SOME CHEMICAL AND BIOLOGICAL CHANGES PRODUCED IN A FOX SANDY LOAM SOIL BY CERTAIN SOIL MANAGEMENT PRACTICES

by

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INTRODUCTION

The effects of cropping systems and fertilizer and lime applications on soils under experimental conditions have been measured chiefly in terms of differences in plant yields. In some of these cases changes in the organic matter content of the soil have been measured by chemical analysis, and quite frequently the quantities of available plant nutrients in the soil of treated and untreated plats have been determined by methods devised for this purpose. Changes in the biological conditions of soils, however, as nitrifying power, numbers of bacteria and fungi, carbon dioxide production, etc. resulting from soil treatments have been studied to a less extent.

In 1917, the Soils Section of Michigan State College placed under experimental treatment a field of Fox sandy loam soil which had fallen to such a low state of fertility that it had been uncropped for several years. During succeeding years many of the treated plats attained a fairly high state of fertility as measured by crop yields, as much as 50 bushels of wheat to the acre having been grown on some plats. Marked differences occurred, however, in the crop yields obtained from plats receiving different treatments. As a matter of interest to the men in charge of the field and to agronomists in general, it was considered important to know if chemical and biological changes had taken place in the soil of the plats receiving different treatments. This investigation was carried on, therefore, to obtain information of this nature and to determine what correlations might exist among the various data collected.

REVIEW OF LITERATURE

Literature dealing with the relationships of chemical and biological activities in soil and the crop producing power of the soil has been thoroughly reviewed elsewhere (32, 33), and hence reference will be made only to those papers most closely related to the studies presented in this paper.

RELATION BETWEEN CROP PRODUCTION AND BACTERIAL ACTIVITY IN THE SOIL

Brown (6) demonstrated a close relationship between bacterial processes as measured by nitrification, ammonification, azofication, and crop production in his studies of some Iowa soils. Russell and Appleyard (22) found curves for carbon dioxide production, nitrate content, and numbers of microorganisms in the soil were similar enough to be called related and that all of these factors exerted an influence on crop production. The results of Noyes and Connor (20) showed a definite relationship between crop yields, nitrification, and bacterial numbers in some acid soils. Neller (18), working with limed and unlimed soils, found a distinct correlation between crop production and bacterial activity as measured by CO_2 production, NO_3 production, and numbers of bacteria. "A close correlation exists between crop production and bacterial activity and both are equally affected by soil treatment" is a summarized statement of the literature by Waksman (32).

NITRIFICATION AS A MEASURE OF SOIL FERTILITY AND THE EFFECTS OF SOIL TREATMENTS

Studies of nitrification by Wahltman (30), Lohnis (17), Kellerman (14), and Russell (23) in soils variously treated and under different experimental conditions, revealed a definite correlation between nitrification and crop production. Where soils were continuously cropped without fertilization, as reported by Allen and Bonazzi (1), nitrification was reduced until no correlation between it and crop production could be made. Harper and Boatman (12) found no increase in nitrification as a result of applying superphosphate and muriate of potash fertilizers to limed soils. Some recent work by Ligon (16) on muck soils differing widely in reaction, showed an excellent correlation between nitrification and the yield of spinach.

The studies of Temple (25), Fred and Graul (8), Barthel, and Beckwith (5), showed that ammonium sulphate, the most commonly used source of nitrogen for nitrification studies, was not suitable for use in such studies in all soils. Halversen (11) demonstrated the value of ammonium sulphate as a source of nitrifiable nitrogen for certain productive soils, but stated that acid soils of humid regions, low in bases, would not be expected to nitrify this material. Kulik (15) and Turchin (28) found a depression of nitrification of urea in podsolized soils by addition of sulphates and especially chlorids. Waksman (31) states from his studies of methods for determining nitrification, that several sources of nitrogen and different methods of procedure should be employed to give a better picture of a soil's power to nitrify nitrogenous materials.

PRODUCTION OF CARBON DIOXIDE AS A MEASURE OF BACTERIAL ACTIVITY IN SOILS

The amount of carbon dioxide produced by aerobic bacterial activity was measured by Van Suchtelen (29) and found to be a better index of soil productivity than the number of microorganisms in a soil. Stocklasa and Ernest (24) found that the greatest amounts of carbon dioxide were produced in fertilé, well aerated soils and that carbon dioxide production paralleled nitrification. Organic matter rich in nitrogen gave a better correlation between carbon dioxide production, ammonification, and nitrification than materials low in this element according to Gainey (10). Carbon dioxide evolution from soils, with and without organic matter additions, may be used as a measure of soil fertility according to Waksman (32).

