2.103	2.46	51.7	132.2	7.753			51
					143.4	7.500	
2.613	3.59	93.8	155.7	7.248			52
3.736	4.34	162.1					53
					132.3	3.174	
2.613	3.93	102.6					54

Table 18a. - Yield and nitrogen uptake of wheat crops in greenhouse experiment

Pot number	1st Crop Yield gms.	%N	Nitrogen uptake mgs.	2nd Crop Yield gms.	%N	Nitrogen uptake mgs.
55						
56						
57	10.255	2.49	255.3	2.690	2.39	64.2
58	11.160	2.63	293.5	4.555	1.63	74.2
59	4.475	1.16	52.0	1.110	1.88	18.9
60	5.630	1.14	64.1	.440	2.75	9.9
61						
62						
63						
64						
17	2.870	1.31	37.5	3.795	1.45	55.0
18	2.370	1.02	27.7	2.100	1.73	36.3
19	.210	.7	1.4	.700	2.01	14.0
20	.260	.9	2.34	1.340	1.97	26.3
21						
22						
23						
24						
25	2.585	1.03	26.6	1.270	1.78	21.6
26	3.060	1.02	31.2	1.460	1.56	22.4
27	.385	1.71	6.5	1.240	1.72	21.3
28	.400	1.63	6.5	1.140	1.78	21.1

Table 18a (Continued)

3rd Crop Yield gms.	3rd Crop %N	3rd Crop Nitrogen uptake mgs.	Totals N mgs.	Totals Yield gms.	Average N mgs.	Average Yield gms.	Pot number
2.813	2.70	75.9					55
					94.1	3.433	
4.043	2.78	112.3					56
2.581	4.16	107.4	413.4	15.378			57
					442.1	16.833	
2.183	3.31	72.7	470.8	18.288			58
1.673	2.98	49.8	122.7	7.588			59
					145.2	8.135	
2.083	3.26	67.9	167.8	8.683			60
2.783	2.66	74.0					61
					96.4	3.058	
3.333	3.57	118.9					62
2.363	3.96	93.5					63
					92.0	2.718	
3.073	2.95	90.6					64
3.243	3.23	104.7	197.2	9.908			17
					180.3	8.450	
2.523	3.94	99.4	163.4	6.993			18
3.083	3.58	110.3	121.5	3.993			19
					104.2	3.703	
1.813	3.22	58.3	86.9	3.413			20
3.643	3.81	138.7					21
					130.1	3.658	
3.673	3.31	121.5					22
3.233	3.98	128.6					23
					133.5	3.388	
3.543	3.91	138.5					24
2.193	4.20	92.1	140.3	6.048			25
					152.8	6.825	
3.063	3.64	111.5	165.4	7.583			26
2.283	3.24	74.0	101.8	3.908			27
					113.7	3.960	
2.423	4.05	98.1	125.7	4.013			28

Table 18a. - Yield and nitrogen uptake of wheat crops in greenhouse experiment

Pot number	1st Crop Yield gms.	%N	Nitrogen uptake mgs.	2nd Crop Yield gms.	%N	Nitrogen uptake mgs.
33	7.000	1.17	81.9	.94	2.19	20.5
34	7.880	1.16	103.0	1.000	1.56	15.6
35	.240	1.46	3.5	.280	1.64	4.6
36	.160	.81	1.3	1.370	1.63	22.3
37						
38						
39						
40						
41	5.525	1.19	65.7	1.245	1.74	21.6
42	6.380	1.14	72.7	1.260	1.82	23.0
43	.380	1.71	6.5	.510	1.92	9.9
44	.765	1.72	13.1	.510	2.34	11.9
45						
46						
47						
48						
65	.240	3.40	8.1	8.550	2.94	251.3
66	.920	4.03	37.0	8.190	1.96	242.4
67	.370	.95	3.5	.395	1.85	7.3
68	.420	1.03	4.3	.400	1.92	7.6

Table 18a (Continued)

