

SOIL NITROGEN AND CARBON FRACTIONS  
AS RELATED TO ORGANIC AMENDMENTS  
AND RESPONSE OF OATS IN THE GREENHOUSE

Thesis for the Degree of Ph. D.  
MICHIGAN STATE UNIVERSITY  
George William Wright  
1964

This is to certify that the

thesis entitled

Soil Nitrogen and Carbon Fractions  
as Related to Organic Amendments  
and Response of Oats in the Greenhouse

presented by

George William Wright

has been accepted towards fulfillment  
of the requirements for

Ph.D. degree in Soil Science



Major professor

Date February 25, 1964

O-169







## ABSTRACT

### SOIL NITROGEN AND CARBON FRACTIONS AS RELATED TO ORGANIC AMENDMENTS AND RESPONSE OF OATS IN THE GREENHOUSE

by George William Wright

Multiple regression analysis was used to relate short term and long term responses of oats in the greenhouse to experimentally imposed variations in several chemical fractions of carbon and nitrogen in soil.

Lignin was isolated from hardwood sawdust by acid treatment to remove carbohydrates. Oshtemo sand was amended with lignin or sawdust at rates calculated to supply  $2\frac{1}{2}$  tons per acre of lignin in each material (9  $\frac{3}{4}$  tons sawdust, containing 25.6 percent lignin). Urea was added to amended and control soil at rates equal to 0, 200 and 400 pounds N per acre. Nitrogen additions were calculated to give C:N ratios for sawdust of 40:1 and 20:1. Mineral salts were added to assure adequate supplies of major nutrients other than nitrogen.

After 5 weeks' incubation in the greenhouse, growth and nitrogen uptake by oats were determined in triplicate short term (11 days) and long term (54 days) experiments. Similar determinations were made again after 15 weeks, using soil from previously cropped and previously uncropped series of pots.

Soil samples were taken just before planting each crop. Soil nitrogen and carbon were fractionated into water-soluble, exchangeable, acid-hydrolyzable and non-hydrolyzable forms.

Nitrate, other water-soluble nitrogen (presumably ammonium), and exchangeable nitrogen were extensively immobilized in the presence of

sawdust. The immobilized nitrogen appeared primarily in the hydrolyzable fraction. Where urea was added with the sawdust, peak immobilization had occurred by the fifth week. Where no nitrogen was added with sawdust, and in the presence of root residues in previously cropped pots, additional increases in hydrolyzable nitrogen were observed at 15 weeks.

Essentially no immobilization of nitrogen occurred with lignin, although small and statistically significant increases in non-hydrolyzable nitrogen, increasing with level of urea addition, occurred with both lignin and sawdust.

Multiple regression analysis indicated that nitrate, other water-soluble and exchangeable forms of nitrogen were utilized by the first long term crop of oats and that increasing levels of hydrolyzable carbon seriously reduced the availability of all three forms. In the second long term crop, variations in growth and nitrogen uptake were determined largely by the level of nitrate present at planting time, although a significant contribution from the hydrolyzable nitrogen fraction was also indicated.

In the short term growth experiments, nitrate and water-soluble materials were removed by leaching just before placing the soil in contact with the root mat of 15-day old, nitrogen deficient oat plants. In the first experiment, 5 weeks after soil amendment, the production of dry matter was stimulated independently of nitrogen uptake by some factor or factors present in both sawdust and lignin. Multiple regression analysis associated the stimulating activity primarily with the non-hydrolyzable carbon fraction. Nitrogen uptake reflected this

stimulus but was additionally influenced by variations in exchangeable forms of nitrogen.

In the second short term experiment, 15 weeks after soil amendment, similar stimulating effects of lignin and sawdust were observed. There also appeared to be a specific inhibitory effect of root residues in previously cropped soil. In this second crop, neither the stimulatory nor the inhibitory effects were clearly associated with any single fraction of carbon or nitrogen by relationships defined in the regression function.

SOIL NITROGEN AND CARBON FRACTIONS  
AS RELATED TO ORGANIC AMENDMENTS  
AND RESPONSE OF OATS IN THE GREENHOUSE

By

GEORGE WILLIAM WRIGHT

A THESIS

Submitted to  
Michigan State University  
in partial fulfillment of the requirements  
for the degree of

DOCTOR OF PHILOSOPHY

Department of Soil Science

1964



1

### ACKNOWLEDGEMENTS

The author wishes to express his sincere thanks to Professor A. R. Wolcott for encouragement and untiring help during this study. His sincere appreciation goes also to Mrs. Arlene King of the M. S. U. Computer Center.

Additional acknowledgement is due Dr. Cook who made it possible for this work to be carried out.

He is also grateful to his family for their patience and cooperation during the course of this work.

## TABLE OF CONTENTS

I.	INTRODUCTION . . . . .	1
II.	REVIEW OF LITERATURE . . . . .	3
III.	EXPERIMENTAL METHODS . . . . .	17
	A. Design of Greenhouse Experiment . . . . .	17
	B. Short Term Oat Response Study . . . . .	21
	C. Fractionation of Soil Carbon and Nitrogen . . . . .	22
	D. Statistical Treatment . . . . .	24
IV.	EXPERIMENTAL RESULTS . . . . .	26
	A. Effects of Treatment on Soil Variables . . . . .	26
	B. Long Term Responses of Oats . . . . .	34
	C. Short Term Responses of Oats . . . . .	39
	D. Functional Relationships among Crop Variables . . . . .	41
	1. Short term relationships . . . . .	41
	2. Long term relationships . . . . .	49
	E. Functional Relationships among Crop and Soil Variables . . . . .	53
	1. Intercorrelations among soil variables . . . . .	53
	2. Short term crop and soil variables . . . . .	58
	3. Long term crop and soil variables . . . . .	64
V.	SUMMARY AND CONCLUSIONS . . . . .	73
VI.	BIBLIOGRAPHY . . . . .	78

# LIST OF TABLES

<u>Table</u>		<u>Page</u>
1	Description of residue and nitrogen treatments . . . . .	20
2	Effects of residue and nitrogen treatments on soil variables measured prior to the first cropping period. . . .	27
3	Main effects of residue treatments on measured soil variables. . . . .	30
4	Main effects of nitrogen treatments on measured soil variables . . . . .	31
5	Effects of previous cropping, residues, and nitrogen treatments on soil variables measured prior to the second cropping period . . . . .	32
6	Effects of residues and nitrogen treatments on long term crop variables for first cropping period . . . . .	35
7	Effects of residue treatments on measured long term crop variables. . . . .	37
8	Main effects of nitrogen treatments on measured long term crop variables . . . . .	37
9	Effects of previous cropping, residues, and nitrogen treatments on long term crop variables measured in the second cropping period . . . . .	38
10	Effects of residue and nitrogen treatments on short term crop variables for first cropping period . . . . .	40
11	Effects of residue treatments on measured short term crop variables. . . . .	42
12	Effects of nitrogen treatments on measured short term crop variables. . . . .	42
13	Effects of previous cropping, residues, and nitrogen treatments on short term crop variables measured in the second cropping period. . . . .	43
14	Linear, semi-log, and log correlations among soil variables measured prior to the first cropping period. . . . .	54
15	Linear, semi-log, and log correlations among soil variables measured prior to second cropping period . . . . .	56
16	Coefficients of multiple determination ( $\bar{R}^2$ ) for short term crop variables as functions of soil variables in different combinations . . . . .	59

TablePage

17	Coefficients of multiple determination ( $\bar{R}^2$ ) for long term crop variables as functions of soil variables in different combinations . . . . .	65
----	--	----

## LIST OF FIGURES

<u>Figure</u>		<u>Page</u>
1	Functional relationships among short term crop variables. . . . .	45
2	Functional relationships among long term crop variables . . . . .	50
3	Functional relationships among first short term crop variables and soil variables . . . . .	61
4	Functional relationships among long term crop variables and soil variables. . . . .	67
5	Dry weight of the first and second long term oat crops vs. nitrate nitrogen of the soil at planting time . . . . .	71

## INTRODUCTION

Agronomists have been attempting to find ways for estimating the availability of soil nitrogen from the time it was learned that most of the nitrogen taken up by plants is obtained from the soil. The mineralization process by which organic nitrogen is converted into available mineral forms has been rather well defined since about 1890, but as yet, no satisfactory method has been devised for determining the nitrogen supplying power of a soil under field conditions. This fact gives mute evidence to the complexity of nitrogen compounds and nitrogen transformation processes occurring in the soil.

There are several reasons why the nitrogen supplying power of a soil is so difficult to assess. If one attempts to isolate the organic from the inorganic fraction of the soil, rather vigorous methods must be employed. These may cause unexplained and often unappreciated changes in the labile organic fractions. Even if the organic fraction could be separated without drastic changes, not enough is known about its chemistry to translate quantitative measurements into expected rates of release of nitrogen under varying soil and climatic conditions.

Another factor that leads to difficulties in estimating the availability of soil nitrogen is the immobilizing effects of root residues which remain in the soil or of surface residues which may be incorporated into the soil. Other effects of added residues may be wholly unrelated to nitrogen supply, such as effects on moisture or oxygen supply, soil structural effects, and growth regulatory effects which may be either stimulatory or inhibitory.

Biological assays are at present the most useful methods for

estimating the nitrogen supplying power of soils. Among these biological assays are various greenhouse procedures for measuring short term growth or uptake of nitrogen by indicator plants. The objective of this investigation was to attempt to relate, empirically by multiple regression analyses, short term and long term responses of oats in the greenhouse to experimentally imposed variations in several chemical fractions of carbon and nitrogen in the soil. It was hoped that, by measuring both carbon and nitrogen in different fractions, it might be possible to estimate both the potential nitrogen supplying power and the immobilizing potential of carbonaceous constituents.



## REVIEW OF LITERATURE

Norman (31) leads off his chapter on lignin with the following statement. "Despite a great volume of research many aspects of the chemistry of lignin remain obscure and controversial".

Part of the difficulties encountered in the study of lignin lies in the fact that it has been difficult to separate lignin from associated plant constituents without seriously altering the lignin itself. Thus the bulk of the work done on lignin has not been carried out on an unaltered pure substance. Norman goes on to criticise the assumption that "lignin found in nature is more or less uniform and homogeneous". He is led to this conclusion since much literature suggests that lignin derived from different plant species has a different character. Even lignin derived from the same species of plant at different physiological ages may be separated into fractions that differ in their properties. The view has been expressed that there is no compound of lignin as such, but that what has been called lignin is the product of methylated carbohydrates that have been altered by the action of organic acids found in the soil.

This view has not received wide acceptance. Lignin is generally considered to be a complex mixture of compounds with similar structure but differing in side chains or substituting groups or in chain length or degree of polymerization.

Lignin is referred to by Fraser (21) as a polymer formed mainly from units of substituted derivatives of phenylpropane. It has been deduced that lignin derived from plant materials as they break down forms the major constituent of humus. This cannot be accepted as an established fact until more is known about the structure of lignin from

various sources.

Ensminger and Pearson (19) make some reference to the composition of organic residues. They suggest that one-third to one-half of the organic matter found in the soil is microbially derived, if plant decomposition takes place under aerobic conditions. It is stated that lignin or lignin-derived materials make up a major portion of the organic fraction of soils. Gottlieb and Hendricks (22) suggest that soil lignin is very similar to "alkali lignin" and that under natural conditions lignin in the soil undergoes transformations similar to lignin treated with alkali.

Brauns (6) has quoted Hagglund as follows: "In no other field of chemistry does there exist such a discrepancy in opinions as to the constitution of a substance as there is on lignin." Concerning the discrepancies that are found in lignin literature, Brauns goes on to say "by far the principal cause is the fact that lignin is one of the most complicated and elusive natural products known to chemists."

In an attempt to clarify some of the confusion, Brauns has defined the terms "lignin building stones" and "lignin building units". The use of these terms is based on the assumption that the lignin molecule is made up of units in a manner somewhat similar to the units which make up starch and related compounds. The "building stones" of lignin are made up of a phenylpropane carbon structure. Four or five of these building stones are linked together to form a lignin "building unit". A number of lignin building units are combined to make up the lignin molecule. Investigators who have attempted to determine the molecular weight of lignin have reported values all the way from 250 to 11,000. These values vary with the type of lignin, and also with the same lignin preparation using different methods for estimation. At the present time,

the most widely accepted molecular weight for lignin is 840. This corresponds to 5 building stones as a lignin building unit. It should be pointed out that this is in no means a final accepted figure, but it does give a basis for further work.

Broadbent (14) has pointed out that as plant residues are added to the soil, the cellulose and hemicelluloses are readily broken down by the soil microorganisms whereas the lignin is quite resistant. For this reason, the relative proportion in the residue increases. This supports the theory that a large part of the soil organic matter is either lignin or lignin-derived. This is further supported by the similarities between a soil fraction and lignin as they are dissolved in alkali and precipitated by the addition of excess acid.

Gottlieb and Hendricks (22) were unable to isolate definitely characterizable products from soil organic matter as they had been able to from wood lignin, using the same techniques. This would indicate that changes had taken place in plant lignin during decomposition processes in the soil. These two workers hypothesized the production of fused ring structures through a condensation of demethylated molecules. Another factor that points to the dissimilarity between wood lignins and soil organic matter is that wood lignin usually contains more than 60% carbon while the lignaceous fraction of surface soils rarely contains more than 52%. The carbon content in the deeper layers is even less than this. Broadbent (14) suggested that more emphasis should be placed on microbial processes involved in altering soil organic matter. However, he feels that the methods that have been used to gain more understanding of wood lignin should continue to be used as an aid to understanding the lignaceous fraction in soil organic matter.

It has been pointed out by Rodrigues (36) and Bremner (10) that

some of the nitrogen in the soil that had been thought to be in organic combinations, was actually present in the form of ammonia trapped in the clay lattice. Waksman and Iyer (43, 44, 45, 46) postulated the existence of resistant nitrogen compounds which they termed "ligno-proteins". Allison (2) stated that the nitrogen found in humus is very heterogeneous and probably consists of proteins, amino-sugars, nucleic acid, chitin, heterocyclic compounds, and complexed ligno-protein compounds.

Mattson and Koutler-Andersson (29) studied the fixation of ammonia by lignin and found that the greatest fixation occurs under aerobic conditions with simultaneous oxidation and that the fixed ammonia is very resistant to acid and fairly resistant to alkaline hydrolysis. They observed this type of fixation of ammonia liberated by bacteria during the breakdown of nitrogenous organic matter.

Parker et al. (33) applied several materials on the surface or incorporated them into the soil with and without nitrogen fertilizer. Incorporation without fertilizer decreased nitrogen uptake while surface application had little effect. From their data using corn as an indicator crop, it seemed that adequate nitrogen fertilizer incorporated with the residue gave good results, however, no better than the surface application.

The use of sawdust on crops was studied by White et al. (49). Sawdust was used both as a mulch and incorporated into the soil. They found that, as a mulch, it improved crop growth due to moisture conservation. However, it did not show beneficial effects when incorporated. Both types of application caused a decrease in soil nitrate, and, in all cases where sawdust was used, extra additions of nitrogen fertilizer were necessary.

Based on the fact that as much as 30% of the total soil nitrogen is resistant to acid or alkali hydrolysis, Bremner (7) suggested that much of the nitrogen present in the soil is non-protein in nature. He was of the opinion that part of the organic nitrogen was present as heterocyclic compounds. In some humic acid preparations, as much as 60% of the nitrogen was not released by hydrolysis with strong acids (9). Work conducted by Dyck and McKibbin (18) indicated that not all organic nitrogen was determined by the Kjeldahl method. Bremner (7) suggested that this discrepancy could be due to heterocyclic nitrogen compounds containing direct nitrogen to nitrogen linkages. Later Bremner (11) studied the Kjeldahl method of nitrogen determination in great detail. He compared a number of the different modifications and found that the method as outlined by the Association of Official Agricultural Chemists, gave results that were from 20 to 37% low as compared with other methods. He further stated that if the digestion time were increased, satisfactory results could be obtained. In this same paper, Bremner suggested that there are few or no highly refractory nitrogen compounds containing N-N or N-O linkages in soils. This refutes some of his earlier statements (7).

Bremner and Shaw (12) reported that in some soil humic acid preparations, nitrogen was released at a rate intermediate between that of lignin-ammonia and that of lignin-protein complexes. They proposed that neither the lignin-ammonia theory of Mattson and Koutler-Andersson (29) nor the lignin-protein theory as advanced by Waksman and Iyer (43, 44) was adequate and that the best explanation of the observed properties of the humic acid complex fractions of the soil could be explained better by combining both of these theories into a single concept.

Stevenson (40) disagreed with Bremner (8) in that he did not

believe that amino acids isolated from the soil were necessarily structural constituents of proteins. He went on to suggest that the amino acids can be protected from microbial decomposition by adsorption on clay surfaces or by association with soil organic colloids. His data were in agreement with the concept that humic acids may fix amino compounds in the soil.