CELLULOSE DECOMPOSITION IN SOILS AND ITS RELATION TO SOIL FERTILITY

Christensen (7) was one of the first investigators to suggest that cellulose deoomposition could be used as a measure of soil productivity. The work of Niklewski (19) showed that cellulose decomposition was controlled chiefly by the supply of available nitrogen. Anderson (2) verified Niklewski's (19) results and showed the effect of nitrogen from different sources on cellulose decomposition. Some cellulose decomposition studies by Holben (13) on soil from the fertility plots at the Pennsylvania station lead to the statement that this process is an excellent measure of soil fertility. The importance of available nitrogen and phosphorus to cellulose decomposition in soils is further stressed by Waksman (32), but soil reaction is considered of little importance in the process. Some recent work by White and co-workers (34) further emphasizes the importance of nitrogen and also demonstrates a relationship between soil reaction and cellulose decomposition.

EXPERIMENTAL

DESCRIPTION OF SOIL TYPES, RECORD OF SOIL TREATMENT AND CROPPING HISTORY OF PLOTS SELECTED FOR THE INVESTIGATION

The Cass County Farm experimental field, near Cassopolis, Michigan, was plotted in 1917 as an experimental area for determining the best methods of management for the lighter soils of southwestern Michigan. For the purposes of this investigation eight plots were selected from this field for study on the basis of the soil treatments used and the crop yields which had been obtained from them.

The Fox sandy loam on which the plots are located is one of the predominating soil types of southwestern Michigan both in area and value of its products. It may be described as a light brown, friable, sandy loam surface soil lying over a pale yellowish soil of similar texture to a depth of 12 to 18 inches. The B horizon consists of sand and gravel cemented together by reddish brown clay and ranging from 10 to 18 inches in thickness. At a depth of 24 to 36 inches, coarse sand and gravel predominate, which affords rapid to excessive drainage. The nature of the B horizon is a distinguishing characteristic and gives to this soil greater retentive powers for moisture and plant nutrients, than similar soils in which this horizon is not so highly developed. Under natural conditions, this soil is low in content of organic matter and plant nutrient elements, which would indicate a greater response to suitable soil management practices than soils well supplied with organic matter and reserve sources of plant nutrients. As the Fox sandy loam is a strongly acid soil, an application of three tons of ground limestone to the acre was made at the beginning of the field experiment to all except the check plots.

Fertilizers were applied at the following rates per acre: Potassium, 100 pounds (50% KCl); Nitrogen, 100 pounds ($NaNO_3$); Phosphorus, 200 pounds (16%) superphosphate); Rock phosphate, 1000 pounds; Gypsum, 86 pounds. All of the fertilizers were applied before the crops were planted with the exception of nitrogen for fall seeded grain. Forty per cent of this was applied in the fall, and sixty per cent in the spring until 1920, when the proportions were changed to twenty per cent in the fall and eighty per cent in the spring. Periodic applications of the fertilizers were made as shown in table 1, and according to the amounts already given.

The cropping history of these plots since they have been under experimentation is as follows: soy beans, 1917; rye, 1918; wheat, 1919; sweet clover, 1920,1921; rye, 1922; corn, 1923; wheat, 1924; sweet clover, 1925; soy beans, 1926; wheat, 1927; corn, 1928; oats, 1929; and wheat, 1930, 1931. The soy bean crop in 1926 was plowed under to supplement the organic matter derived from crop residues. The yield was determined before plowing under the crop. Other crops were removed; no straw, stover, or similar material being added to the soil.

METHOD OF TAKING SOIL SAMPLES

Soil samples of sufficient quantity for all the laboratory studies were taken from the plots in the fall of 1931.