3rd Crop Yield gms.	3rd Crop %N	3rd Crop Nitrogen uptake mgs.	Totals N mgs.	Totals Yield gms.	Average N mgs.	Average Yield gms.	Pot number
1.743	2.59	45.2	147.5	9.683	201.2	10.463	33
2.353	5.80	136.4	254.8	11.244			34
3.193	2.84	90.6	98.9	3.713	97.5	3.793	35
2.343	3.10	72.6	96.2	3.873			36
3.663	3.80	139.2			146.4	3.793	37
3.843	4.00	153.7					38
3.323	2.73	90.7			79.2	3.018	39
2.713	2.50	67.8					40
2.223	3.35	74.4	161.7	8.993	168.6	9.388	41
2.143	3.73	79.9	175.6	9.783			42
1.863	2.68	49.9	66.3	2.753	74.5	3.105	43
2.183	2.65	57.7	82.7	3.458			44
2.353	2.91	68.4			82.8	2.463	45
2.573	3.78	97.2					46
4.703	3.65	152.2			108.8	3.658	47
2.613	2.51	65.5					48
2.243	4.43	99.3	358.7	11.033	387.1	11.658	65
3.173	4.29	136.1	415.5	12.283			66
1.103	2.09	23.0	33.8	1.868	31.6	1.875	67
1.063	1.65	17.5	29.4	1.883			68

Table 18a. - Yield and nitrogen uptake of wheat crops in greenhouse experiment

Pot number	1st Crop Yield gms.	%N	Nitrogen uptake mgs.	2nd Crop Yield gms.	%N	Nitrogen uptake mgs.
69	.510	.80	4.0	.170	1.29	2.1
70	.395	.87	3.4	.220	1.13	2.5
71	.300	.90	2.7	.430	2.44	10.4
72	.260	.70	1.8	.780	1.57	12.2
73	.215	.70	1.5	.220	1.90	4.1
74	.210	.70	1.5	.280	1.98	5.5
75	.890	.95	8.4	.145	.80	1.1
76	1.290	.91	11.7	.160	.63	1.0
77	.135	.93	1.2	.115	.87	1.0
78	.255	.64	1.6	.160	.80	1.2
79	.240	.55	1.3	.245	.60	1.4
80	.215	.45	.9	.185	.75	1.3
81	.170	.53	.9	.460	1.20	5.5
82	.165	.51	.8	.155	.78	1.2
83	8.045	1.14	92.3	1.530	.95	14.5
84	6.640	1.16	77.0	1.590	1.28	20.3
85	9.000	3.41	306.9	9.625	2.33	224.0
86	7.700	3.43	264.1	10.505	1.79	188.0
87						
88						
89	9.700	2.67	258.9	5.315	1.19	63.2
90	9.530	2.57	244.9	5.070	1.33	67.4

Table 18a (Continued)

3rd Crop			Totals		Average		
Yield gms.	%N	Nitrogen uptake mgs.	N mgs.	Yield gms.	N mgs.	Yield gms.	Pot number
.623	2.13	13.2	19.3	1.303	20.6	1.340	69
.763	2.11	16.0	21.9	1.378			70
2.201	2.95	64.7	77.8	2.904	78.3	2.978	71
2.203	2.95	64.9	78.9	3.054			72
1.923	2.52	48.4	54.0	2.143	43.5	1.878	73
1.123	2.32	26.0	33.0	1.613			74
.173	1.15	2.0	11.5	1.208	15.0	1.465	75
.273	1.83	5.0	17.5	1.723			76
.243	2.05	5.0	7.5	.493	6.2	.525	77
.143	1.39	2.0	4.9	.558			78
1.613	2.82	45.4	48.1	2.098	55.4	2.531	79
2.563	2.37	60.7	62.8	2.963			80
.923	2.34	21.5	27.9	1.553	19.5	1.213	81
.533	1.73	9.2	11.2	.873			82
1.763	2.90	51.1	157.9	11.337	140.1	10.420	83
1.273	2.00	25.4	122.4	9.503			84
4.073	4.11	167.4	698.5	22.698	635.4	22.038	85
3.173	3.76	119.3	571.4	21.378			86
3.513	4.11	144.3			151.1	3.208	87
2.903	5.44	157.9					88
1.343	3.91	52.5	374.6	16.358	385.7	16.787	89
2.613	3.24	84.6	396.9	17.217			90