Mortland and Wolcott (30) have recently made an extensive review of the literature dealing with the fixation of inorganic nitrogen by the soil constituents. A number of methods have been used in the investigation of soil nitrogen compounds, including X-ray diffraction, kinetic and thermodynamic adsorption studies, and infra-red adsorption. From these studies, a number of concepts have been formulated.

The ammonium ion is formed when ammonia is adsorbed by either hydrogen or base-saturated clay minerals. Coordinate covalent amine compounds may be formed if good electron acceptors are present on the exchange complex. Ammoniate combinations, analogous to hydrates, may also be formed. Another way in which ammonia or ammonium may be held in the soil is by hydrogen bonding. Simple physical bonding may be active in the adsorption of ammonia, but this is of a transitory nature and a stronger type bonding is soon formed.

The stability of these nitrogen compounds ranges from water soluble forms to complex heterocyclic compounds from which nitrogen is not released by hydrolysis with strong acids at high temperatures.

A large number of the soil organic matter reactions, including the fixation of nitrogen, are responsive to the oxidation-reduction status of the soil. Relationship between redox potentials and decomposing soil organic matter are very difficult to follow due to the complexity of decomposition processes and the many different factors

that cause the redox potential to vary.

Mortland and Wolcott went on to suggest that it was probable that the incorporation of nitrogen into complex heterocyclic compounds was accidental in that, when nitrogen was present, nitrogen bonds might form between adjacent molecules during condensation and polymerization where, in the absence of nitrogen, oxygen bonds would be expected.

Under alkaline conditions, ammonia fixation takes place rapidly and extensively. The rate and amount of fixation is markedly reduced by lowering the pH of the system. At a low pH the slow fixation that continues is probably due to the continuing exposure of active sites by enzymatic or mineral catalysis of oxidative reactions.

Gaseous nitrogen compounds at an intermediate stage of oxidation-reduction, such as  $N_2O$  and  $N_2$ , have been observed in systems involving the breakdown of organic matter. It has been suggested that a reaction similar to the Van Slyke reaction may take place in the soil. Such an overall reaction might proceed through step-wise reactions, involving intermediate complexes with clays or organic matter, so that the reaction normally observed under drastic laboratory conditions could take place in a field soil. A factor which may promote such step-wise reactions is the polarizing and catalytic effect that clay mineral surfaces exert on organic and inorganic reactants in soil. Such catalytic reactions have been demonstrated under controlled conditions using silica and hydrated iron oxides.

In the breakdown of organic matter, as described by Waksman and Iyer (43), the carbon is used as a source of energy by soil organisms, with part of the carbon and a corresponding proportion of nitrogen being combined in the synthesis of microbial tissue. Part of the nitrogen is thus held in the soil in the form of proteins. As the

organisms die and are subject to decomposition, the protein nitrogen is released as ammonia, which is oxidized to nitrites and nitrates.

Harmsen and Van Schreven (23) pointed out that the liberation in mineral form of organically combined nitrogen by microbial processes in the soil has been recognized since 1893, but these processes have undergone continuing study in an effort to better understand their impact on plant nutrition.

The overall process by which organically combined nitrogen in the soil becomes available to plants has been termed "mineralization". It has been considered to take place in two steps. The first step, termed "ammonification", involves the degradation of protein to give rise to ammonia. The second step, called "nitrification", is the bacterial oxidation of the ammonia, first to nitrites and then to nitrates.

Pinck et al. (35) studied nitrogen availability in soils treated with from 0 to 4 tons of straw per acre. They found that residue addition reduced nitrogen availability. Applied nitrogen fertilizer completely counteracted the effect of the residue. An incubation period following the addition of the residue increased the availability of the soil nitrogen. Similar effects have been reported by others (4, 13, 33).

The recovery of fertilizer nitrogen from soils to which corn and alfalfa residues had been added was studied by Bartholomew and Hiltbold (5). Both materials were ground to pass a 10 mm. sieve and  $4\frac{1}{2}$  g. of plant material were added to pots containing 1800 g. of soil. Part of the pots were incubated and plants were grown on others. After the incubation or growth period, the entire 1800 g. of soil were extracted with normal sodium chloride at pH of 1.0. Aliquots of the extract were analyzed for mineral nitrogen. From their study,



they concluded that plant yields and nitrogen uptake were reduced by the addition of the corn residue. The total recovery of added fertilizer nitrogen ranged from 27 to 54%.

Bartholomew (4) presented information showing that decomposition of organic matter and the release of available nitrogen was dependent on such things as crop, cropping sequence, soil texture, structure, and moisture conditions during the actual growing season of the crop.

It has been reported by Woodruff (52) that the nitrogen uptake from a particular soil was influenced by the type of crop grown. He reported twice as much nitrogen delivered to corn as to a small grain. One might consider here that these two crops grow at different seasons, and that corn growing during the warm season would be expected to have a greater uptake than a cool season crop such as the small grain due to seasonal differences in rates of mineralization and plant metabolism.

Smith (39) found that soil texture was an important modifier of nitrogen release from the soil. He reported that a clay or clay loam would release  $1\frac{1}{4}$  to  $2\frac{1}{2}\%$  of its total nitrogen in one season, a silt loam  $1\frac{1}{2}$  to  $3\%$ , and a sand or sandy loam 4 to 5%. He also stressed the important influence of moisture and temperature.

Harmson and Van Schreven (23) reviewed the work of Norman and Werkman (1943), Broadbent and Norman (1947), Broadbent (1948), Broadbent and Bartholomew (1949), and Thornton (1946) in their discussion concerning the claims that the stable portions of the soil organic matter were subjected to fresh attack by microorganisms upon the addition of a fresh organic residue such as a green manure crop. Löhnis had reported such findings as early as 1926. The conclusion drawn by Harmson and Van Schreven was that the addition of fresh energy material to the soil caused such an increase in numbers and activity of

the soil microbial population that some of the "stable" organic matter was broken down along with the fresh material. They suggested that the "stable" humus was much less stable than had been supposed, and that its rate of decomposition was due in part to insufficient microbial activity. The addition of fresh material was spoken of as a "forced draft on the smoldering bacterial fire". These conclusions support the opinion of Broadbent (13) that a large part of the nitrogen taken up by a crop following a green manure crop has its origin in the "stable" soil organic fraction rather than the freshly added plant residue.

Harmson and Van Schreven (23) cited the work of Barnes (1953) on the mineralization rate of plant residues added to the soil. During the incubation in soil of mustard, vetch, and grain straw, only straw, with its wide carbon-to-nitrogen ratio, inhibited the mineralization of nitrogen for the entire 89 weeks of the incubation experiment.

Waksman and Tenney (47) criticised the use of only the carbon-to-nitrogen ratio when predicting the mineralization rate of applied organic amendments. They carried out a study comparing lignin, cellulose, and sugars as organic amendments. The lignin had only a slight effect on mineralization rates whereas both sugar and cellulose had an inhibitory influence. Waksman and Tenney (48) also studied mineralization rates as affected by the physiological age of plants making up organic residues. Older plants retarded the mineralization rate more than younger plants. Two reasons for this were given: the older plants had a higher lignin content and, also, a wider carbon-to-nitrogen ratio. They concluded that if a plant residue had a nitrogen content of 1.7%, it was just sufficient to cover the nitrogen requirements of microorganisms during the period of decomposition. If the nitrogen content were below 1.7%, nitrogen would have to be taken

from other sources and would cause a serious shortage of available nitrogen in the immediate area.

Harmsen and Van Schreven (23) have considered the nitrogen content required in plant residues added to the soil if tie-up of soil nitrogen is to be averted. They have suggested that the organic material added should contain from 1.5 to 2.5% nitrogen. They further stated that if organic matter added to the soil has a carbon-to-nitrogen ratio wider than 20 to 1, then the release of mineral nitrogen will not occur until the carbon-to-nitrogen ratio reaches approximately 20 to 1. This would imply that the nitrogen content of added residue should be about 2.5% if mineralized nitrogen is to be released during the early stages of decomposition.

Seasonal variations in the mineral nitrogen content of a heavy loam soil in Holland were reported by Harmsen and Van Schreven. In a fallow soil, the mineral nitrogen content rose in the spring, stayed at a high level during the summer, and decreased in the fall. The decrease in the fall was explained by slower production of mineral nitrogen during the cooler season, and by leaching caused by fall precipitation. In the case of a soil under grass vegetation, the mineral nitrogen content remained at a very low level throughout the entire year. This was caused by the continued crop use of the nitrogen as it was made available.

The correlation of nitrogen uptake by plants with various soil tests for available nitrogen was studied by Peterson et al. (34). They found that, under greenhouse conditions, initial nitrate nitrogen content accounted for 94% of the variation in nitrogen uptake in the first cropping period. This reliability did not hold for a second crop.

An inverse relationship between the ratio of hydrolyzable carbon to hydrolyzable nitrogen and the ratio of total nitrogen to hydrolyzable nitrogen was reported by Kamerman and Klintworth (27).

Lyon et al. (28) reviewed reports of various workers and observed that as much as 45 pounds of nitrogen per acre per year could not be accounted for by crop removal, leaching, or erosion. They suggested that at least a part of this was lost through the formation of volatile nitrogen compounds.

Nitrogen losses have been studied by Hiltbold and Adams (24). They worked on soil acidity changes caused by applied nitrogen fertilizer. Among other things, they found that when urea was applied to soils having a pH above 7.0, a large loss of nitrogen occurred during a three-month incubation period. In this investigation, glucose was added as an energy source for soil microorganisms.

Clark et al. (16) also found that soils with a pH range from 7.0 to 7.5 were subject to losses of nitrogen added as urea. They reported losses as high as 40% of the applied nitrogen.

Harmson and Van Schreven (23) considered that the loss of nitrogen through the volatilization of ammonia might be quite high in some instances. They pointed out that the loss was greatest in waterlogged soils with a high pH. It might be noted here that these conditions often occur in greenhouse culture, and that losses probably occur. They also reported that the risk of nitrogen loss increased with temperature, pH, calcium carbonate content, and a decrease in soil moisture. Mineral or organic colloids, if present in substantial amounts, could prevent or limit these losses due to their ability to adsorb ammonia.

Mann and Barnes (1951), as reviewed by Harmson and Van Schreven,

found that no more than 40 to 51% of added nitrogen could be accounted for at the end of an experiment in which two successive crops of barley were grown on well aerated soils under greenhouse conditions. Their experiments covered a total time of 18 months.

Allison (1) has given a comprehensive review of a large number of investigations in which attempts have been made to account for all nitrogen in a system during crop production. In some of these experiments, only about half of the nitrogen could be accounted for. Allison considered that at least a part of this nitrogen was lost by volatilization. Several mechanisms for volatile nitrogen loss were discussed. Ammonia losses from soils with a pH of 6 to 7 were barely detectable, however, losses at a higher pH were marked. Wet soils lost little ammonia but losses were very high as the soil dried if the pH was above 7. Temperature increases were found to increase volatilization rates. Soils with a low exchange capacity lost more ammonia than a soil with a high exchange capacity under similar physical conditions. Ammonia losses tended to be high, even in acid soils, where nitrogenous organic matter was undergoing rapid decomposition.

Chemical reduction of nitrogenous compounds and the loss of nitrogen as nitric oxide or nitrogen gas was also considered by Allison. He concluded that this type of loss was of minor importance. The recent review by Mortland and Wolcott (30) suggests that some elemental nitrogen may be lost from the soil. Further work is necessary to evaluate this pathway of nitrogen loss from the soil.

Bacterial denitrification and nitrogen loss was mentioned by Allison. The presence of free oxygen greatly reduces bacterial denitrification and it has been thought that this type of nitrogen loss was completely inhibited in moderately well aerated soils. Recent work has

indicated that bacterial denitrification may continue in the presence of molecular oxygen and that this type of nitrogen loss may be of some importance. Proper evaluation of this type of nitrogen loss will require techniques with smaller sampling errors than those currently in use.

Schwartzbeck et al. (37) measured the loss of gaseous nitrogen from soil in the forms of  $N_2$  and  $N_2O$  using infrared and mass spectroscopy. They found that there were substantial losses from saturated soils, and smaller losses from soils at field capacity. Nitrogen fertilizer was added in both nitrate and ammonia forms, and it was found that losses were markedly influenced by the type of fertilizer added.  $N_2O$  losses were highest when a 50-50 mixture of nitrate and ammonia nitrogen were added. Different soil types lost different amounts of nitrogen under similar conditions.

## EXPERIMENTAL METHODS

### Design of Greenhouse Experiment

The objective of the experiment was to relate short term and long term response of oats to various chemical fractions of nitrogen and carbon in the soil. Johnston (26) had shown that large variations in soluble, acid-hydrolyzable and non-hydrolyzable forms of nitrogen in soil resulted from the addition of sawdust and lignin isolated from sawdust, together with varying proportions of urea nitrogen. Large differences associated with treatment were still apparent after 40 week' incubation in the greenhouse. Additional variations resulted when wheat was grown during the incubation period. Similar amendments and a cropping variable were used in the present study to impose a suitable range of variation in measured carbon and nitrogen fractions for multiple correlation with various response measurements on short term and long term crops of oats. It was hoped that, by measuring fractional forms of carbon as well as nitrogen, it would be possible to identify forms of nitrogen which contribute uniquely to the available supply and forms of carbon which contribute uniquely to immobilization and reduced availability of nitrogen.

Mixed hardwood sawdust was obtained from the Michigan State University experimental sawmill. It was dried at 50° C. and ground in a Wiley mill to pass a 20-mesh screen.

Lignin was prepared from an aliquot of this sawdust by acid extraction of cellulose and other carbohydrates, as described by Brauns (6). Two-hundred-fifty grams of ground sawdust were added to 1 liter of 72 percent sulfuric acid at 15° C. The mixture was stirred to avoid the formation of lumps. The mass first turned green, then

black. After a reaction time of 15 minutes, the mass became fluid. The digestion mixture was then diluted with 2 liters of distilled water and filtered onto hardened filter paper in a Buchner funnel. The residue was washed with 500 ml. of 3 percent sulfuric acid and then taken up in 1 liter of 3 percent sulfuric acid and refluxed on a hot plate for four hours. During this period, the color changed from nearly black to a brown color. The residue in the reflux chamber was then filtered, washed free of acid, and resuspended in 1 liter of 0.5 percent hydrochloric acid. After heating for 12 hours on a water bath, it was again filtered, washed with distilled water and dried at 105° C. The resulting cake of lignin was ground in a Wiley mill, using a 20 mesh screen, and stored for later use.

A soil low in organic matter was used so that changes in nitrogen and carbon due to treatment would be more readily detected through use of larger soil aliquots than would be possible with a soil already high in carbon and nitrogen. The soil chosen was an Oshtemo sand with an exchange capacity of 3.5 me. per 100 g., and containing 0.4 percent carbon and 0.023 percent nitrogen. Soil was obtained in the fall of the year, dried, put through a 1/4-inch screen, thoroughly mixed and stored for future use.

Soil tests showed the following nutrient status: pH, by glass electrode in a 1:1 water suspension, was 5.8; available P using Bray's weak acid extractant ( $.025 \text{ N HCl}$ ,  $.03 \text{ N NH}_4\text{F}$ ) was 2.5 ppm.; K at 48 ppm., Ca at 350 ppm., and Mg at 8 ppm. - all exchangeable to  $\text{N NH}_4\text{OAc}$  at pH 7.0. At potting time, mineral amendments were made to insure adequate supplies of major nutrients other than nitrogen. To 12,000 g. of soil in each 3-gal. pot, the following were added:



Ca (OH) <sub>2</sub>	6.0 g.
Ca HPO <sub>4</sub> • 2H <sub>2</sub> O	3.0 g.
MgO	1.2 g.
KCl	3.0 g.

At the same time, organic amendments and urea were added according to the schedule in table 1. Immediately after potting, the soil moisture level was brought to about 80 percent of field capacity with distilled water. This moisture level was maintained by bringing the pots to the constant predetermined weight with distilled water every other day, or more frequently as needed.

The treatments in table 1 were established in two series of triplicate pots for each treatment. After incubating for 37 days, one series of pots was planted to oats. Sixty-eight day later (105 days after treatment) oats were planted again, this time in both series of pots. Populations were adjusted shortly after emergence to 15 plants per pot. Both crops were grown for 54 days, at which time the oats were fully headed. The above-ground portions of the plants were harvested. Fresh weights were taken, and dry weights after drying at 60° C. The dry material was ground and its nitrogen content determined by macro-Kjeldahl procedure, using salicylic acid to include nitrate.

Following the removal of the first crop, the soil in the 27 pots of the first series was removed. The roots were separated, dried, crumbled by hand and remixed with the soil. The soil was returned to the pots and allowed to incubate two weeks before the second crop was planted.

Just prior to planting each crop, a 500-g. sample of soil was taken from each pot for the short term oat response studies and for

Table 1. - Description of residue and nitrogen treatments.