Table 1. Periodic Application of Fertilizers to the Designated Plots

Soil		Year of Application										
No.	Field * Treatment	1917	1918	1919	1922	1923	1924	1926	1927	1928	1929	1930
301N	None											
301S	Lime	L										
302	LNPK	LNPK	N	PN	PNK	N	PNK	P	N	PNK	NP	N
305	LPK	LPK		P	PK		PK	P		PK	P	
307	LN	LN	N	N	N	N	N		N	N	N	
308	LK	LK			K		K			K		
309	LP	LP		P	P		P	P		P	P	
310	L RP. C	L RP.C			RP.C					RP.C		

* L Lime in the form of ground limestone
K Potassium as potassium chloride
RP. Phosphorus as rock phosphate

N Nitrogen as sodium nitrate
P Phosphorus as superphosphate
C Calcium sulphate as gypsum

(This legend will be used throughout this paper)

To secure a representative compositive sample, five samples were taken at systematically located points on each plot and thoroughly mixed. Samples were taken by removing and discarding the surface inch of soil and then taking with a spade the desired amount of plow soil to a depth of 6 to 8 inches. After thorough mixing and screening to remove stones and large particles of undecomposed organic matter, aliquots were taken, air dried, and stored in sterilized containers.

LABORATORY METHODS

Nitrogen was determined by the method given for soils in the Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists (2). Organic matter was determined by loss on ignition, since the soils contained no carbonates. The reaction in terms of pH, was determined on air dry soil by the quinhydrone method as described by Bauer (4). Available phosphorus in pounds per acre was obtained by the method of Truog (26). Fred and Waksman's (9) reduction method was used to determine nitrate nitrogen. Numbers of bacteria and fungi were obtained by taking the average count from six agar plates of the respective media used. Nitrification and carbon dioxide production methods are described in the respective studies dealing with these substances.

RELATION BETWEEN SOIL TREATMENT, CROP PRODUCTION, SOIL REACTION, AND SOME AVAILABLE NUTRIENTS

Because of the influence of seasonal variations on crop production, which tend to modify differences due to fertilizer treatment and other factors affecting productivity, it was deemed advisable to express the yields of the different plots in terms of pounds of dry plant material produced during the entire period they have been under experimentation. Since the problem deals primarily with the chemical and biological changes which may have taken place in the soil, during the period of field treatment and their relation to crop production, information on soil reaction, quantities of available phosphorus, total organic matter, and nitrogen furnishes a basis for comparing soil changes with crop yields. An analysis of the data given in table 2 and presented graphically in figure I, brings out the following points.

Although the supply of available phosphorus is low in all the soils, those receiving phosphate applications have the largest amounts. This is especially true of soils 310N and 309, which were treated with rock phosphate and superphosphate, respectively. Since the available supply of phosphorus is low, any difference due to soil treatment may be expected to have a marked influence on the chemical and biological conditions in these plots. Content of organic matter and total nitrogen are closely related for all soils except number 301N, which is low in organic matter and relatively high in total nitrogen. The relatively low content of organic matter in soil 301N is doubtless due to the fact that very low yields of crops were obtained from this plot and as a result a small amount of crop roots and stubble was left in the soil.

The high content of nitrogen in soil 301N, in view of the small quantities of nitrogen-containing organic material left in it, suggests that another source of nitrogen must have existed.

Table 2. Chemical Analyses of Soils and Crop Yields in Pounds Per Acre and in Per Cent Based on the Results from the Untreated Soil, in Relation to Soil Treatment.

Soil		Reaction		Crop Yields		Available P_2O_5		Total Nitrogen		Organic Matter	
No.	Field Treatment	pH	Per Cent	Pounds of Dry Matter	Per Cent	Pounds Per Acre	Per Cent	Pounds Per Acre	Per Cent	Pounds Per Acre	Per Cent
301N	No Treatment	4.71	100	8,857	100	23	100	1380	100	34,200	100
301S	Lime	5.15	109	26,399	298	26	112	1300	94	35,600	104
302	LNPK	5.59	118	42,253	478	33	144	1420	103	41,200	120
305	LPK	5.45	116	37,425	423	34	148	1420	103	41,400	121
307	LN	5.67	120	32,738	370	29	126	1200	87	36,200	106
308	LK	5.74	121	29,288	331	30	131	1220	89	36,600	107
309	LP	5.61	119	25,692	290	32	139	1200	87	34,600	101
310	L RP. C	5.59	118	27,363	309	40	174	1120	81	34,000	100