Table 18a. - Yield and nitrogen uptake of wheat crops in greenhouse experiment

Pot number	1st Crop Yield gms.	1st Crop %N	1st Crop Nitrogen uptake mgs.	2nd Crop Yield gms.	2nd Crop %N	2nd Crop Nitrogen uptake mgs.
91						
92						

Table 18a (Continued)

3rd Crop Yield gms.	3rd Crop %N	3rd Crop Nitrogen uptake mgs.	Totals N mgs.	Totals Yield gms.	Average N mgs.	Average Yield gms.	Pot number
3.973	3.76	149.3					91
					154.7	4.008	
4.043	3.96	160.1					92

Table 18b. - Yield and nitrogen uptake of alfalfa and succeeding wheat crop *

Pot number	Alfalfa Cuttings 1st Crop	2nd Crop	3rd Crop	Total N uptake mgs.	Total yield gms.
101	2.250	1.895	3.390	217.2	7.212
102	2.300	1.670	2.920	165.9	6.890
103	1.860	1.485	1.885	143.4	5.230
104	2.070	1.805	2.870	201.1	6.745
105	2.610	2.095	3.240	218.5	7.945
106	2.950	2.085	3.350	238.8	8.385
107	2.480	2.485	4.250	260.6	9.215
108	2.340	2.300	2.740	228.8	7.380
109	2.350	2.435	4.690	304.0	9.447
110	1.870	2.070	4.505	252.2	8.445
111	2.600	2.580	4.777	327.3	9.957
112	3.730	3.600	6.350	429.5	13.680
113	2.880	2.140	4.090	250.4	9.110
114	2.280	1.890	3.510	223.6	7.680
115	3.070	2.865	5.540	340.1	11.475
116	2.950	2.695	4.830	301.2	10.475
117	1.720	1.730	3.180	183.7	6.630
118	2.740	2.430	4.715	236.5	9.885
119	2.190	1.805	3.605	203.7	7.600
120	2.410	2.155	3.445	205.0	8.010

* Three cuttings of alfalfa followed by one crop of wheat

Table 18b (Continued)

Alfalfa - Ave.		Wheat		Average		
N. uptake mgs.	Yield gms.	Yield gms.	N. uptake mgs.	Yield gms.	N. uptake mgs.	Pot number
		1.723	39.9			101
191.5	7.001			.968	22.5	
		.213	5.0			102
		2.543	73.4			103
172.2	5.987			2.988	87.5	
		3.433	101.6			104
		1.833	45.4			105
228.0	8.165			2.028	48.4	
		2.223	51.5			106
		2.763	76.8			107
245.7	8.297			2.693	95.3	
		3.163	113.8			108
		2.333	59.2			109
278.1	8.960			2.290	58.1	
		2.253	57.0			110
		3.363	104.2			111
378.4	11.818			3.368	96.2	
		3.373	88.0			112
		1.543	33.7			113
237.0	8.395			2.108	54.0	
		2.673	74.3			114
		3.693	122.2			115
320.6	10.975			3.113	107.2	
		2.533	92.2			116
		.263	7.1			117
210.1	8.258			.318	8.4	
		.373	9.6			118
		1.253	30.8			119
204.4	7.855			1.813	49.0	
		2.373	67.3			120

Table 19. - Average yield and nitrogen uptake by three crops of wheat grown upon various soil amendments

Treatment		1st Crop Yield gms.			1st Crop Nitrogen uptake, mgs		
		N_0	N_1	N_2	N_0	N_1	N_2
Lignified sawdust	H	1.09	9.76	6.99	10.1	237.8	269.9
	L	.39	9.13	6.56	3.9	180.9	247.5
Sawdust	H	.19	4.61	2.38	1.4	60.8	106.9
	L	.45	5.05	10.70	3.7	58.1	274.4
Straw	H	.28	.23	2.62	2.1	11.8	32.6
	L	.20	.39	2.82	2.2	6.5	28.9
Corn stalks	H	.16	.20	7.94	.9	2.4	92.4
	L	.21	.57	5.95	1.5	9.8	69.2
Alfalfa	H			8.35			285.5
	L			9.61			251.9
Check	—	7.34		*.59	84.6		*3.7

* Check plus nitrogen

H=100 grams per pot of residue = 25 tons/acre

L= 50 grams per pot of residue = 12.5 tons/acre

N_0= no nitrogen, N_1 = low level N,

N_2= High level of N, (see Table 17).