Treatment		Residue		Carbon added	Nitrogen Added			C/N Ratio
No.	Code	Material	Rate of addition ppm.		In residue ppm.	As urea ppm.	Total ppm.	
1	R <sub>1</sub> N <sub>1</sub>	Control	-	-	-	0	0	-
2	R <sub>1</sub> N <sub>2</sub>	Control	-	-	-	106	106	-
3	R <sub>1</sub> N <sub>3</sub>	Control	-	-	-	213	213	-
4	R <sub>2</sub> N <sub>1</sub>	Lignin*	2500	1500	6	0	6	242
5	R <sub>2</sub> N <sub>2</sub>	Lignin	2500	1500	6	100	106	14
6	R <sub>2</sub> N <sub>3</sub>	Lignin	2500	1500	6	207	213	7
7	R <sub>3</sub> N <sub>1</sub>	Sawdust	9750	4202	16	0	16	269
8	R <sub>3</sub> N <sub>2</sub>	Sawdust	9750	4202	16	90	106	40
9	R <sub>3</sub> N <sub>3</sub>	Sawdust	9750	4202	16	197	213	20

\* Lignin added in quantities equivalent to that added in sawdust, which yielded 25.6 percent lignin by sulfuric acid digestion.

the fractionation of soil carbon and nitrogen, which are described in succeeding sections. Soil tests for pH and nutrient status were also made on these samples. Soil tests after the first crop averaged 55 ppm. P, 60 ppm. K, 325 ppm. Ca and 150 ppm. Mg. Soil tests at the same time in the uncropped series averaged 60 ppm. P, 110 ppm. K, 440 ppm. Ca and 150 ppm. Mg. Differential declines in P and K with different treatments due to differences in crop removal or other treatment effects were adjusted for by appropriate additions of these nutrients to the first series of pots prior to planting the second crop.

Initial addition of bases exceeded exchange capacity and gave rise to a soil pH of 7.5 in unamended soil. Release of  $\text{NH}_3$  from urea resulted in additional pH increases up to 8.0. Soil pH declined rapidly as ammonium was nitrified. However, these declines were less rapid in sawdust-treated soil and in the absence of cropping. After the first crop, pH in control and lignin-treated soils ranged from 6.6 to 7.0; with sawdust pH ranged from 7.0 to 7.4. The lower values in each case were associated with previously cropped pots and those receiving the higher additions of urea.

Pots were randomly located in the greenhouse and rotated periodically to minimize position effects and allow for statistical analyses in accordance with a completely random design.

#### Short Term Oat Response Study

The short term responses of oats were observed, using fresh soil samples taken just prior to the planting of each long term crop.

The availability of nitrogen in non-water-soluble fractions was of primary concern in these short term studies. For this reason, 200-g. aliquots of soil were thoroughly leached (soil: leachate = 1:6)

before being exposed to the root mat of 15-day-old, nitrogen-deficient oat plants, in accordance with the procedure described by De Ment et al. (17). The above-ground portions of the plants were harvested after 11 days' contact with the soil. Green weight was determined, and dry weight after drying at 50° C. Kjeldahl nitrogen was determined, using salicylic acid to include nitrate.

#### Fractionation of Soil Carbon and Nitrogen

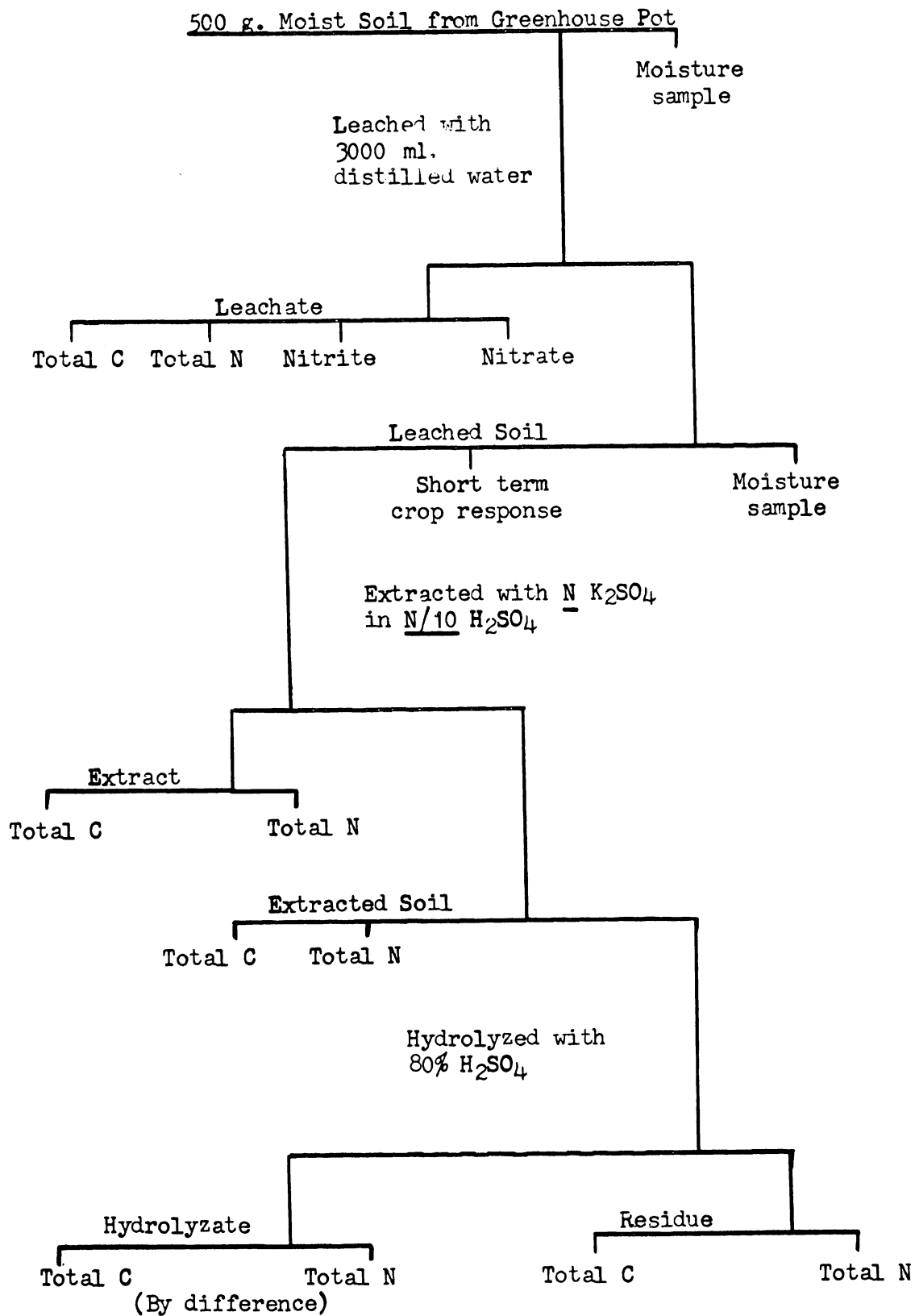
A flow diagram for the soil fractionation procedure will be found on the following page. Total carbon, total nitrogen, nitrite and nitrate were determined in a 1:6 water leachate of the fresh soil. The leachate was brought to a boil, cooled quickly and stored at 4° C. until the analyses could be completed.

Aliquots of the leached soil were used for moisture determination and for the short term response study. The balance was divided into duplicate samples and quick frozen until the remainder of the fractionation could be undertaken according to procedures described by Singh (38).

"Extractable" nitrogen and carbon represent compounds exchangeable to  $\underline{N}$   $K_2SO_4$  at pH 1.5 to 2.0 ( $\underline{N}/10 H_2SO_4$ ). Two-hundred ml. of this extracting solution were added to each of duplicate 110 g. aliquots of the frozen soil and shaken at room temperature for 30 minutes. The extract was recovered by filtration and combined with three distilled water washes to give a final volume of 500 ml. Total carbon and nitrogen were determined.

The extracted soil was dried at 70° C. for 10 hours and its total carbon and nitrogen determined. Twenty-five g. of the dried soil, in duplicate, was digested in 80 ml. of 80% sulfuric acid at room

## FLOW DIAGRAM FOR SOIL FRACTIONATION



temperature for 2 hours, with frequent shaking. The volume was then made up to 350 ml. with distilled water and autoclaved at 15 pounds pressure for four hours in a flask fitted with a Bunsen valve. After cooling, the residue was collected on filter paper in a Buchner funnel. "Non-hydrolyzable" nitrogen and carbon were determined in the residue. "Hydrolyzable" carbon and nitrogen were calculated by difference.

Total carbon in the whole soil and in the various fractions was determined by wet combustion and gravimetric measurement of  $\text{CO}_2$  as described by Allison (3). Total nitrogen was determined by macro-Kjeldahl methods described by Jackson (25), using  $\text{CuSO}_4$  and  $\text{HgO}$  as catalysts. Thiosulfate was added with the alkali during distillation to reduce mercuric compounds. Methyl purple was used as indicator.

Analyses for nitrate in the water leachate were made, using the phenoldisulfonic acid procedure as described by Jackson (25). Nitrite analyses followed the naphthylamine-sulfanilic acid method described by Fraps and Sterges (20).

Duplicate samples of soil from each pot were carried through the entire fractionation and analyzed separately. The mean of duplicate determinations was used as the unit observation for each pot.

#### Statistical Treatment

All experimental values were subjected to analyses of variance in accordance with a completely random design with three replications. The significance of mean differences was tested by multiple range and multiple F tests according to Duncan.<sup>1</sup> Multiple correlation and

---

<sup>1</sup> Duncan, D. B. Multiple range and multiple F tests. Biometrics. Vol. II. pp. 1-42. 1955.

regression analyses made use of an exponential-power function described by Halter et al. (15) and proposed for use in fertilizer input-output studies by Sundquist and Robertson (41). This function is of the following form:

$$\text{Log } Y = a + b_1 \log X_1 + c_1 X_1 + b_2 \log X_2 + c_2 X_2 + \dots + b_i \log X_i + c_i X_i$$

The use of this function has some advantages over the use of a polynomial. It is, in reality, a cross-product function:

$$Y = aX_1^{b_1} c_1 X_2^{b_2} c_2 \dots X_i^{b_i} c_i$$

As a result, a degree of interaction between independent variables is expressed without the loss of degrees of freedom which occurs when separate cross-product terms are introduced in polynomial functions. The function also allows for polymodal changes in direction of the response curve. For these reasons it was anticipated that more useful multiple correlations and more highly significant regression coefficients might be obtained among a relatively large number of variables using relatively few observations.

## EXPERIMENTAL RESULTS

### Effects of Treatment on Soil Variables

The first soil samples were taken five weeks after treatment, just before planting the first crop of oats. Soil fractionation data for this sampling are presented in Table 2.

Increasing additions of urea nitrogen were reflected by increases in nitrate, other water-soluble and salt-extractable forms of nitrogen in control soil and soil amended with lignin.

In the presence of sawdust, these forms of nitrogen were sharply reduced. This was likely due to microbial immobilization, since corresponding increases occurred in the hydrolyzable fraction. Changes in the non-hydrolyzable nitrogen fraction were small, but significantly higher levels were obtained with lignin and sawdust than in the control. This suggests that chemical complexing of ammonia by lignaceous constituents may have occurred to a limited extent.

Carbon added as lignin appeared primarily in the non-hydrolyzable fraction, whereas the addition of sawdust was reflected in both the hydrolyzable and non-hydrolyzable carbon fractions. Changes in C:N ratio reflected both residue and nitrogen treatments.

The nature of water-soluble nitrogen other than nitrate in table 2 was not determined. Only traces of nitrite (less than 2 ppm.) were found. No carbon was found in the water leachate, so it is assumed that this fraction was principally ammonium. It is unlikely that any urea would have remained after five weeks. The salt-extractable nitrogen was largely exchangeable ammonium, although some organic compounds were included, since a small amount of carbon appeared in the potassium sulfate extract. Nitrogen in these two fractions had been extensively



Table 2. - Effects of residue and nitrogen treatments on soil variables

Treatment*	NO <sub>3</sub> -N	Water Sol.	K <sub>2</sub> SO <sub>4</sub> Ext.		Hydrolyzable	
	N**	N**	N	C	N	C
	ppm.	ppm.	ppm.	ppm.	ppm.	ppm.
Control						
N <sub>1</sub>	19 abc	4 c	10 de	9 ab	198 b	1426 f
N <sub>2</sub>	17 abcd	25 bc	14 bcd	6 c	218 b	1469 ef
N <sub>3</sub>	26 a	80 a	32 a	7 bc	211 b	1321 fg
Lignin						
N <sub>1</sub>	12 bcd	3 c	7 e	10 a	209 b	1826 d
N <sub>2</sub>	25 a	21 bc	16 bc	7 bc	212 b	1213 g
N <sub>3</sub>	22 ab	76 a	30 a	6 c	207 b	1598 e
Sawdust						
N <sub>1</sub>	1 e	2 c	9 de	8 bc	200 b	2336 b
N <sub>2</sub>	7 de	7 c	11 cde	7 bc	258 a	2818 a
N <sub>3</sub>	11 cd	46 b	19 b	8 abc	257 a	2137 c

\* N<sub>1</sub> = no nitrogen. N<sub>2</sub> and N<sub>3</sub> = nitrogen added to give C:N ratios for

\*\* Other than nitrate or nitrite.

a, b, c, --- g. Ranges of equivalence. For a given soil variable, different at 5 percent.

measured prior to the first cropping period.

Treatment	Non Hydrolyzable		Total Non Water Soluble		
	N	C	N	C	C/N
	ppm.	ppm.	ppm.	ppm.	
Control					
N <sub>1</sub>	45 e	1248 f	250 e	2584 d	10.6 e
N <sub>2</sub>	51 d	1510 e	282 bcd	2986 d	10.6 e
N <sub>3</sub>	51 d	1472 e	294 b	2801 d	9.5 e
Lignin					
N <sub>1</sub>	58 bc	2704 cd	274 cd	4541 c	16.6 bc
N <sub>2</sub>	59 bc	2917 bc	288 bc	4136 c	14.4 d
N <sub>3</sub>	60 abc	2586 d	296 b	4190 c	14.2 d
Sawdust					
N <sub>1</sub>	56 c	3240 a	266 de	5585 ab	21.0 a
N <sub>2</sub>	62 ab	3156 ab	332 a	5981 a	18.0 b
N <sub>3</sub>	65 a	3160 ab	341 a	5305 b	15.6 cd

sawdust of 40:1 and 20:1 respectively.

numerical values with a common literal subscript are not significantly

converted to nitrate 68 days later when the second sampling was made at the beginning of the second cropping period (tables 3 and 4). Some additional immobilization of nitrogen in the hydrolyzable fraction also occurred with sawdust during this period (table 3). As a result there was little change in total non-water soluble nitrogen with sawdust, whereas some loss of insoluble forms occurred in the controls and in lignin-amended soil.

There was a marked change in the nature of carbonaceous soil materials during this time interval as shown by net decreases in the non-hydrolyzable fraction and net increases in the less resistant hydrolyzable forms. Extensive losses of carbon with the sawdust treatment occurred primarily from the non-hydrolyzable fraction (table 3), and these losses were enhanced by increasing levels of applied nitrogen (table 4). In the case of the control and lignin treatments, net increases in total carbon reflected additions in the form of root residues from oats grown, and these additions appeared primarily in the hydrolyzable fraction.

Removal of nitrogen by the first crop of oats was reflected in levels of nitrate remaining for the second crop (table 5). A portion of the nitrogen removed by oats remained in the soil in the form of root residues and appeared primarily in the hydrolyzable fraction. The apparent contribution of both carbon and nitrogen to this fraction from oat root residues was greater in soil amended with lignin or sawdust and increased with increasing level of applied nitrogen.

The greatest residual accumulations of nitrogen in organic forms at this time were found in the sawdust treated soil. The principal mineral form at this time was nitrate which was depressed in the presence of sawdust as compared with the control and lignin treatments.

Table 3. - Main effects of residue treatments on measured soil variables.

Treatment	NO <sub>3</sub> -N Water Sol.		K <sub>2</sub> SO <sub>4</sub> Extr.		Hydrolyzable		Non Hydrolyzable		Total Non Water Soluble	
	ppm.	N* ppm.	N ppm.	C ppm.	N ppm.	C ppm.	N ppm.	C ppm.	N ppm.	C/N
First Cropping Period										
Control	21 a	36 a	19 a	7 a	209 b	1405 c	49 b	1410 c	275 c	2790 c
Lignin	20 a	33 a	18 a	8 a	209 b	1546 b	59 a	2736 b	286 b	4289 b
Sawdust	6 b	18 b	13 b	8 a	238 a	2430 a	61 a	3185 a	313 c	5624 a
Second Cropping Period										
Control	61 a	6 a	11 a	7 b	189 b	1710 b	49 b	1364 c	249 c	3086 b
Lignin	67 a	6 a	10 a	6 c	202 b	1724 b	57 a	2620 a	267 b	4356 a
Sawdust	33 b	6 a	9 a	8 a	254 a	2494 a	56 a	2079 b	319 a	4582 a

\* Other than nitrate or nitrite.

a, b, c. Ranges of equivalence. For a given soil variable, numerical values within a cropping period with a common literal subscript are not significantly different at 5 percent.