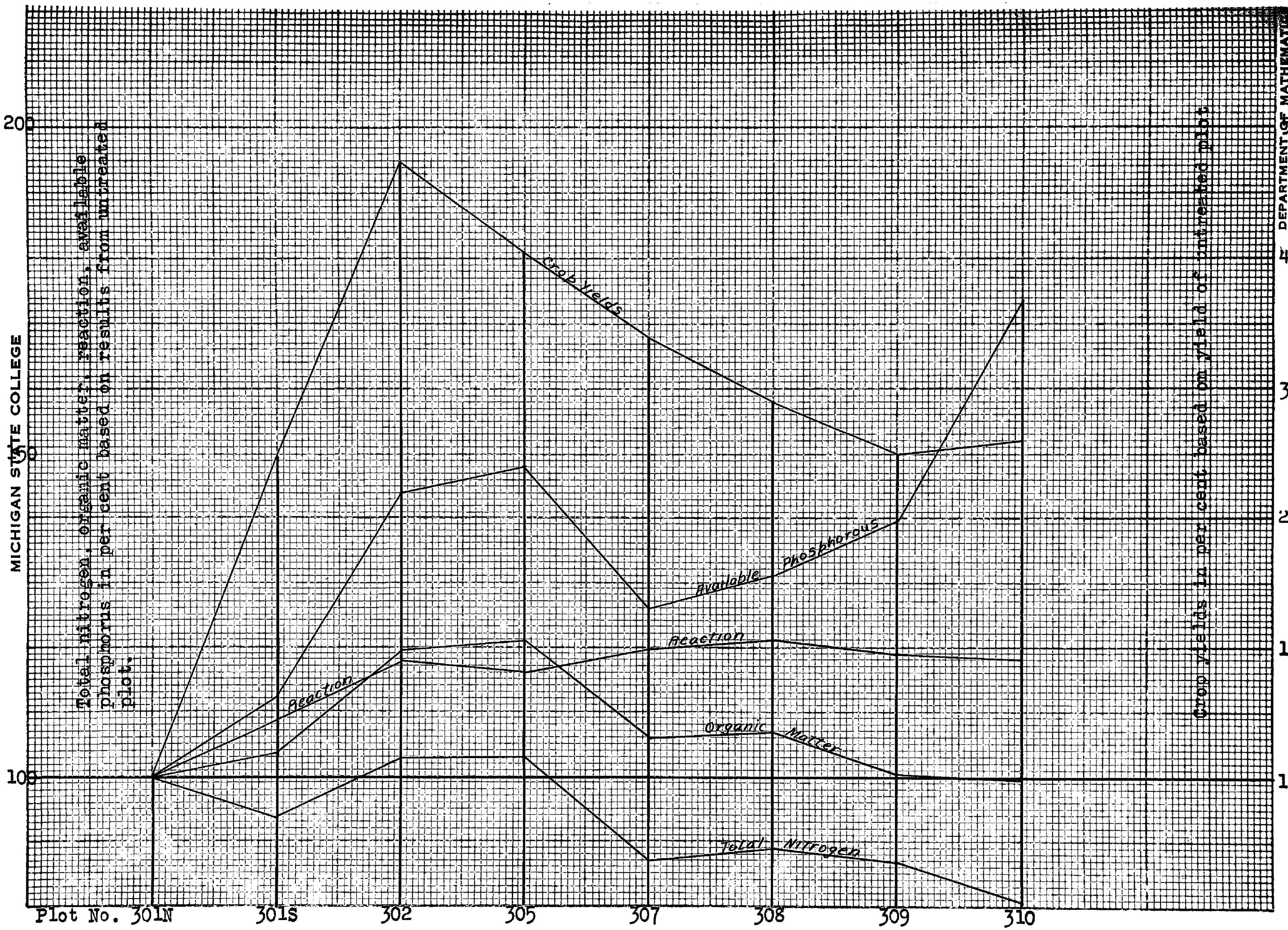

FIG. I

Interrelations Between Soil Treatment, Crop Yields, Soil Reaction, and Content of Available Phosphorus, Organic Matter, and Total Nitrogen.

Some unpublished data on nitrogen fixation show a very active nitrogen fixing flora especially by anaerobic organisms in soil 301N. It may be concluded from this that free nitrogen fixation was instrumental in raising the total nitrogen content of this soil.

The soils receiving combinations of lime, nitrogen, and phosphorus were higher in nitrogen and organic matter than those receiving lime and single element treatment. Since all the soils were comparatively low in nitrogen and organic matter, additions of these substances should have some effect on the biological activities in these soils as measured by nitrification and carbon dioxide production.