Table 19 (Continued)

Treatment		2nd Crop Yield gms.			Nitrogen uptake, mgs.		
		N_0	N_1	N_2	N_0	N_1	N_2
Lignified sawdust	H	.15	5.04	7.20	2.0	73.3	206.1
	L	.39	2.11	6.94	7.5	22.4	186.2
Sawdust	H	.14	.53	4.62	1.1	10.4	154.4
	L	.19	1.00	3.62	2.3	14.1	69.2
Straw	H	.21	1.02	2.94	1.4	20.2	45.6
	L	.60	1.22	1.36	11.3	21.2	22.0
Corn stalk	H	.31	.82	.97	3.4	13.2	18.0
	L	.25	.51	1.25	4.8	10.9	22.3
Alfalfa	H			10.06			206.0
	L			5.19			65.3
Check		1.56		8.37	17.4		246.8

Table 19. - Average yield and nitrogen uptake by three crops of wheat grown upon various soil amendments

Treatment		3rd Crop Yield gms.			3rd Crop Nitrogen uptake mgs.		
		N_0	N_1	N_2	N_0	N_1	N_2
Lignified sawdust	H	.23	.86	3.73	3.7	26.9	166.6
	L	1.13	.76	2.23	20.2	17.8	95.2
Sawdust	H	.19	2.35	2.50	3.7	72.7	98.5
	L	.69	1.88	2.38	14.6	48.9	88.8
Straw	H	2.08	2.45	2.88	53.1	84.3	102.1
	L	2.20	2.31	2.62	64.9	86.1	101.8
Corn stalks	H	.74	2.77	2.04	15.3	81.6	90.0
	L	1.07	2.02	2.18	37.2	53.8	77.2
Alfalfa	H			3.62			143.4
	L			2.98			68.5
Check		1.07		2.71	38.2		117.7

Table 19 (Continued)

	Uncropped Pots*				Total three crops					
	Yield gms		N-uptake		Yield gms			Nitrogen uptake mgs		
	N_1	N_2	N_1	N_2	N_0	N_1	N_2	N_0	N_1	N_2
H	2.82	3.19	134.9	150.2	1.4	16.6	17.8	15.0	338.0	641.5
L	3.63	3.58	147.9	141.3	1.8	12.0	15.7	31.6	221.1	501.7
H	3.42	3.17	99.1	132.3	.5	7.5	9.5	6.2	143.4	359.8
L	2.72	3.06	92.1	96.5	1.3	8.1	16.8	20.6	145.2	442.4
H	3.39	3.65	133.5	130.1	2.5	3.7	8.5	55.4	104.2	180.3
L	3.19	2.66	108.1	78.1	3.0	3.9	6.8	78.9	133.7	152.8
H	3.02	3.75	79.2	146.4	1.2	3.7	10.4	19.5	97.5	201.2
L	3.16	2.46	108.1	78.1	1.8	3.1	9.3	43.5	74.5	168.6
		3.20		151.1			12.0			635.4
		2.01		104.7			16.7			385.7
					10.4		11.6	140.4		387.1

* Treatments cropped only once, after 40 weeks of decomposition.

Table 20. - Daily rate of CO_2 evolution by soils during 10 day incubation period at 35°C.