Table 5. - Effects of previous cropping, residues, and nitrogen treat-

Treatment *	NO <sub>3</sub> -N		Hydrolyzable N	
	Uncropped	Cropped	Uncropped	Cropped
	ppm.	ppm	ppm.	ppm.
Control				
N <sub>1</sub>	23 ef	6 ef	196 cde	177 e
N <sub>2</sub>	86 bc	20 ef	194 de	181 de
N <sub>3</sub>	163 a	66 cd	196 cde	188 de
Lignin				
N <sub>1</sub>	16 ef	8 ef	193 de	180 de
N <sub>2</sub>	111 b	17 ef	213 cde	218 cd
N <sub>3</sub>	181 a	67 cd	208 cde	197 cde
Sawdust				
N <sub>1</sub>	1 f	2 f	233 bc	215 cde
N <sub>2</sub>	45 e	13 ef	259 ab	284 a
N <sub>3</sub>	103 bc	31 ef	260 ab	270 ab
Means for Cropping	81 a	24 b	217 a	212 a

\* N<sub>1</sub> = no nitrogen. N<sub>2</sub> and N<sub>3</sub> = nitrogen added to give C:N ratios for a, b, c, --- e. Ranges of equivalence. Within a pair of columns for a significantly different at the 5 percent level.

ments on soil variables measured prior to the second cropping period.

Treatments	Hydrolyzable C		Non Water Soluble N	
	Uncropped	Cropped	Uncropped	Cropped
	ppm.	ppm.	ppm.	ppm.
Control				
N <sub>1</sub>	1710 bcd	1891 bcd	254 de	241 e
N <sub>2</sub>	1713 bcd	1783 bcd	257 cde	240 e
N <sub>3</sub>	1455 d	1710 bcd	252 de	248 de
Lignin				
N <sub>1</sub>	1628 bcd	1567 cd	260 cde	243 e
N <sub>2</sub>	1342 d	2247 abcd	284 cd	285 cd
N <sub>3</sub>	1646 bcd	1917 bcd	272 cde	260 cde
Sawdust				
N <sub>1</sub>	3095 a	2216 abcd	294 bc	274 cde
N <sub>2</sub>	2481 abc	2117 bcd	326 ab	347 a
N <sub>3</sub>	2473 abc	2586 ab	333 a	337 a
Means for Cropping	1949 a	2004 a	281 a	275 a

sawdust of 40:1 and 20:1 respectively.

given soil variable, numerical values with a common subscript are not

Nevertheless, the peak immobilization effect of sawdust had been expressed earlier where nitrogen had been applied. Nitrate was now accumulating where the initial C:N ratio had been 40:1 ( $N_2$ ), as well as at C:N = 20:1 ( $N_3$ ).

#### Long Term Responses of Oats

Two successive crops of oats were planted on one series of pots. A second series was uncropped during the first cropping period. The first crop was planted five weeks after addition of residue and nitrogen amendments. The second crop was planted 68 days later. Both crops were harvested 54 days after planting when the oats were fully headed out.

Determinations made on above-ground portions of the first crop are presented in table 6. In sawdust treated soil, there was very little growth of oats after germination where no nitrogen was applied. Nitrogen added with sawdust to give a C:N ratio of 40:1 produced yields equal to those in the control without nitrogen. Yields were practically doubled when additional nitrogen was used to give a C:N ratio of 20:1. Nevertheless, yields were significantly less with sawdust at all levels of nitrogen than in controls or with lignin. Lignin had no effect on yields, but it did reduce percent nitrogen and nitrogen uptake at the higher level of nitrogen addition. Increasing nitrogen additions increased succulence (decreased percent dry matter) and increased percent nitrogen. Both sawdust and lignin reduced succulence when added without nitrogen. Sawdust markedly reduced percent nitrogen and nitrogen uptake at all levels of applied nitrogen.

The depressing effect of sawdust on yields, nitrogen content and



Table 6. - Effects of residues and nitrogen treatments on long term crop variables for first cropping period.

Treatment*	Green Weight g.	Dry Weight mg.	Dry Matter %	Nitrogen %	Nitrogen Uptake mg.
Control					
N <sub>1</sub>	95 d	19.1 c	20.1 bc	1.09 d	.209 f
N <sub>2</sub>	212 abc	39.4 a	18.5 bc	2.09 b	.823 c
N <sub>3</sub>	226 a	39.8 a	17.7 c	2.60 a	1.035 a
Lignin					
N <sub>1</sub>	87 d	19.1 c	22.1 ab	1.08 d	.207 f
N <sub>2</sub>	209 bc	39.6 a	18.9 bc	2.13 b	.842 c
N <sub>3</sub>	223 ab	41.8 a	18.8 bc	2.29 b	.957 b
Sawdust					
N <sub>1</sub>	3 e	0.8 d	24.5 a	0.75 e	.006 g
N <sub>2</sub>	96 d	19.9 c	20.6 abc	1.35 c	.270 e
N <sub>3</sub>	200 c	35.2 b	17.6 c	2.19 b	.768 d

\* N<sub>1</sub> = no nitrogen. N<sub>2</sub> and N<sub>3</sub> = nitrogen added to give a C:N ratio for sawdust of 40:1 and 20:1 respectively.

a, b, c, --- e. Ranges of equivalence. For a given crop variable, numerical values with a common literal subscript are not significantly different at 5 percent.

nitrogen uptake were still apparent in the second crop (table 7). Residual responses to the initially applied nitrogen were also expressed on the second crop (table 8). The general level of yields in the second crop was about one-half that in the first crop. This appears to have been due to the fact that the second crop was grown during the long, hot days of summer. These are conditions unfavorable for a cool season crop such as oats. Nitrogen does not appear to have been limiting at the higher levels of nitrogen addition.

The nitrogen responses shown in table 8 represent overall averages. The response of the second crop to residual nitrogen from the initial applications was strongly influenced both by residue treatment and by previous cropping. Dry matter yields and nitrogen uptake for previously cropped and uncropped pots are presented in table 9.

Soil tests showed that phosphorus and potassium had been differentially removed by differential growth of oats in the first crop. These variations were evened out by application of additional phosphorus and potassium before planting the second crop. As will be seen in a later section, variations in yield and nitrogen uptake in table 9 were essentially a function of nitrogen availability. Removal of nitrogen in the tops and immobilization in the presence of roots from the first crop sharply reduced yields and nitrogen uptake with most treatments.

Of primary interest in table 9 is the fact that the immobilizing effect of sawdust had completely disappeared at both levels of nitrogen addition ( $N_2$  and  $N_3$ ), although growth was still completely inhibited where no nitrogen had been applied ( $N_1$ ).

In both cropped and uncropped soils, yields of dry matter leveled off at the  $N_2$  level of addition with all residue treatments. However,

Table 7. - Effects of residue treatments on measured long term crop variables.

Treatment	Green Weight g.	Dry Weight mg.	Dry Matter %	Nitrogen %	Nitrogen Uptake mg.
First Cropping Period					
Control	178 a	32.8 a	18.8 a	1.93 a	.689 a
Lignin	173 a	33.5 a	19.9 a	1.83 a	.669 a
Sawdust	100 b	18.6 b	20.9 a	1.20 b	.348 b
Second Cropping Period					
Control	75 a	4.4 a	19.9 a	2.26 a	.346 a
Lignin	80 a	14.2 a	18.9 ab	2.27 a	.341 a
Sawdust	57 b	10.8 b	18.2 b	2.18 a	.258 b

Table 8. - Main effects of nitrogen treatments on measured long term crop variables.

Treatment*	Green Weight g.	Dry Weight mg.	Dry Matter %	Nitrogen %	Nitrogen Uptake mg.
First Cropping Period					
N <sub>1</sub>	62 c	13.0 c	22.2 a	0.97 c	.141 c
N <sub>2</sub>	172 b	33.0 b	19.3 b	1.86 b	.645 b
N <sub>3</sub>	216 a	38.9 a	18.0 b	2.03 a	.920 a
Second Cropping Period					
N <sub>1</sub>	40 c	8.2 b	19.9 a	1.52 c	.116 c
N <sub>2</sub>	81 b	15.6 a	19.8 a	2.20 b	.358 b
N <sub>3</sub>	92 a	15.7 a	17.3 b	2.99 a	.472 a

\* N<sub>1</sub> = no nitrogen. N<sub>2</sub> and N<sub>3</sub> = nitrogen added to give a C:N ratio for sawdust of 40:1 and 20:1 respectively.

Table 9. - Effects of previous cropping, residues, and nitrogen treatments on long term crop variables measured in the second cropping period.

Treatment*	Dry Weight		Nitrogen Uptake	
	Uncropped	Cropped	Uncropped	Cropped
	mg.	mg.	mg.	mg.
Control				
N <sub>1</sub>	16.4 abcd	8.4 h	237 de	99 f
N <sub>2</sub>	17.8 ab	12.8 g	576 a	209 e
N <sub>3</sub>	16.9 abc	14.3 defg	536 ab	418 c
Lignin				
N <sub>1</sub>	13.9 efg	8.6 h	249 e	104 f
N <sub>2</sub>	17.1 ab	14.4 cdefg	526 ab	199 e
N <sub>3</sub>	15.5 bcdef	15.8 abcde	535 ab	462 bc
Sawdust				
N <sub>1</sub>	0.8 i	1.0 i	15 g	18 g
N <sub>2</sub>	18.0 ab	13.3 fg	423 c	213 e
N <sub>3</sub>	18.1 a	13.6 efg	575 a	305 d
Means for Cropping	14.9 a	11.4 b	405 a	225 b

\* N<sub>1</sub> = no nitrogen. N<sub>2</sub> and N<sub>3</sub> = nitrogen added to give C:N ratios for sawdust of 40:1 and 20:1 respectively.

a, b, c, --- g. Ranges of equivalence. Within a pair of columns for a given crop variable, numerical values with a common subscript are not significantly different at 5 percent.

in previously uncropped soil, nitrogen uptake was additionally increased following the  $N_3$  level of nitrogen addition. With sawdust, there was a similar increase in nitrogen uptake at the  $N_3$  over the  $N_2$  level in previously uncropped soil. Thus, residual effects of initially applied nitrogen were reflected in nitrogen uptake beyond the point where yields were influenced. Nitrogen was apparently taken up in excess of plant needs at high levels of available nitrogen in the soil.

#### Short Term Response of Oats

It was recognized that long term growth of oats would be influenced primarily by soluble forms of nitrogen. To differentiate between responses to soluble and insoluble forms of nitrogen, 200 gram aliquots of soil which had been leached with 1200 ml. of distilled water were placed in containers. Oats previously germinated in nitrogen-free sand cultures were grown for 14 days, according to the procedure described by DeMent et al. (17). This was done at the beginning of each cropping period and is referred to here as the "short term crop". Data for the first short term crop are presented in table 10.

In contrast to the long term response where yields were sharply depressed by sawdust (tables 6 and 7), both green and dry weight yields were significantly increased by both sawdust and lignin. This was true, even where no nitrogen was added. The top growth with lignin and sawdust was less succulent (higher in dry matter content). Although nitrogen content was lower than in the controls, nitrogen uptake was greater. It would appear that both lignin and sawdust provided some factor or factors which stimulated the synthesis of dry matter by oats during this early stage of growth. The principal stimulating

Table 10. - Effects of residue and nitrogen treatments on short term crop variables for first cropping period.

Treatment*	Green Weight	Dry Weight	Dry Matter	Nitrogen	Nitrogen Uptake
	g.	mg.	%	%	mg.
Control					
N <sub>1</sub>	4.050 c	444 d	11.0 cd	1.64 c	7.3 e
N <sub>2</sub>	4.076 c	375 e	9.2 e	1.87 b	7.0 e
N <sub>3</sub>	4.969 ab	518 c	10.4 d	2.07 a	10.7 b
Lignin					
N <sub>1</sub>	5.030 ab	654 ab	13.0 a	1.40 d	9.1 cd
N <sub>2</sub>	5.037 ab	640 ab	12.7 ab	1.52 cd	9.7 bc
N <sub>3</sub>	5.320 a	631 ab	11.9 bc	1.90 ab	11.9 a
Sawdust					
N <sub>1</sub>	5.110 ab	638 ab	12.5 ab	1.38 d	8.8 cd
N <sub>2</sub>	4.667 b	600 b	12.9 ab	1.39 d	8.3 d
N <sub>3</sub>	5.387 a	689 a	12.8 ab	1.41 d	9.7 bc

\* N<sub>1</sub> = no nitrogen. N<sub>2</sub> and N<sub>3</sub> = nitrogen added to give C:N ratio for sawdust of 40:1 and 20:1 respectively.

a, b, c, ---e. Ranges of equivalence. For a given crop variable, numerical values with a common literal subscript are not significantly different at 5 percent.

factor does not appear to have been nitrogen in any form, since there were no consistent relationships between dry matter yields and the amount of nitrogen which had been applied two weeks before leaching. The level of nitrogen applied was reflected rather consistently in nitrogen content and nitrogen uptake, so that nitrogen supply, as a secondary factor, did influence response to some extent.

The stimulating effect of sawdust and lignin on yields and nitrogen uptake were still apparent in the second short term planting made 68 days later (table 11). The nitrogen effect, however, had completely disappeared (table 12).

Previous cropping of the soil reduced growth and nitrogen uptake of the second short term crop, except where sawdust had been incorporated without nitrogen (table 13). The nature of this cropping effect is not clear. It may reflect a toxic effect of root residues from the preceding crop of oats. There would have been a negligible amount of such residues in the soil where sawdust was applied without nitrogen, since there had been essentially no growth during the first cropping period (table 6).

#### Functional Relationships Among Crop Variables

##### Short term relationships

It appeared from the short term response data that dry matter production may have been affected by factors other than nitrogen uptake. Dry weight and nitrogen uptake were positively correlated ( $r = .593$  in the first crop,  $r = .840$  in the second). Dry weight was negatively correlated with percent nitrogen and positively correlated with percent dry matter in the first crop, however, in the second crop the reverse

Table 11. - Effects of residue treatments on measured short term crop variables.

Treatment	Green Weight g.	Dry Weight mg.	Dry Matter %	Nitrogen %	Nitrogen Uptake mg.
First Cropping Period					
Control	4.37 b	446 b	10.2 b	1.86 a	8.3 c
Lignin	5.13 a	642 a	12.5 a	1.61 b	10.2 a
Sawdust	5.06 a	629 a	12.7 a	1.39 c	8.9 b
Second Cropping Period					
Control	4.60 c	630 a	13.9 ab	1.43 a	9.0 b
Lignin	4.91 b	688 a	14.2 a	1.36 a	9.4 ab
Sawdust	5.23 a	704 a	13.5 b	1.43 a	10.1 a

Table 12. - Effects of nitrogen treatments on measured short term crop variables.

Treatment*	Green Weight g.	Dry Weight mg.	Dry Matter %	Nitrogen %	Nitrogen Uptake mg.
First Cropping Period					
N <sub>1</sub>	4.73 b	579 a	12.2 a	1.47 c	8.4 b
N <sub>2</sub>	4.60 b	525 b	11.6 b	1.59 b	8.3 b
N <sub>3</sub>	5.23 a	613 a	11.7 b	1.79 a	10.8 a
Second Cropping Period					
N <sub>1</sub>	4.93 a	679 a	13.9 a	1.45 a	9.8 a
N <sub>2</sub>	4.87 a	667 a	13.9 a	1.40 a	9.4 a
N <sub>3</sub>	4.94 a	674 a	13.8 a	1.37 a	9.2 a

\* N<sub>1</sub> = no nitrogen. N<sub>2</sub> and N<sub>3</sub> = nitrogen added to give C:N ratios for sawdust of 40:1 and 20:1 respectively.



Table 13. - Effects of previous cropping, residues, and nitrogen treatments on short term crop variables measured in the second cropping period.

Treatment*	Dry Weight		Nitrogen Uptake	
	Uncropped	Cropped	Uncropped	Cropped
	mg.	mg.	mg.	mg.
Control				
N <sub>1</sub>	702 a	588 a	10.2 abc	8.5 de
N <sub>2</sub>	696 a	563 a	10.1 abc	7.6 e
N <sub>3</sub>	681 a	551 a	9.8 abc	7.6 e
Lignin				
N <sub>1</sub>	768 a	573 a	11.2 ab	7.6 e
N <sub>2</sub>	747 a	640 a	11.1 ab	7.8 e
N <sub>3</sub>	775 a	623 a	11.4 a	7.3 e
Sawdust				
N <sub>1</sub>	695 a	750 a	10.5 abc	11.0 ab
N <sub>2</sub>	709 a	657 a	10.7 abc	8.8 cde
N <sub>3</sub>	735 a	677 a	10.1 abc	9.2 bcde
Means for Cropping	723 a	625 b	10.6 a	8.4 b

\* N<sub>1</sub> = no nitrogen. N<sub>2</sub> and N<sub>3</sub> = nitrogen added to give C:N ratios for sawdust of 40:1 and 20:1 respectively.

a, b, c, --- f. Ranges of equivalence. Within a pair of columns for a given crop variable, numerical values with a common literal subscript are not significantly different at 5 percent.

was true. There was no significant correlation between nitrogen uptake and either nitrogen or dry matter content in the first crop, whereas in the second crop nitrogen uptake was positively correlated with percent nitrogen and negatively correlated with percent dry matter.