Due to the low buffering capacity of these soils, the reaction fluctuates over a considerable range, depending upon the moisture and carbon dioxide supply. Despite this tendency to vary, samples of all the soils taken at the same time of the year, should give comparative reaction values reflecting the influence of plot treatment. Lime alone raised the pH over that of the untreated soil, but not as much as has lime and fertilizers together. While the difference in reaction between soils receiving lime alone and those receiving neither lime or fertilizer and between those treated with lime alone and lime with fertilizers may be of sufficient magnitude to influence biological and chemical relationships, the differences in reaction between soils receiving different combinations of lime and fertilizersare not large enough to have any appreciable effect on these processes.

All of the field treatments have increased the yield of crops over those from the untreated soil. Lime alone has given a very marked increase, while lime with combinations of nitrogen, phosphorus, and potassium has given the greatest increases. Combinations of lime and either superphosphate or rock phosphate have shown the smallest increases in crop yields over the untreated soil, being about the same or slightly greater than the yields from lime alone.

In this part of the paper, the relationship between soil treatment, reaction, crop yields, available phosphorus, total nitrogen, and organic matter has been discussed, but no attempt has been made to correlate these factors. These relationships will be discussed in detail following presentation of the biological investigations.

NUMBERS OF BACTERIA AND FUNGI IN RELATION TO SOIL TREATMENT AND SOME AVAILABLE NUTRIENTS

If available nutrients and numbers of microorganisms in soils are related and are equally affected by soil treatment as stated by Waksman (32), then a determination of the number of the more important groups of microorganisms and the quantities of available nutrients should show some definite relationships for these soils. The number of fungi developing on acid agar media and bacteria developing on Brown's aliuminate agar media from air dry samples of the different soils were determined. The results given in table 3 show the actual numbers of organisms present and also the numbers as per cent based on the number found in the untreated soil. The results of chemical tests are expressed in per cent based on the results for the untreated soil. The curves are constructed from the percentage data.

Table 3. Numbers of Bacteria and Fungi in Relation to Soil Treatment and Available Nutrients

Soils		Microorganisms				Chemical Factors			
		Bacteria		Fungi		Available Phosphorus	Organic Matter	Reaction	Nitrogen
No.	Treatment	Number	Per Cent	Number	Per Cent	Per Cent	Per Cent	Per Cent	Per Cent
301N	None	567,000	100	347,000	100	100	100	100	100
301S	Lime	1,388,000	245	443,000	128	112	104	109	94
302	LNPK	1,659,000	293	624,000	180	144	120	118	103
305	LPK	2,028,000	358	444,000	128	148	121	116	103
307	LN	1,725,000	305	433,000	125	126	106	120	87
308	LK	1,614,000	285	905,000	261	131	107	121	89
309	LP	1,902,000	336	964,000	277	139	101	119	87
310N	L RP.C	1,917,000	338	438,000	126	174	100	118	81