Treatment or pot number	Production of CO_2 by days of incubation Mgs CO_2/day/100 gm soil*						
	1	2	3	4	5	8	10
			Ferden farm samples				
Check	26.1	14.0	10.5	7.5	7.2	5.1	4.2
1st year	51.8	56.5	60.8	67.3	66.3	52.5	50.8
3rd year	45.0	26.2	17.6	14.7	13.3	10.8	9.5
5th year	53.2	25.4	19.6	14.9	12.5	10.4	8.6
			Greenhouse samples				
1	7.7	6.2	5.3	3.6	3.8	2.7	2,5
2	10.3	7.3	5.1	3.8	6.4	3.1	3.2
3	16.7	12.6	11.2	9.3	6.7	10.5	10.1
4	8.0	6.5	5.7	4.4	4.6	2.8	2.4
5	4.6	3.8	1.5	1.8	1.6	1.8	1.3
6	5.6	4.0	3.5	1.7	2.0	2.4	2.0
33	13.6	8.6	5.0	5.7	5.9	5.5	5.6
37	10.7	6.9	6.4	6.0	5.5	4.1	3.8
49	12.2	7.3	5.2	5.0	3.8	4.0	3.4
50	9.8	6.9	4.3	3.8	4.3	2.9	2.1
51	11.6	8.1	5.3	5.0	4.5	4.5	4.1
52	12.6	7.7	6.7	6.9	5.3	4.2	3.7
53	9.6	5.2	2.2	3.2	2.8	2.2	1.6
54	7.6	4.9	2.2	1.8	2.1	1.7	1.1

* All figures represent average of duplicate determinations

Table 20 (cont.). - Daily and cumulative CO_2 production
Average of replicates

Treatment or pot number	Mgs CO_2/day/100 gms of soil — Days of incubation							Cumulative totals
	1	2	3	4	5	8	10	
Check								89.1
1st year								579.0
3rd year								167.5
5th year								173.7
1								
2	9.0	6.8	5.2	3.7	5.1	3.0	2.9	44.5
3								
4	12.4	9.5	8.4	6.8	5.6	6.6	6.2	75.0
5								
6	5.1	3.9	2.5	1.8	1.8	2.0	1.6	24.0
33								67.5
37								55.5
49								
50	11.0	7.1	4.7	4.4	4.1	3.4	2.8	47.2
51								
52	12.1	7.9	6.0	5.9	4.9	4.3	3.9	57.6
53								
54	8.6	5.1	2.2	2.5	2.4	1.9	1.3	29.2

Table 20 (Cont.) - Daily rate of CO_2 evolution by soils during 10 day incubation period at 35°C.

Treatment or pot number	Production of CO_2 by days of incubation Mgs CO_2/day/100 gm soil*						
	1	2	3	4	5	8	10
65	5.0	2.7	1.6	2.4	3.2	2.0	1.1
66	5.5	3.8	1.5	2.4	2.9	1.5	1.3
75	12.5	8.4	8.5	8.7	9.5	8.2	7.5
76	9.6	8.5	8.2	8.6	8.8	8.2	10.7
77	20.1	15.4	14.8	12.1	12.2	12.2	12.2
78	24.0	18.2	16.2	14.9	15.8	14.5	10.8
81	18.8	15.2	13.6	11.7	11.7	9.2	7.1
83	6.4	3.8	2.7	2.8	2.5	1.7	1.4
84	6.7	3.8	2.6	2.9	2.5	1.6	1.3
85	14.3	7.1	4.9	4.8	4.2	4.1	3.6
86	10.9	8.3	5.2	4.6	4.0	4.1	6.8
87	12.1	5.9	5.2	4.9	4.6	4.2	3.7
88	14.6	6.8	3.8	4.4	4.1	5.6	4.4
103	18.4	15.8	13.6	12.1	11.3	10.9	10.9
113	13.7	12.2	9.7	8.7	8.5	7.4	8.1
115	10.3	7.8	6.2	5.3	4.9	4.4	4.1

* All figures represent average of duplicate determinations

Table 20 (Cont.) - Daily and cumulative CO_2 production
Average of replicates

Treatment or pot number	Mgs CO_2/day/100 gms of soil — Days of incubation							Cumulative totals
	1	2	3	4	5	8	10	
65	5.2	3.2	1.6	2.4	3.1	1.9	1.2	23.5
66								
75	10.9	8.5	8.3	8.6	9.1	8.2	9.1	88.2
76								
77	22.0	16.8	15.5	13.5	14.0	13.3	11.4	144.9
78								
81								112.7
83	6.6	3.8	2.7	2.8	2.5	1.7	1.4	26.1
84								
85	12.6	7.7	5.1	4.7	4.1	4.1	5.2	56.9
86								
87	13.3	6.3	4.5	4.6	4.3	4.9	4.1	55.9
88								
103								123.9
113								91.2
115								56.1