For these reasons, it seemed desirable to investigate functional relationships among the measured crop variables, using the exponential Carter-Halter function. These are shown for the two short term crops in figure 1. The plotted functions were fitted to unit observations. Treatment means for three replicate observations are also shown to give visual evidence of the extent to which the functions accounted for variability in the dependent variable plotted on the vertical axis.

In figure 1-A it is apparent that increasing dry weight was essentially a function of increasing percent dry matter, although a significant positive relationship with percent nitrogen was also expressed. Both responses were curvilinear. Dry weight increased more rapidly with increasing nitrogen and dry matter contents above their mean values. The  $\bar{R}^2 = .865$  indicates that 86.5 percent of the total variation in dry weight was accounted for by these functional relationships with nitrogen and dry matter contents. It is significant that the simple negative correlation observed between dry weights and nitrogen percentages by themselves was reversed when percent dry matter was also considered.

The equation for figure 1-B accounted for only 35 percent of the variation in dry weight of the second crop. Nevertheless, regression coefficients for the log and semi-log terms for dry matter content were statistically significant. The plotted function shows an increasingly rapid decline in dry weight with increasing percent dry matter.

If figures 1-A and 1-B are considered together, it would appear

Figure 1. - Functional relationships among  
short term crop variables.

Figure 1-A. - Dry weight (Dwt.) of first crop of oats vs. dry matter (DM) and nitrogen (N) in tissue:

$$\begin{aligned}\text{Log Dwt.} = & - 7.0013 - 4.16^* \log N + 117.^* N + .339 \log DM \\ & + 5.94 DM\end{aligned}$$

$$\bar{R}^2 = .865$$

Figure 1-B. - Dry weight (Dwt.) of second crop of oats vs. dry matter (DM) and nitrogen (N) in tissue:

$$\begin{aligned}\text{Log Dwt.} = & + 14.629^* - .426 \log N + 16.8 N + 9.69^* \log DM \\ & - 32.5^* DM\end{aligned}$$

$$\bar{R}^2 = .346$$

Figure 1-C. - Nitrogen uptake (NUpt.) of first crop of oats vs. nitrogen (N) and dry matter (DM) in tissue:

$$\begin{aligned}\text{Log NUpt.} = & - 7.00 - 3.16 \log N + 117.^* N + .339 \log DM \\ & + 5.94 DM\end{aligned}$$

$$\bar{R}^2 = .809$$

Figure 1-D. - Nitrogen uptake (NUpt.) of second crop of oats vs. nitrogen (N) and dry matter (DM) in tissue:

$$\begin{aligned}\text{Log NUpt.} = & + 14.625^* + .574 \log N + 16.8 N + 9.69^* \log DM \\ & - 32.5^* DM\end{aligned}$$

$$\bar{R}^2 = .720$$

---

\* Regression coefficient significant at 5 percent.

\*\* Regression coefficient significant at 1 percent.

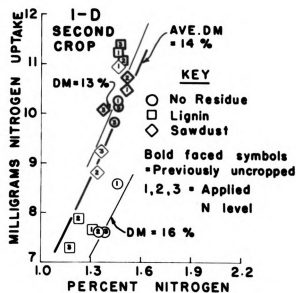
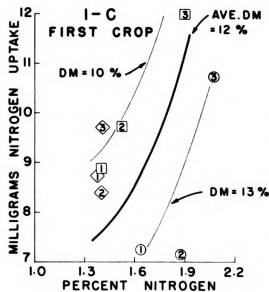
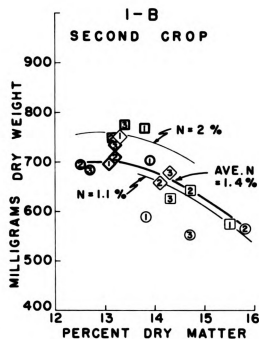
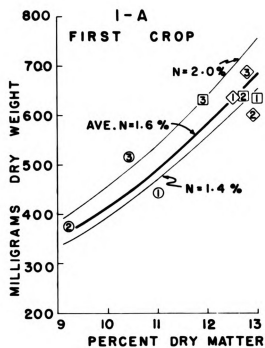


Figure 1

that an optimum for dry matter synthesis by oats at this stage of growth might occur at 13 to 14 percent dry matter. In the first crop, some factor or factors in lignin or sawdust promoted dry matter synthesis and decreasing succulence over the positive leg of the response curve. In the second crop, decreasing synthesis of dry matter was associated with some factor in previously cropped soil which reduced succulence excessively.

This depression in previously cropped soil was essentially unrelated to nitrogen content of tissue or to nitrogen treatment. In previously uncropped soil, dry weight increased with both nitrogen and dry matter contents, as in the first crop. The Carter-Halter function did not give expression to this obviously different relationship in previously uncropped soil as compared with that in previously cropped soil.

In figure 1-C, the scatter of mean values for nitrogen uptake was rather well covered by functional values calculated over the observed range of mean values for dry matter and nitrogen content. Nitrogen uptake increased with both nitrogen and dry matter contents, as had dry matter in figure 1-A. In the second crop (figure 1-D), the variation in nitrogen uptake in previously cropped soil was fairly well accounted for by a positive relationship with nitrogen content and a negative relationship with dry matter. In previously uncropped soil, however, the response to unknown factors in sawdust and lignin exceeded the observed limits of the function.

Functional relationships in figure 1 support the earlier inference that dry matter synthesis was specifically stimulated by non-nitrogenous constituents in sawdust and lignin. Thus, oats in residue amended pots in the first crop were segregated by the function into a separate

population from oats in control pots on the basis of differences in dry matter percentage (or succulence). Nevertheless, in both populations there was a similar increase in dry weight and nitrogen uptake with increasing nitrogen content (figures 1-A and 1-C).

In the second crop, there appeared to be an inhibitory effect of root residues from the previous crop of oats. This inhibitory effect on dry matter production exceeded the functional limits imposed by the range of mean nitrogen and dry matter contents actually observed (figure 1-B). Thus, oats on previously cropped and previously uncropped soils appear to belong to two populations differentiated by some factor or factors other than nitrogen content or dry matter content. Dry matter contents appeared to be excessively high in the previously cropped population which suggests that water uptake or normal tissue hydration was interfered with. Nitrogen uptake associated with these excessively high dry matter contents was reduced, and the reduction was fully accounted for within the functional limits actually observed (figure 1-D).

In figure 1-D nitrogen uptake in excess of the functional limits was associated with sawdust and lignin treatments which had stimulated dry matter production in a direction opposite to the general trend expressed by the function in figure 1-B. It is apparent that the inclusion of data for both cropped and uncropped pots in one function was not fully justified. Also, the functional form used allows for an inflexible, additive type of interaction in which inflexions or peaks associated with one independent variable are not free to vary with variations in another. Thus, a maximum dry weight in figure 1-B is expressed at 13 percent dry matter, regardless of the level of tissue nitrogen. A quadratic function with interaction terms would allow for

flexibility in expression of this maximum at different levels of nitrogen.

In spite of these shortcomings, the Carter-Halter function led to useful inferences and is employed in succeeding sections.

### Long term relationships

Functional relationships among long term crop variables in figure 2 were fitted to unit observations. Experimental means of triplicate observations are also shown.

The functional relationships in the first crop between dry weight (figure 2-A) and nitrogen uptake (figure 2-C), on the one hand, and tissue nitrogen and dry matter content, on the other, were very similar. The curves suggest that optimum production of dry matter occurred at a nitrogen content of 2.0 percent. Optimum nitrogen uptake occurred at a somewhat higher nitrogen content (about 2.2 percent). Expected dry weight and nitrogen uptake, as defined by the function, declined rapidly at nitrogen contents above this optimum. These declines, however, were opposed by increasing dry matter content. The regression coefficients for tissue nitrogen content in both equations were highly significant and 83 and 90 percent, respectively, of the total variation in dry weight and nitrogen uptake was accounted for.

In the second crop (figures 2-B and 2-D), no optimum level of tissue nitrogen was expressed for either dependent variable. Only 36 to 54 percent of the total variation was accounted for and only the semi-log term for tissue nitrogen acquired a statistically significant regression coefficient in either equation. Nevertheless, the functional relationships are revealing.

In figure 2-B, increasing percent nitrogen was accompanied by

Figure 2. - Functional relationships among  
long term crop variables.

Figure 2-A. - Dry weight (Dwt.) of first crop of oats vs. nitrogen (N)  
and dry matter (DM) in tissue:

$$\text{Log Dwt.} = + 24.441 + 10.7^{**}\log N - 2.32^{**}N + .508 \log DM + 2.23 DM$$

$$\bar{R}^2 = .827$$

Figure 2-B. - Dry weight (Dwt.) of second crop of oats vs. nitro-  
gen (N) and dry matter (DM) in tissue:

$$\text{Log Dwt.} = +2.439 - 3.87 \log N + 112.^*N + 10.2 \log DM - 16.0 DM$$

$$\bar{R}^2 = .358$$

Figure 2-C. - Nitrogen uptake (NUpt.) of first crop of oats vs.  
nitrogen (N) and dry matter (DM) in tissue:

$$\text{Log NUpt.} = +24.439 + 11.7^{**}\log N - 232.^{**}N + .507 \log DM + 2.25 DM$$

$$\bar{R}^2 = .898$$

Figure 2-D. - Nitrogen uptake (NUpt.) of second crop of oats vs.  
nitrogen (N) and dry matter (DM) in tissue:

$$\text{Log NUpt.} = + 2.439 - 2.87 \log N + 112.^*N + 10.2 \log DM - 16.0 DM$$

$$\bar{R}^2 = .542$$

---

\* Regression coefficient significant at 5 percent.

\*\* Regression coefficient significant at 1 percent.



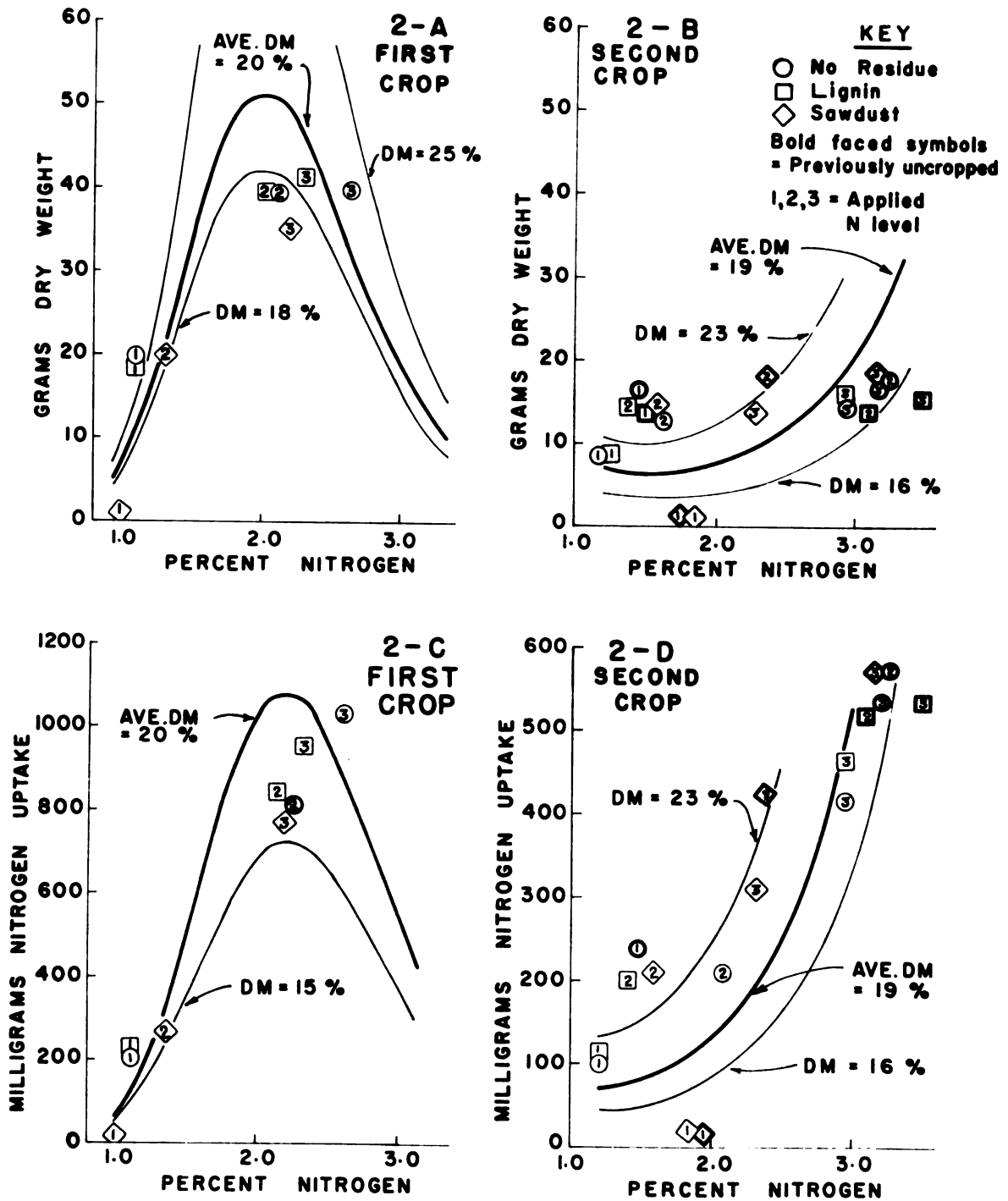


Figure 2

decreasing percent dry matter. The expected increases in yield of dry matter failed to materialize. It appears likely that this was due to respiratory losses of photosynthate promoted by unfavorably high temperatures and long days during the summer months when this crop was grown.

In the case of nitrogen uptake (figure 2-D), the essential functional relationships were similar to those for dry weight, but large increases in nitrogen uptake were actually obtained over the whole range of observed values. Maximum uptake of nitrogen was associated with maximum succulence (minimum dry matter percentage).

Comparing figures 2-B and 2-D, it is apparent that nitrogen taken up in excess of 300 mg. was in excess of the crop's potential for increased growth. Such excessive accumulations of nitrogen occurred at nitrogen percentages in excess of about 2.0. This approximates the optimum nitrogen content expressed for the first crop by the functions in figures 2-A and 2-C.

It was inferred from patterns of response for the short term crops in figure 1 that dry matter synthesis and nitrogen uptake can be influenced independently by soil or environmental factors. This inference is supported by response patterns for the long term crops in figure 2. It is apparent that neither dry matter yields nor nitrogen uptake, individually, are adequate to characterize crop responses to soil treatments or environmental variables.

In spite of the limitations of the mathematical model used, it was useful in bringing out meaningful relationships among the various crop measurements. Useful information was obtained even where regression coefficients fell short of statistical significance or where  $\bar{R}^2$  values indicated that only a portion of the variation in the dependent variable was described by the function.

Functional Relationships  
among Crop and Soil Variables

Intercorrelations among soil variables

The interpretation of multiple regression analyses becomes difficult where independent variables are highly intercorrelated. Simple correlation coefficients among soil variables measured prior to the first cropping period are given in table 14. Four correlation coefficients are given for each pair of factors, since each factor appears in linear and log form in the functions used. Coefficients not significant at the 5 percent level of probability are not listed.

Nitrate and other water soluble forms of nitrogen were positively correlated only with extractable nitrogen. Nitrate was negatively correlated with hydrolyzable and non-hydrolyzable carbon. Other water-soluble and extractable nitrogen were negatively correlated with extractable and hydrolyzable carbon. Hydrolyzable and non-hydrolyzable nitrogen and carbon were all positively correlated among themselves.

Simple correlation coefficients for soil variable measured prior to the second cropping period are given in table 15. Much of the intercorrelation observed in the earlier sampling had disappeared. Significant correlations involving water-soluble nitrogen and extractable carbon are of little consequence because these were present in very small quantities (tables 3 and 4). Positive correlations between hydrolyzable nitrogen and carbon, and between non-hydrolyzable nitrogen and carbon in both samplings tend to be prejudicial to some of the inferences to be made in the following sections.

However, all pairs fall short of perfect correlation, and most have a large degree of independence. Thus, correlations of the order

Table 14. - Linear, semi-log, and log correlations among soil variables measured prior to the first cropping period.

Soil Variable	Function	H <sub>2</sub> O-sol. N <sup>1</sup>		Extractable C		Hydrolyzable N		Non-hydrolyzable C	
				N	C	N	C	N	C
NO <sub>3</sub> -N	Linear	NS		+ .455*	NS	NS	-.684**	NS	-.548**
	Linear-log	+ .514**		+ .477*	NS	NS	-.702**	NS	-.511**
	Log-linear	+ .393*		+ .421*	NS	NS	-.625**	NS	-.557**
	Log-log	+ .574**		+ .446*	NS	NS	-.646**	NS	-.517**
H <sub>2</sub> O-sol. N	Linear			+ .962**	-.439*	NS	NS	NS	NS
	Linear-log			+ .909**	-.483*	NS	NS	NS	NS
	Log-linear			+ .878**	-.507**	NS	-.381*	NS	NS
	Log-log			+ .915**	-.546**	NS	-.391*	NS	NS
Extr. N	Linear				-.456*	NS	-.387*	NS	NS
	Linear-log				-.487**	NS	-.394*	NS	NS
	Log-linear				-.522**	NS	-.387*	NS	NS
	Log-log				-.543**	NS	-.402*	NS	NS
Extr. C	Linear					NS	NS	NS	NS
	Linear-log					NS	NS	NS	NS
	Log-linear					NS	NS	NS	NS
	Log-log					NS	NS	NS	NS

Table 14. - (Continued).

Soil Variable	Function	H <sub>2</sub> O-sol. N <sup>1</sup>	Extractable		Hydrolyzable		Non-hydrolyzable	
			N	C	N	C	N	C
Hyd. N.	Linear				+ .574**		+ .508**	+ .418*
	Linear-log				+ .524**		+ .494**	+ .405*
	Log-linear				+ .550**		+ .511**	+ .414*
	Log-log				+ .503**		+ .498**	+ .403*
Hyd. C	Linear						+ .496**	+ .585**
	Linear-log						+ .492**	+ .551**
	Log-linear						+ .506**	+ .582**
	Log-log						+ .502**	+ .549**
Non-hyd. N	Linear							+ .857**
	Linear-log							+ .867**
	Log-linear							+ .864**
	Log-log							+ .877**

<sup>1</sup> Other than nitrate or nitrite.

\* Significant at 5 percent.

\*\* Significant at 1 percent.

Table 15. - Linear, semi-log, and log correlations among soil variables measured prior to second cropping period.

Soil Variable	Function	H <sub>2</sub> O-sol. N <sup>1</sup>	Extractable		Hydrolyzable		Non-hydrolyzable	
			N	C	N	C	N	C
NO <sub>3</sub> -N	Linear	+ .368**	NS	NS	NS	--.295*	NS	NS
	Linear-log	+ .470**	NS	NS	NS	--.295*	NS	NS
	Log-linear	+ .311*	NS	NS	NS	--.398**	NS	NS
	Log-log	+ .484**	NS	NS	NS	--.369**	NS	NS
H <sub>2</sub> O-sol. N	Linear		NS	NS	NS	NS	NS	NS
	Linear-log		NS	NS	NS	NS	NS	NS
	Log-linear		NS	NS	NS	NS	NS	NS
	Log-log		NS	NS	NS	NS	NS	NS
Extr. N	Linear			NS	NS	NS	NS	NS
	Linear-log			NS	NS	NS	NS	NS
	Log-linear			NS	NS	NS	NS	NS
	Log-log			NS	NS	NS	NS	NS
Extr. C	Linear				+ .361**	NS	NS	--.414**
	Linear-log				+ .336*	NS	NS	--.375**
	Log-linear				+ .346*	NS	NS	--.430**
	Log-log				+ .320*	NS	NS	--.390**

Table 15. - (Continued).

Soil Variable	Function	$H_2O$ -sol. N <sup>1</sup>	Extractable		Hydrolyzable		Non-hydrolyzable	
			N	C	N	C	N	C
Hyd. N	Linear					+ .508**	NS	NS
	Linear-log					+ .513**	NS	NS
	Log-linear					+ .509**	+ .283*	NS
	Log-log					+ .514**	+ .272*	NS
Hyd. C.	Linear						NS	NS
	Linear-log						NS	NS
	Log-linear						NS	NS
	Log-log						NS	NS
Non-hyd. N	Linear							+ .545**
	Linear-log							+ .557**
	Log-linear							+ .549**
	Log-log							+ .559**

<sup>1</sup> Other than nitrate or nitrite.

\* Significant at 5 percent.

\*\* Significant at 1 percent.

of  $r = -.7$  between nitrate-nitrogen and hydrolyzable carbon (table 14) correspond to coefficients of determination of the order  $r^2 = .49$ . In other words, only about 50 percent of the variation in nitrate-nitrogen was associated with inverse variation in hydrolyzable carbon. The higher correlations between water-soluble nitrogen and extractable nitrogen and between non-hydrolyzable nitrogen and carbon in the first sampling (table 14) are more damaging. With such a high degree of interdependence, a functional relationship shown for one independent variable may actually reflect a more fundamental relationship involving the other.

#### Short term crop and soil variables

For each short term crop variable, five separate functions employed. In the first three listed across the top of table 16, nitrogen and carbon were used as log and semi-log terms in separate functions for the extractable, hydrolyzable and non-hydrolyzable fractions. In the fourth function, all these forms of nitrogen and carbon were used as separate log and semi-log terms in a single function. In the fifth, the sums of nitrogen and carbon in these three non-water-soluble fractions were used. Nitrate and other water-soluble forms of nitrogen were not included, since these were removed by leaching prior to placing the germinated oats in contact with the experimental soil aliquots.

In the first cropping period (table 16), 45 to 69 percent of the variation in green weight, dry weight and percent dry matter was accounted for by variations in non-hydrolyzable nitrogen and carbon. The amount of information provided by this fraction alone was greater than for the total non-water-soluble nitrogen and carbon and almost as great as in the fourth function involving all three fractions as separate



Table 16. - Coefficients of multiple determination ( $\bar{R}^2$ ) for short term crop variables as functions of soil variables in different combinations.

Y Short term crop variables	$\bar{R}^2$ = variability in Y accounted for by functions of soil variables.				
	Extractable	Hydrolyzable	Non-hydrolyzable	Combined *	Total
	N and C	N and C	N and C	function	non-water-sol. N and C
	First cropping period				
Green weight	-.015	-.082	+.452	+.581	+.429
Dry weight	-.157	-.026	+.694	+.725	+.567
Nitrogen uptake	+.454	-.131	+.273	+.779	+.322
Percent nitrogen	+.534	+.225	+.590	+.923	+.654
Percent dry matter	-.052	+.064	+.663	+.732	+.547
	Second cropping period				
Green weight	+.090	+.152	+.045	+.143	+.130
Dry weight	+.045	+.164	+.108	+.190	+.170
Nitrogen uptake	-.007	+.125	+.038	+.092	+.062
Percent nitrogen	-.022	-.019	-.004	-.033	-.037
Percent dry matter	+.087	+.031	-.021	+.094	+.079

\* Extractable, hydrolyzable and non-hydrolyzable nitrogen and carbon as separate terms in a single function.

terms. The extractable and hydrolyzable fractions were completely without effect on green or dry weight or percent dry matter.

Nitrogen uptake and percent nitrogen, on the other hand, were influenced by variations in nitrogen and carbon in both the extractable and non-hydrolyzable fractions. Percent nitrogen also reflected some influence of the hydrolyzable fractions. For these two plant variables, the fourth function provided a substantial increase in information over that supplied by the individual fractions or by the sum of non-water-soluble nitrogen and carbon.

In the second crop, functional relationships with soil variables were much reduced in their contribution to variation in short term crop variables (table 16).

Functional relationships calculated for unit observations in the first crop are depicted graphically in figure 3. Experimentally observed means for triplicate observations are also shown.

The function for figure 3-A accounted for 73 percent of the variation in dry matter percentage and provided statistically significant regression coefficients for non-hydrolyzable carbon and hydrolyzable nitrogen. The major response indicated was to carbonaceous constituents supplied by both lignin and sawdust and appearing in the non-hydrolyzable fraction. The function, therefore, substantiates the inference made from inspection of the data in tables 10 and 11. The basic response was modified by nitrogenous constituents in the hydrolyzable fraction, although the observed range of this effect was inadequate to cover the range of observed means. Additional increases above the mean function would have been associated with extractable nitrogen, but these would have been countered by a depressing effect associated with extractable carbon. These moderating effects were not statistically significant but

Figure 3. - Functional relationships among first short term crop variables and soil variables.

Figure 3-A. - Dry matter content (DM) of oats vs. non-hydrolyzable carbon (NHC) and hydrolyzable nitrogen (HN) in soil:

$$\begin{aligned}\text{Log DM} = & + 14.1399 - .145 \log \text{EXN} + .0033 \text{EXN} + .916 \log \text{EXC} \\ & - .0368 \text{EXC} - 7.47^* \log \text{HN} + .0145^* \text{HN} - .166 \log \text{HC} \\ & - .00001 \text{HC} - 3.46 \log \text{NHN} + .0259 \text{NHN} + 1.31^* \log \text{NHC} \\ & - .0002 \text{NHC}\end{aligned}$$

$$\bar{R}^2 = .732$$

Figure 3-B. - Dry weight (Dwt.) of oats vs. non-hydrolyzable carbon (NHC) in soil:

$$\begin{aligned}\text{Log Dwt.} = & + 17.780 - .243 \log \text{EXN} + .0088 \text{EXN} + 1.10 \log \text{EXC} \\ & - .0434 \text{EXC} - 7.78 \log \text{HN} + .0150 \text{HN} + .0381 \log \text{HC} \\ & - .00004 \text{HC} - 4.69 \log \text{NHN} + .0340 \text{NHN} + 1.84 \log \text{NHC} \\ & - .0002 \text{NHC}\end{aligned}$$

$$\bar{R}^2 = .725$$

Figure 3-C. - Nitrogen content (N) of oats vs. extractable nitrogen (EXN) in soil:

$$\begin{aligned}\text{Log N} = & - 13.067^* - .132 \text{EXN} + .0070^{**} \text{EXN} - .533 \log \text{EXC} + .0174 \text{EXC} \\ & + 4.80 \log \text{HN} - .0092 \text{HN} - .0293 \log \text{HC} + .000003 \text{HC} \\ & + 2.27 \log \text{NHN} - .0184 \text{NHN} - .165 \log \text{NHC} - .00002 \text{NHC}\end{aligned}$$

$$\bar{R}^2 = .923$$

Figure 3-D. - Nitrogen uptake (NUpt.) of oats vs. extractable nitrogen (EXN) and non-hydrolyzable carbon (NHC) in soil:

$$\begin{aligned}\text{Log NUpt.} = & + 4.714 - .375 \log \text{EXN} + .0157^{**} \text{EXN} + .570 \log \text{EXC} \\ & - .0260 \text{EXC} + 2.97 \log \text{HN} + .006 \text{HN} + .067 \log \text{HC} \\ & - .00001 \text{HC} - 2.42 \log \text{NHN} + .0156 \text{NHN} + 1.68^* \log \text{NHC} \\ & - .0003 \text{NHC}\end{aligned}$$

$$\bar{R}^2 = .779$$

---

\* Regression coefficient significant at 5 percent.

\*\* Regression coefficient significant at 1 percent.

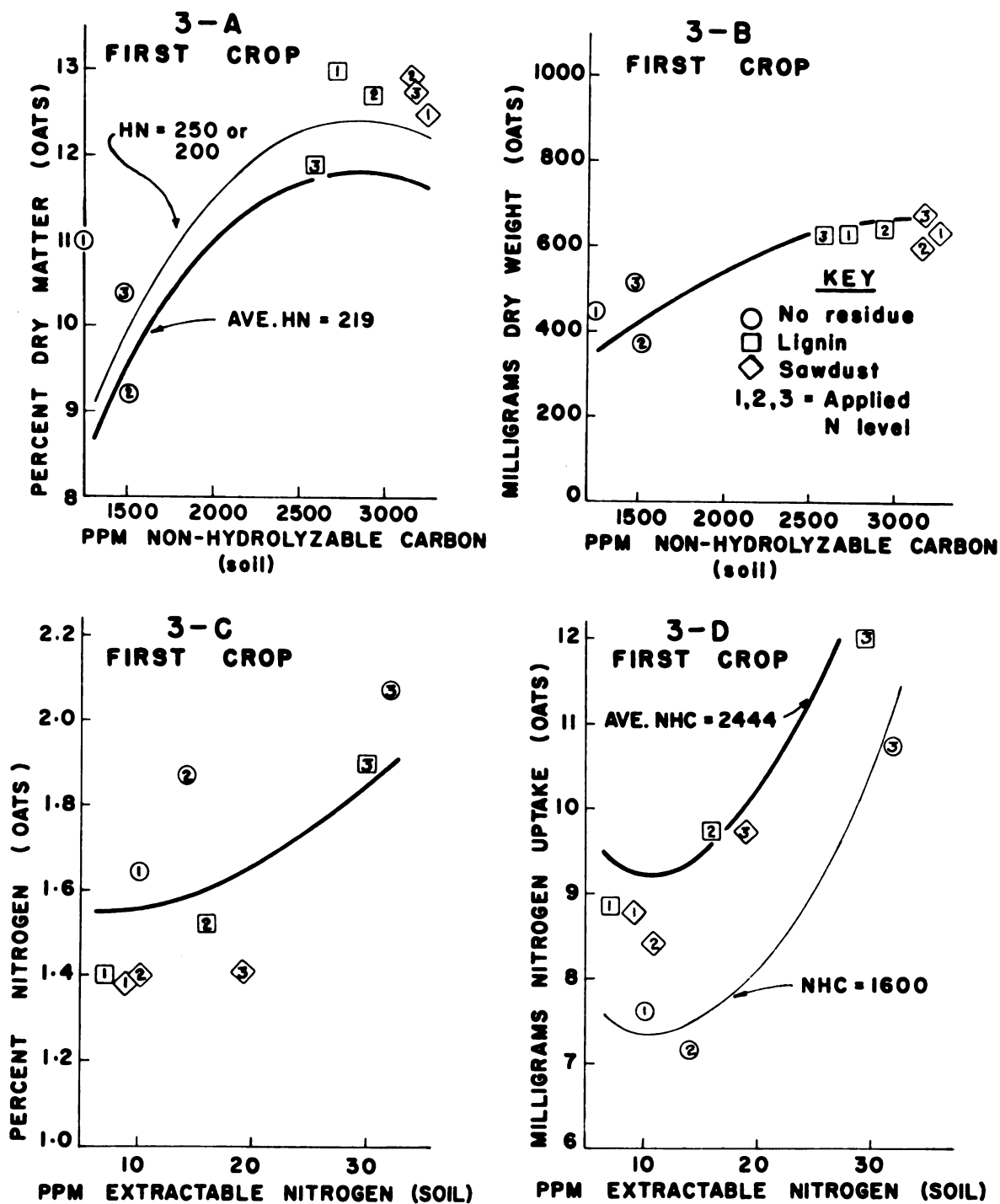


Figure 3

are theoretically realistic.

As was observed in figure 1-A, dry weight was essentially a function of percent dry matter, whereas the effect of nitrogen content was minor. It is not surprising, therefore, that dry weight was also related primarily to non-hydrolyzable carbon by the function in figure 3-B. Other soil variables contributed very little to explaining the deviation of experimental points away from this line.

The functional relationships in figure 3-C suggest that percent nitrogen was influenced primarily by extractable nitrogen. Other soil variables, in their combined effect, accounted for much of the deviation of experimental points away from this line. However, their individual effects were small, and no attempt was made to show them in the figure.

Nitrogen uptake in figure 3-D was influenced by soil factors which affected both dry matter production and nitrogen percentage. Thus, a major part of the variation in observed experimental means was encompassed within the functional limits established by observed values for extractable nitrogen and non-hydrolyzable carbon. This figure is strikingly similar to figure 1-C where relationships between nitrogen uptake and tissue dry matter and nitrogen contents are shown.

The relationships in figure 3 clearly suggest that a primary stimulus to early growth of oats was expressed by some constituent in the non-hydrolyzable carbon fraction. The stimulating factor was present in both sawdust and lignin obtained from the sawdust by acid extraction of cellulose and other carbohydrates. This stimulus was of the order of a hormonal response and was expressed specifically on processes of dry matter synthesis.

In addition to this regulatory effect on dry matter production,

nitrogen uptake also reflected a greater availability of nitrogen in the extractable fraction. Nitrogen in the hydrolyzable fraction may have influenced the early response of oats, also, as indicated by the function for percent dry matter in figure 3-A.

These relationships in the first short term crop of oats were associated with early stages of decomposition of the added residues. Similar relationships in the second crop were much less marked and were less effective in accounting for variation in the measured crop variables (table 16). It appeared that effects associated with initial residue treatments were confounded with effects of root residues from the previous oat crop. As noted in discussion of figure 1-B, these root residues appeared to have an inhibitory influence on early growth of the second crop.