Table 21. - Nitrates and nitrifiable nitrogen in experimental soils by the Iowa test

Treatment or pot number	Nitrate-nitrogen* (Initial extraction)			Nitrifiable nitrogen* (Final extraction)		
	Rep. I	Rep. II	$\bar{x}$	Rep. I	Rep. II	$\bar{x}$
			Ferden Farm Samples			
Check	4	2	3	78	56	67
1st year	4	4	4	0	0	0
3rd year	6	2	4	126	126	126
5th year	1	1	1	99	122	112
			Greenhouse Experiment			
1	58	56	57	28	32	30
2	89	111	100	30	57	44
3	1	3	2	24	28	26
4	57	66	62	44	42	43
5	800	672	736	30	32	31
6	278	288	283	8	3	6
33	32	24	28	48	42	45
37	102	93	98	43	54	49
49	118,118	146,126	129	42,43	33,30	34
50	169	174	172	31	27	29
51	84	81	83	38	43	41
52	74	66	70	35	33	34
53	340,266	360,380	336	42,38	35,31	39
54	408,400	366,460	408	42,30	26,23	40

* Values give in lbs/acre NO_3-N

Table 21 (Continued)

Treatment or pot number	Nitrate-nitrogen* (Initial extraction)			Nitrifiable nitrogen* (Final extraction)		
	Rep. I	Rep. II	$\bar{x}$	Rep. I	Rep. II	$\bar{x}$
65	342,305	288,373	329	32,23	21,24	25
66	204,254	256,236	238	16,6	8,5	9
75	3	0	2	0	0	0
76	0	0	0	0	0	0
77	0	0	0	0	0	0
78	0	0	0	0	0	0
81	3	1	2	42	44	43
83	62,34	50,41	47	36,24	26,25	28
84	38	34	36	25	24	25
85	201,192	136,159	172	63,54	57,60	59
86	83,114	87,114	99	52,54	64,53	56
87	156,114	136,102	129	54,57	48,47	52
88	100	84	92	58	56	57
101	8	6	7	38	41	40
113	53	50	52	38	37	38
115	12	14	13	44	42	43

* Values given in lbs/acre NO_3-N

Table 22: Numbers of bacteria and fungi in Oshtemo sand at various time intervals after treatment

Treatment	Bacteria x 10^6*				Fungi x 10^5*			
	2 wks.	15wks.	25wks.	40wks.	2wks.	15wks.	25wks.	40wks.
LS-W-N_0	56	9	11	21	20	3	4	7
LS-W-N_1	51	34	28	39	17	11	14	14
LS-W-N_2	41	47	11	12	7	2	3	3
LS-W_0-N_2	36	23	63	42	2	9	14	4
SD-W-N_0	36	51	14	18	33	12	5	6
SD-W-N_1	44	29	38	28	5	13	17	8
SD-W-N_2	26	6	20	12	4	2	3	4
SD-W_0-N_2	38	34	35	32	4	3	25	4
ST-W-N_1	246	99	37		16	17	29	
ST-W-N_2	168	88	40		35	4	18	
ST-W_0-N_2					34	5	22	
CS-W-N_1	170	85	36		25	9	3	
CS-W-N_2	153	129	56		27	3	12	
CS-W_0-N_2	179	65	63		40	25	27	
ALF-W-N_0	300	201	300	119	24	23	36	6
ALF-W_0-N_0	294	248	202	142	38	28	25	9
CK-W-N_0	15	15	33	17	1	2	2	1
CK-W plus N	6	2	19	18	.3	.1	4	6

* Counts represent averages of duplicate plates from each of duplicate pots of each treatment (4 plates per count).

Table 23. - A comparison of ignition and dry combustion determinations of carbon is soil containing plant residues *

Treatment or pot number	Dry Combustion ** %C	Ignition Method *** Actual %C	Ignition Method *** Corrected %C
		Ferden Farm	
Check	4.40	3.32	2.64
1st year	4.01	4.34	3.46
3rd year	3.24	4.08	3.25
5th year	3.06	3.99	3.18
		Greenhouse Samples	
1	1.28	1.73	1.38
2	1.51	1.73	1.38
3	2.85	1.69	1.35
4	2.14	1.96	1.57
5	2.19	2.10	1.68
6	2.09	1.72	1.38
33	1.00	1.25	1.00
37	1.08	1.29	1.03
49	1.33	1.36	1.08
50	1.30	1.42	1.14
51	1.34	1.63	1.30
52	1.34	1.43	1.14
53	1.31	1.43	1.14
54	1.32	1.57	1.25

Table 23 (Cont.). - A comparison of ignition and dry combustion determinations of carbon is soil containing plant residues *

Treatment or pot number	Dry Combustion %C	Ignition Method *** Actual %C	Ignition Method *** Corrected %C
65	.73	.95	.76
66	.81	1.00	.80
75		1.71	1.36
76		1.75	1.40
77		1.49	1.19
78		1.56	1.24
81	1.08	1.28	1.02
83	.78	1.04	.83
84	.76	1.04	.84
85	1.07	1.25	1.00
86	1.04	1.27	1.01
87		1.26	1.01
88		1.26	1.01

* Notes on carbon determination:

By selection of a rather uniform sample (in this case number 33) the following method was used for the correction of the carbon content by the ignition method. Sample number 33 was run six times by the dry combustion method so as to obtain as accurately as possible a sample that could be a standard to samples run by the ignition method. This sample was then included in all determinations of carbon run by the ignition method and the actual value for the test samples was corrected by the use of this standard formula:

$$\frac{\text{Wt loss of Standard}}{\text{\% carbon in standard}} : \frac{\text{Wt loss of unknown}}{\text{\% carbon of unknown}}$$

Table 23 (Cont.). - A comparison of ignition and dry combustion determinations of carbon is soil containing plant residues *

An example is given:

$$\frac{1.2539}{1.00} : \frac{4.3498}{x} \qquad x = 3.46\ \%\ \text{carbon}$$

The soil was first dried at 105°C before ignition to eliminate the variation due to water held by the soil and crop residues.

This method was chosen for carbon analysis because of the wide variation encountered with samples taken from the sawdust treatments. With dry combustion only 1-2 gram samples can be used for a determination, but in the ignition method 25-50 grams of soil could be analyzed. The use of such large sample was thought to minimize the great variations of non-uniform residues encountered in the samples. A reference sample has limitations in that it is not desirable when changing from one soil type to another. There is little doubt that the dry combustion method is one of the most accurate for determining carbon content when a sample can be uniformly sampled. For most of the samples taken in this experiment one gram was too small for accurate sampling. Both methods have limitations but the use of a larger sample was thought to eliminate more error in relative values for different treatments than with the use of a smaller sample and the dry combustion method.

** Dry combustion of 1-2 gm sample at 950°C in carbon train

*** Ignition of 50 gm sample at 500°C in muffle furnace

Table 24. - Description of media used for bacterial and fungal plate counts

Modified Formula of Soil Extract - Tryptone Agar (See Ref. 49)
(for bacteria and actinomycetes)

Magnesium Sulfate ($MgSO_4 \cdot 7H_2O$)	0.2 grams
Dibasic Potassium Phosphate (K_2HPO_4)	0.5 grams
Ferric Chloride ($FeCl_3$)	10 mgs.
Tryptone	0.5 grams
Glucose (Dextrose)	1.0 grams
Agar	15.0 grams
Soil Extract Solution	500 mls.
Tap Water	500 mls.

Reaction of media: pH 7.2

Martin's Medium for Fungi (See Ref. 92)

Dextrose	10.0 gm.
Peptone	5.0 gm.
Potassium dihydrogen phosphate	1.0 gm.
Magnesium sulfate	0.5 gm.
Rose bengal	1 part in 30,000 parts of medium
Agar	20.0 gm.
Streptomycin solution	30γ per ml.
Distilled water	1000.0 ml.

ROOM USE ONLY