#### Long term crop and soil variables

Coefficients of multiple determination ( $\bar{R}^2$ ) for functions applied to data for the long term oat crops are present in table 17. As seen in the first column, most of the variation in growth and nitrogen uptake in both crops was accounted for by variations in nitrate and other water soluble forms of nitrogen. There was little change in  $\bar{R}^2$  for these three crop measurements when water-soluble nitrogen was replaced in successive functions involving extractable, hydrolyzable or non-hydrolyzable N and C. The fit was somewhat improved when all soil variables were included in a single function. In the first cropping period the use of total non-water-soluble nitrogen and carbon in a function with nitrate tended to detract slightly from the information obtained with nitrate and water-soluble nitrogen.

Variations in percent nitrogen were well accounted for in the first crop by nitrate and water-soluble nitrogen. Some effect of the

Table 17. - Coefficients of multiple determination ( $\bar{R}^2$ ) for long term crop variables as functions of soil variables in different combinations.

Y	$\bar{R}^2$ - variability in Y accounted for by functions of soil variables.				
	Nitrate, water-sol. N	Nitrates, extractable N and C	Nitrates, hydrolyzable N and C	Nitrates, Combined function N and C	Nitrates, total non-water-soluble N and C
Long term crop variables					
			First cropping period		
Green weight	.978	.970	.918	.943	.957
Dry weight	.975	.966	.934	.954	.964
Nitrogen uptake	.972	.951	.866	.901	.942
Percent nitrogen	.931	.858	.557	.614	.823
Percent dry matter	.511	.524	.382	.319	.489
			Second cropping period		
Green weight	.918	.912	.914	.919	.911
Dry weight	.885	.871	.881	.876	.876
Nitrogen uptake	.971	.968	.972	.968	.971
Percent nitrogen	.646	.633	.676	.663	.666
Percent dry matter	.274	.217	.330	.218	.294

\* Nitrates, water soluble nitrogen, extractable, hydrolyzable, and non-hydrolyzable nitrogen and carbon as separate terms in a single function.

extractable fraction was apparent, but the hydrolyzable and non-hydrolyzable fractions contributed little information beyond that which would have been supplied by nitrate alone. In the second crop, percent nitrogen was essentially a function of nitrate, although the hydrolyzable and non-hydrolyzable fractions tended to contribute additional information.

Percent dry matter was influenced by soil variables to a much lesser extent than the other crop variables, particularly in the second crop. Nevertheless, there was evidence that water-soluble and extractable fractions contributed to variability in the first crop. In the second crop, there was a marked increase in  $\bar{R}^2$  for dry matter when all fractions were combined in a single function (column 5, table 17).

Functional relationships between soil variables and dry weight and nitrogen uptake of both long term crops are presented in figure 4. Again the functions were fitted to unit observations. Experimental means for triplicate observations are shown for reference.

Essentially all of the variation in dry weight was accounted for by the function for figure 4-A. Statistically significant regression coefficients in the function suggest that the most influential soil factors were nitrate and hydrolyzable carbon. Examination of the curves for low and high levels of hydrolyzable carbon would suggest that deviations from the average curve for nitrate were due to increasing immobilization of nitrogen in the presence of increasing carbonaceous energy supplies represented by hydrolyzable forms of carbon. This would not, however, explain the two low points for the control and lignin treatment without nitrogen, since both of these were relatively low in hydrolyzable carbon (table 2).



Figure 4. - Functional relationships among long term crop variables and soil variables.

Figure 4-A. - Dry weight (Dwt.) of first crop of oats vs. nitrate ( $\text{NO}_3$ ), hydrolyzable carbon (HC), soluble nitrogen (SN) and extractable nitrogen (EXN) in soil:

$$\begin{aligned}\text{Log Dwt.} = & + 6.534 + 1.33^{**} \log \text{NO}_3 - .0257^{**} \text{NO}_3 + .206 \log \text{SN} \\ & - .0043 \text{SN} - .957 \log \text{EXN} + .0274 \text{EXN} - .440 \log \text{EXC} \\ & - .0257 \text{EXC} - 5.35 \log \text{HN} + .0139 \text{HN} + 1.18^* \log \text{HC} \\ & - .0004^* \text{HC} - 2.91 \log \text{NHN} + .0443 \text{NHN} + 1.48 \log \text{NHC} \\ & - .0004 \text{NHC}\end{aligned}$$

$$\bar{R}^2 = .993$$

Figure 4-B. - Dry weight (Dwt.) of second crop of oats vs. nitrate ( $\text{NO}_3$ ) and hydrolyzable nitrogen (HN) in soil:

$$\begin{aligned}\text{Log Dwt.} = & + 32.789^{**} + .854^{**} \log \text{NO}_3 - .0046^{**} \text{NO}_3 + .0443 \log \text{SN} \\ & - .0189 \text{SN} + .464 \log \text{EXN} - .0054 \text{EXN} + 2.56 \log \text{EXC} \\ & - .193 \text{EXC} - 9.17^{**} \log \text{HN} + .0187^{**} \text{HN} + 1.23 \log \text{HC} \\ & - .0002 \text{HC} - 9.56 \log \text{NHN} - .0728 \text{NHN} - 2.64 \log \text{NHC} \\ & + .0006 \text{NHC}\end{aligned}$$

$$\bar{R}^2 = .910$$

Figure 4-C. - Nitrogen uptake (NUpt.) of first crop of oats vs. nitrate ( $\text{NO}_3$ ), hydrolyzable carbon (HC) and extractable nitrogen (EXN) in soil:

$$\begin{aligned}\text{Log NUpt.} = & + 13.187 + 1.21^{**} \log \text{NO}_3 - .0178^{**} \text{NO}_3 + .416^* \log \text{SN} \\ & - .0067 \text{SN} - 1.38 \log \text{EXN} + .0402 \text{EXN} - 1.37 \log \text{EXC} \\ & - .0043 \text{EXC} - 9.86 \log \text{HN} + .0251 \text{HN} + 1.73^* \log \text{HC} \\ & - .0007^{**} \text{HC} - 6.25 \log \text{NHN} + .0845 \text{NHN} + .248 \text{NHC} \\ & - .0007^* \text{NHC}\end{aligned}$$

$$\bar{R}^2 = .995$$

Figure 4-D. - Nitrogen uptake (NUpt.) of second crop of oats vs. nitrate ( $\text{NO}_3$ ), and hydrolyzable nitrogen (HN) in soil:

$$\begin{aligned}\text{Log NUpt.} = & + 16.891^{**} + .932^{**} \log \text{NO}_3 - .0032^{**} \text{NO}_3 + .0430 \log \text{SN} \\ & - .0070 \text{SN} + .112 \log \text{EXN} + .0020 \text{EXN} + .592 \log \text{EXC} \\ & - .0610 \text{EXC} - 4.16^{**} \log \text{HN} + .0092^{**} \text{HN} + .642 \log \text{HC} \\ & - .0001 \text{HC} - 7.53 \log \text{NHN} + .0584 \text{NHN} - 1.11 \log \text{NHC} \\ & + .0002 \text{NHC}\end{aligned}$$

$$\bar{R}^2 = .974$$

---

\* Regression coefficients significant at 5 percent.

\*\* Regression coefficients significant at 1 percent

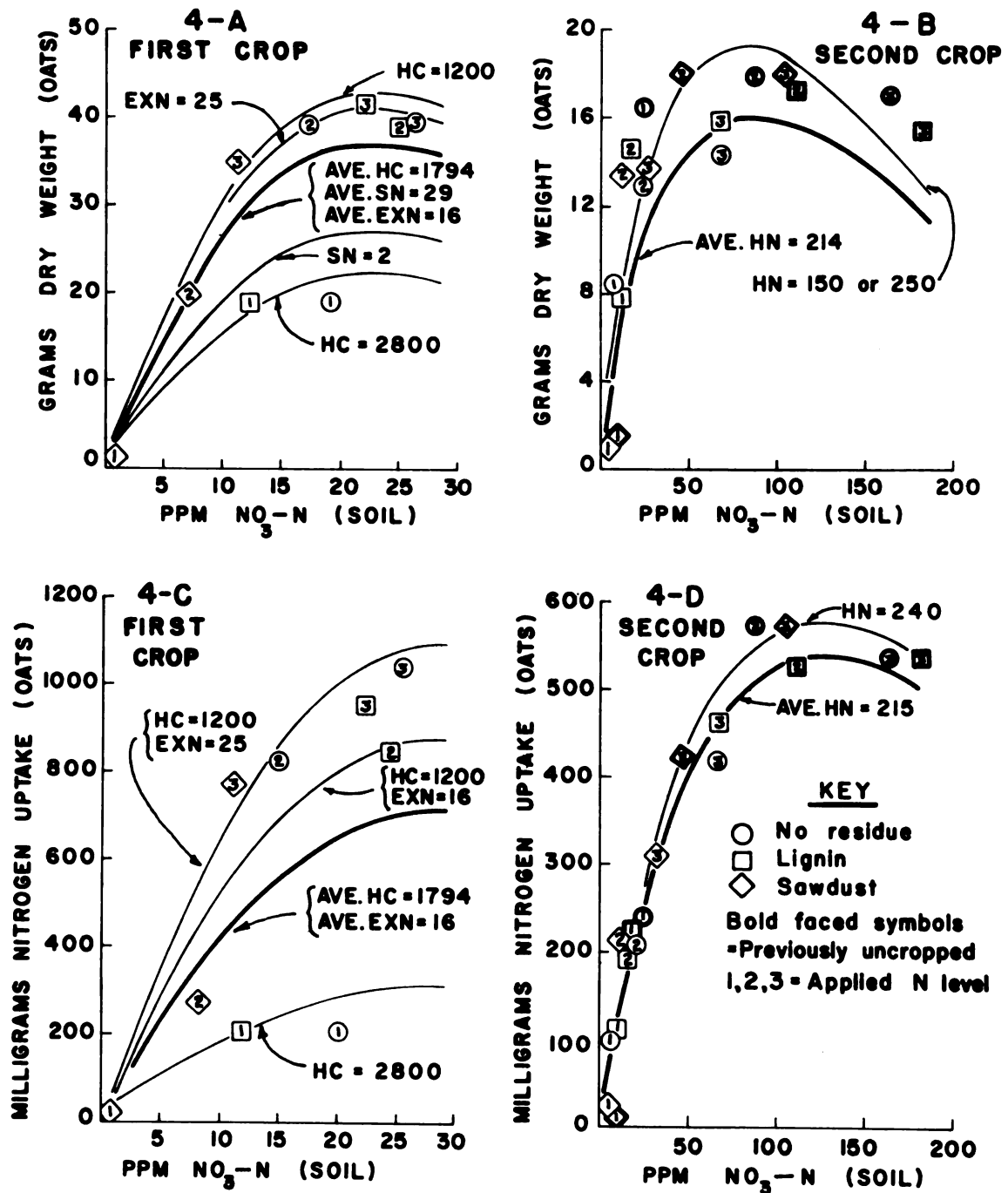


Figure 4

The effect of varying other soil variables one at a time in the function for figure 4-A revealed that deviations below the average function were associated primarily with variations in water-soluble nitrogen other than nitrate. Deviations above the average function were associated with variations in extractable nitrogen. As seen in table 14, these forms of nitrogen, as well as nitrate, were negatively correlated with hydrolyzable carbon. Because of these and other intercorrelations, the depressing effect of hydrolyzable carbon was exaggerated by transfer to hydrolyzable carbon terms in the function of effects associated with other soil variables.

It is likely that a function which would permit more flexibility in expression of interaction effects would be more useful in segregating out specific effects of individual variables. Nevertheless, relationships brought out by the function are meaningful and reasonable. Distortions can be rationalized by reference to observed experimental values.

Functional relationships for nitrogen uptake by the same crop (figure 4-C) were essentially similar. The major response to nitrate was influenced by a depressing effect of hydrolyzable carbon. A portion of this depressing effect, however, was transferred to non-hydrolyzable carbon by reason of the high positive correlation between these two forms of carbon and their negative correlation with nitrate. Deviations below the average curve were again associated with variations in soluble nitrogen, deviations above the curve with extractable nitrogen.

In the second crop (figures 4-B and 4-D) the depressing effect of hydrolyzable carbon was greatly minimized by the function. This was due in part to a much reduced intercorrelation between nitrate and

hydrolyzable carbon (cf. tables 14 and 15). More significantly, however, nitrogen which had accumulated residually in the hydrolyzable fraction was released during the second cropping period and added appreciably to the supply of available nitrogen represented by nitrate present at planting time. Some experimental means fall outside the functional limits set by observed mean values for nitrate and hydrolyzable nitrogen, indicating that minor effects were also associated with other soil or environmental variables.

In the first crop, an optimum for dry matter production was expressed at a soil nitrate-nitrogen level of about 22 ppm. (figure 4-A), whereas the optimum for nitrogen uptake was somewhat higher, about 28 ppm. (figure 4-C). In the second crop, the functional optimum for dry weight was about 85 ppm. (figure 4-B), and the optimum expressed for nitrogen uptake was about 130 ppm. nitrate-nitrogen (figure 4-D). These differences reflect the greater efficiency of dry matter accumulation during the short days and cool greenhouse temperatures of late winter and spring when the first crop was grown, as compared with greater respiratory dissipation of products of photosynthesis under the high summertime temperatures to which the second crop was exposed.

The larger quantities of nitrogen available to the second crop tended to compensate for the unfavorable environmental conditions. However, as may be seen in figure 5, the major increases in dry weight in both crops occurred at soil nitrate levels less than 30 ppm. nitrate-nitrogen. On this basis, nitrogen taken up by the second crop (figure 4-D) in excess of about 300 mg. exceeded the capacity of the crop to efficiently assimilate it. As noted in the discussion of figure 2-D, such excessive accumulations of nitrogen in tissue occurred at nitrogen contents in excess of 2.2 percent. In the first crop a distinct optimum

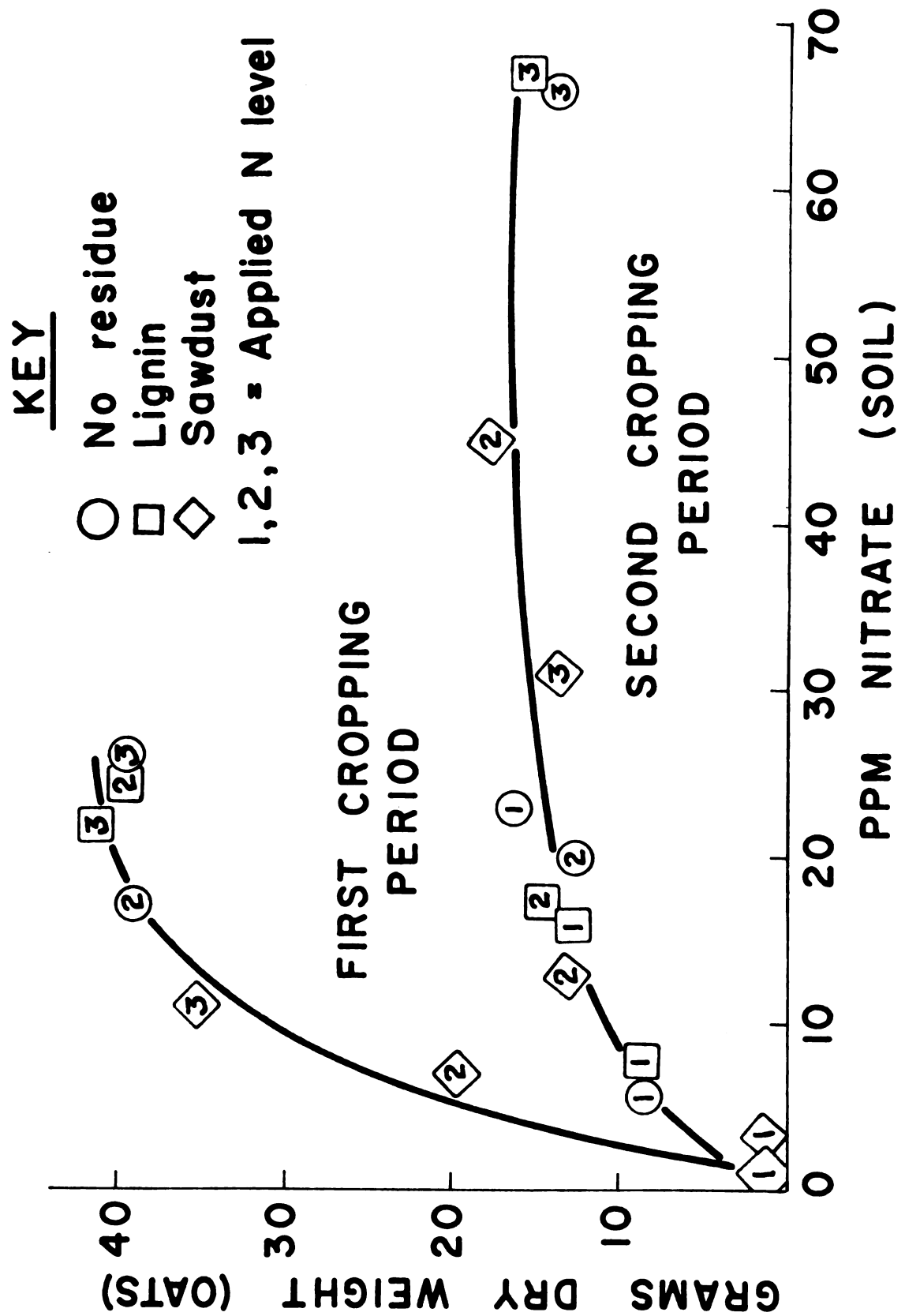


Figure 5 - Dry weight of the first and second long term oat crops vs. nitrate nitrogen in the soil at planting time.

for dry matter production was expressed at 2.0 percent nitrogen (figure 2-A).

Thus it would appear that 20 to 30 ppm. nitrate-nitrogen in soil and 2.0 to 2.2 percent nitrogen in tissue may represent critically optimum levels for growth of oats under widely varying environmental conditions. Functional analysis as employed in this study provides a tool for identifying such critical optima with greater precision than is possible by examination of means by conventional analysis of variance.

## SUMMARY AND CONCLUSIONS

Large changes in the chemical nature of carbon and nitrogen compounds in soil were observed during the first three month's decomposition of added sawdust. Water-soluble and salt-extractable forms of nitrogen were initially immobilized, appearing primarily in the acid-hydrolyzable fraction after five weeks. This initial immobilization was due almost entirely to hydrolyzable forms of carbon and did not occur with lignin isolated from the sawdust by acid hydrolysis and removal of cellulose and other carbohydrates.

Small, but statistically significant, increases in non-hydrolyzable nitrogen were observed after 5 weeks with both sawdust and lignin. These increases were greater with increasing levels of added urea nitrogen.

It appeared likely that immobilization of nitrogen in non-hydrolyzable forms was due to the formation of chemical complexes between lignin degradation products and ammonia. Immobilization in hydrolyzable forms was probably due mainly to incorporation of mineral nitrogen into microbial proteins.

Where urea nitrogen was added to give C:N ratios for sawdust of 40:1 and 20:1, peak immobilization of nitrogen occurred prior to the sampling made 5 weeks after amendment. During the next 10 weeks, additional immobilization of nitrogen in this fraction occurred where no nitrogen was used with sawdust, and in the presence of roots of the first long term crop of oats. In sawdust amended soil, this continued immobilization was associated with extensive conversion of non-hydrolyzable forms of carbon to hydrolyzable forms.

Growth and uptake of nitrogen in 54 days by the first long term

crop of oats was influenced primarily by levels of nitrate and hydrolyzable carbon found at planting time, 5 weeks after amendment. Multiple regression analyses revealed that, in addition to nitrate, other forms of nitrogen (primarily ammonium), which were soluble in water or extractable with  $\text{N K}_2\text{SO}_4$  at pH 1.5 to 2.0, contributed extensively to nitrogen availability. The effect of increasing levels of hydrolyzable carbon was to depress the availability of these three forms of nitrogen.

Growth and uptake of nitrogen in a similar period by a second oat crop planted 105 days after amendment was essentially a function of nitrate present at planting time, although a significant contribution of nitrogen from the hydrolyzable fraction was indicated.

In both long term crops, 91 to 99 percent of the observed variation in dry weight and nitrogen uptake was accounted for by functional relationships defined by an exponential-power function in which all measured forms of nitrogen and carbon were included as separate terms. In both crops, near optimum production of dry matter was associated with soil tests of 20 to 30 ppm. nitrate-nitrogen and tissue nitrogen contents of about 2.0 percent. Optimum levels of soil nitrate and tissue nitrogen for nitrogen uptake were higher but represented nitrogen taken up in excess of the crop's ability to assimilate the nitrogen.

An unexpected result was encountered 5 weeks after amendment when nitrate and other soluble materials were removed by leaching with water before placing the roots of nitrogen-deficient oat plants in contact with the soil. Dry matter synthesis in the first 11 days of growth was stimulated independently of nitrogen uptake by some factor or factors present in both lignin and sawdust. Multiple regression analysis associated the stimulating activity primarily with the non-hydrolyzable carbon fraction. Reduced percent nitrogen in harvested tissue and



increases in nitrogen taken up in the same period reflected increased dry matter production and increased vigour, but were additionally influenced by the supply of nitrogen present in salt-extractable forms in the soil. When nitrogen uptake was plotted against percent nitrogen in tissue, the distribution of experimental points and the expressed functional relationships with percent dry matter were analogous to those obtained when nitrogen uptake was plotted against extractable nitrogen and varying levels of non-hydrolyzable carbon in the soil. In both cases, about 80 percent of the total variation in nitrogen uptake was accounted for by functional relationships defined in the mathematical model.

Similar stimulating effects of sawdust and lignin on short term growth and nitrogen uptake were observed in the second short term oat planting made 105 days after amendment. However, this stimulating action was accompanied by what appeared to be an inhibitory effect of root residues from the first long term crop of oats in previously cropped pots. Neither the stimulatory activity of sawdust and lignin nor the inhibitory activity in root residues was clearly associated with any measured fraction of carbon or nitrogen by functional relationships defined in the mathematical model.

In spite of shortcomings in the exponential-power function which was employed as a mathematical model, its use did lead to meaningful inferences regarding the contribution to plant response of various fractional forms of carbon and nitrogen in the soil. The fractions measured were particularly meaningful at early stages of decomposition of sawdust incorporated into the soil. After decomposition had proceeded for three months, the contribution of carbon and nitrogen fractions, other than nitrate, was only weakly expressed by the function.

This is not surprising, since the hydrolytic fractions studied represent gross fractions in which forms of carbon or nitrogen with high specific activity would have been masked by mixture with forms of little or no significance for crop response. Nevertheless, the degree of functional association which was encountered indicated that multiple correlation and regression analysis is a useful tool for isolating activity in chemically characterizable fractions of carbon and nitrogen in soil. In future work, more precisely characterizable fractions or groups of organic compounds should be considered.

Not all of the responses of crops to additions of carbonaceous residues are to be understood in terms of their effects on nutrient availability. The data reported here indicate that specific growth regulatory effects are associated with early stages of decomposition of crop residues remaining or added to the soil. These effects may be inhibitory as well as stimulatory and may involve carbon compounds which contain little or no nitrogen. These hormonal effects of carbon compounds may be as important as the immobilizing effects of carbonaceous energy materials in influencing the effective nitrogen supplying power of soils. Short term growth or nitrogen uptake of plants may be very misleading when used as a bioassay for the availability of insoluble forms of nitrogen in soil recently enriched with crop residues.

Of direct practical importance was the observation that inhibitory effects on long term growth of oats, due to nitrogen immobilization, had completely disappeared 3 months after addition of sawdust at the rate of 10 tons per acre when 200 pounds per acre of urea nitrogen was also applied. This quantity of nitrogen was calculated to give a C:N ratio for the sawdust of 40:1. No reduction in yield occurred after

5 weeks when nitrogen was applied at the rate of 400 pounds per acre to give a C:N ratio of 20:1.

## BIBLIOGRAPHY

1. Allison, F. E. The enigma of soil nitrogen balance sheets. In A. G. Norman, ed. *Advances in Agronomy* Vol. VII. Acad. Press, Inc., New York. pp. 213-237. 1955.
2. Allison, F. E. Estimating the ability of soils to supply nitrogen. *Agr. Chem.* 11 (No. 4):46-48. 1956.
3. Allison, L. E. Wet-combustion apparatus and procedure for organic and inorganic carbon in soil. *Soil Sci. Soc. Amer. Proc.* 24:36-40. 1960.
4. Bartholomew, W. V. Availability of organic nitrogen and phosphorus from plant residues, manures and soil organic matter. *Papers of the Soil Microbiology Conference.* Perdue University. 1954.
5. \_\_\_\_\_, and Hiltbold, A. E. Recovery of fertilizer nitrogen by oats in the greenhouse. *Soil Sci.* 73:193-201. 1952.
6. Brauns, F. E. *The Chemistry of Lignin.* Acad. Press Inc., New York. p. 157. 1952.
7. Bremner, J. M. A review on recent work on soil organic matter: I. *J. Soil Sci.* 2:67-82. 1951.
8. \_\_\_\_\_. The nature of soil nitrogen complexes. *J. Sci. Food Agr.* 3:497-500. 1952.
9. \_\_\_\_\_. Studies on humic acids. *J. Agr. Sci.* 48:352-360. 1956.
10. \_\_\_\_\_. Determination of fixed ammonium in soil. *J. Agr. Sci.* 52:147-160. 1959.
11. \_\_\_\_\_. Determination of nitrogen in soil by the Kjeldahl method. *J. Agr. Sci.* 55:11-33. 1960.
12. \_\_\_\_\_, and Shaw, K. The mineralization of some nitrogenous materials in soils. *J. Sci. Food Agr.* 8:341-347. 1957.
13. Broadbent, F. E. Nitrogen release and carbon loss from soil organic matter during decomposition of added plant residues. *Soil Sci. Soc. Amer. Proc.* 12:246-249. 1947.
14. \_\_\_\_\_. The soil organic fraction. In A. G. Norman, ed. *Advances in Agronomy* Vol. V. Acad. Press Inc., New York. pp. 153-181. 1953.
15. Halter, A. N., Carter, H. O., and Hocking, J. G. A note on the transcendental production function. *Jour. of Farm Econ.* 39:966-974. 1957.

16. Clark, F. E., Beard, W. F., and Smith, D. H. Dissimilar nitrifying capacities of soil in relation to losses of applied nitrogen. *Soil Sci. Soc. Amer. Proc.* 24:50-54. 1960.
17. DeMent, J. D., Stanford, G., and Hunt, C. M. A method for measuring short-term nutrient absorption by plants: III. Nitrogen. *Soil Sci. Soc. Amer. Proc.* 23:371-374. 1959.
18. Dyck, A. W. J., and McKibbin, R. R. The non-protein nature of a fraction of soil organic nitrogen. *Can. J. Res.* 13:264-268. 1935.
19. Ensminger, L. E., and Pearson, R. W. Soil nitrogen. In A. G. Norman, ed. *Advances in Agronomy Vol. II.* Acad. Press, Inc., New York. pp. 81-109. 1950.
20. Fraps, G. S., and Sterges, A. J. Estimation of nitric and nitrous nitrogen in soils. *Texas Agr. Exp. Sta. Bul.* 439, pp. 19-21. 1931.
21. Fraser, G. K. Soil organic matter. In F. E. Bear, ed. *Chemistry of the Soil.* Reinhold Pub. Corp., New York. pp. 149-176. 1955.
22. Gottlieb, S., and Hendricks, S. B. Soil organic matter as related to newer concepts of lignin chemistry. *Soil Sci. Soc. Amer. Proc.* 10:117-125. 1945.
23. Harmsen, G. W., and Van Schreven, D. A. Mineralization of organic nitrogen in soil. In A. G. Norman, ed. *Advances in Agronomy Vol. VII.* Acad. Press, Inc., New York. pp. 300-383. 1935.
24. Hiltbold, A. E., and Adams, F. Effects of nitrogen volatilization on soil acidity changes due to applied nitrogen. *Soil Sci. Soc. Amer. Proc.* 24:45-47. 1960.
25. Jackson, M. L. *Soil Chemical Analysis.* Prentice-Hall, Inc., Englewood Cliffs, New Jersey. pp. 183-201. 1958.
26. Johnston, H. H. Studies on the chemical, physical and biological properties of soil organic matter. Ph. D. Thesis, Department of Soil Science, Michigan State University. 1958.
27. Kamerman, P., and Klintworth, H. Influence of fertilizer on the nitrogen and carbon cycles in soils. *Union of South Africa, Department of Agr. Sci. Bul.* 137:1-26. 1934.
28. Lyon, T. L., Buckman, O. H., and Brady, N. C. *The Nature and Properties of Soils.* The Macmillan Company, New York. pp. 455-458. 1952.
29. Mattson, S., and Koutler-Andersson, E. The acid-base condition in vegetable litter and humus: VI. Ammonia fixation and humus nitrogen. *Annals Agr. College Sweden* 11:107-134. 1943.

30. Mortland, M. M., and Wolcott, A. R. Sorption of inorganic nitrogen compounds by soil materials. (MSS. submitted for publication in Soil Nitrogen. Monograph series. American Society of Agronomy.) 1963.
31. Norman, A. G. The Biochemistry of Cellulose, the Polyuronides, and Lignin. Oxford, at the Clarendon Press. 1937.
32. Norman, A. G. Problems in the chemistry of soil organic matter. Soil Sci. Soc. Amer. Proc. 7:7-15. 1942.
33. Parker, D. T., Parson, W. E., and Bartholomew, W. V. Studies on nitrogen tie-up as influenced by location of plant residues in soils. Soil Sci. Soc. Amer. Proc. 21:608-611. 1957.
34. Peterson, L. A., Attoe, O. J., and Ogden, W. B. Correlation of nitrogen soil tests with nitrogen uptake by the tobacco plant. Soil Sci. Soc. Amer. Proc. 24:205-209. 1960.
35. Pinck, L. A., Allison, F. E., and Gaddy, V. L. The nitrogen requirements in the utilization of carbonaceous residues on soils. Agron. Jour. 38:410-420. 1946.
36. Rodrigues, G. Fixed ammonia in tropical soils. J. Soil Sci. 5:264-274. 1954.
37. Schwartzbeck, R. A., MacGregor, J. M., and Schmidt, E. L. Gaseous nitrogen losses from nitrogen fertilized soils measured with infrared and mass spectroscopy. Soil Sci. Soc. Amer. Proc. 25:186-189. 1961.
38. Singh, B. N. Mineral and organic forms of nitrogen in some Michigan soils and an agro-economic evaluation of their potential usefulness for advisory purposes. Ph. D. Thesis, Department of Soil Science, Michigan State University. 1960.
39. Smith, G. E. Soil fertility and corn production. Missouri Agr. Exp. Sta. Bul. 583. 1952.
40. Stevenson, F. J. Effects of some long-time rotations on the amino acid composition of the soil. Soil Sci. Soc. Amer. Proc. 20: 204-208. 1956.
41. Sundquist, W. B., and Robertson, L. S., Jr. An economic analysis of some controlled fertilizer input-output experiments in Michigan. Michigan State Univ. Tech. Bul. 269. 1959.
42. Waksman, S. A. Soil Microbiology. John Wiley & Sons, Inc., New York. 1952.
43. \_\_\_\_\_, and Iyer, K. R. N. Contributions to our knowledge of the chemical nature and origin of humus: I. On the synthesis of the "humus nucleus". Soil Sci. 34:43-69. 1932.

44. \_\_\_\_\_, \_\_\_\_\_. Contributions to our knowledge of the chemical nature and origin of humus: II. The influence of "synthesized" humus compounds and of "natural humus" upon soil microbiological processes. Soil Sci. 34:71-79. 1932.
45. \_\_\_\_\_, \_\_\_\_\_. Contributions to our knowledge of the chemical nature and origin of humus: III. The base exchange capacity of "synthesized humus" (ligno-protein and "natural humus" complexes). Soil Sci. 36:57-67. 1933.
46. \_\_\_\_\_, \_\_\_\_\_. Contributions to our knowledge of the chemical nature and origin of humus: IV. Fixation of proteins by lignins and formation of complexes resistant to microbial decomposition. Soil Sci. 36:69-82. 1933.
47. Waksman, S. A., and Tenney, F. G. On the origin and nature of the soil organic matter or soil "humus": IV. The decomposition of the various ingredients of straw and of alfalfa meal by mixed and pure cultures of microorganisms. Soil Sci. 22:395-406. 1926.
48. \_\_\_\_\_, \_\_\_\_\_. Composition of natural organic materials and their decomposition in the soil: II. Influence of age of plant upon the rapidity and nature of its decomposition--rye plants. Soil Sci. 24:317-333. 1927.
49. White, A. W., Jr., Giddens, J. E., and Morris, H. D. The effect of sawdust on crop growth and physical and biological properties of Cecil soil. Soil Sci. Soc. Amer. Proc. 23:365-368. 1959.
50. White, W. C., and Pesek, J. Nature of residual nitrogen in Iowa soils. Soil Sci. Soc. Amer. Proc. 23:39-42. 1959.
51. Wolcott, A. R., and Johnston, H. H. Immobilization and mineralization of nitrogen in soils following organic amendments. Agron. Abstr. p. 17. 1958.
52. Woodruff, C. M. Estimating the nitrogen delivery of soil from the organic matter determination as reflected by the Sanborn field. Soil Sci. Soc. Amer. Proc. 14:208-212. 1949.

FOR USE ONLY



MICHIGAN STATE UNIVERSITY



3 1293 03178 5