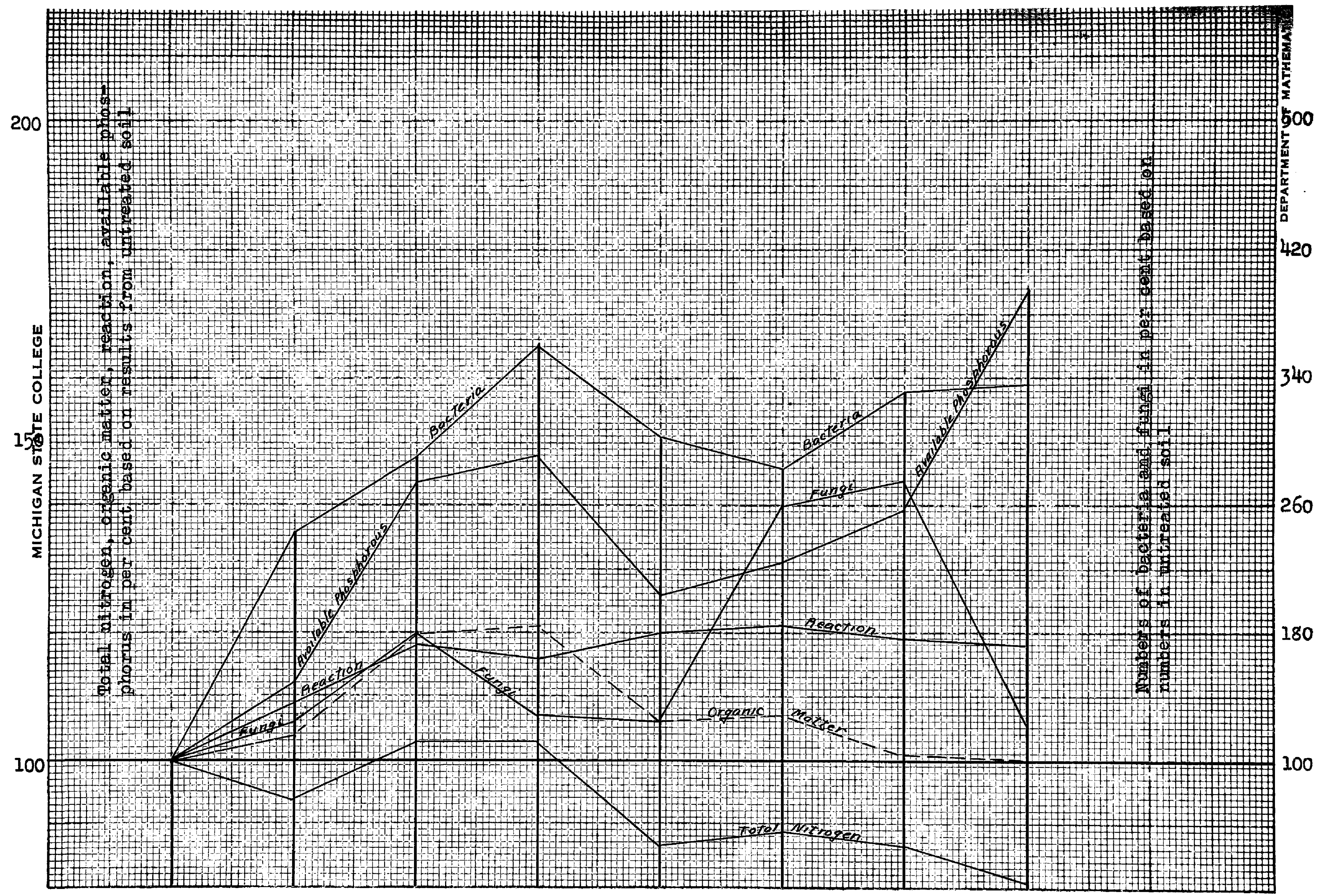

FIG. II

Numbers of Bacteria and Fungi in Relation to Soil Treatment, Soil Reaction, and Content of Total Nitrogen, Organic Matter, and Available Phosphorus.

Lime alone greatly increased the number of bacteria over the number found in the untreated soil, and lime in combination with fertilizing elements enhanced this increase. This increase in numbers was especially marked for those soils receiving phosphorus with lime or with lime and other fertilizing elements. The evidence points to the important part played by phosphorus in bacterial development in these soils.

All field treatments increased the number of fungi developing on agar plates over that from untreated soil. This increase was exceedingly great for soils 308 and 309, which received lime plus potassium and lime plus superphosphate, respectively. Additions of lime alone, lime plus nitrogen, lime plus rock phosphate, and combinations of lime, phosphorus, and potassium gave only small increases in numbers of fungi in the soil. These results indicate no consistent relationship between soil treatment and number of fungi present.

A comparison of bacterial numbers and available nutrients show some relationships, which may be correlated with soil treatment. Phosphorus applications increased numbers of bacteria to a greater extent than any other soil treatment. Except in case of soil 310N, which is unusually high in available phosphorus, a distinct relationship was found between the supply of available phosphorus and numbers of bacteria. Since all combinations of lime and fertilizer treatment had equal effect on soil reaction and since these same combinations increased the numbers of bacteria unequally, little if any correlation exists between numbers of bacteria and soil reaction for these soils. Organic matter content correlates well with the number of bacteria, except for soils 309 and 310N, which are comparatively low in organic matter and high in numbers of bacteria. Total nitrogen content shows the same correlation with numbers of bacteria as does organic matter except in the case of soil 301N, which is relatively higher in total nitrogen, but contained the smallest number of bacteria.

Numbers of fungi showed little relation to soil treatment although the other factors discussed did, so it is evident that little or no correlation could be made between numbers of fungi, organic matter, total nitrogen, available phosphorus, soil reaction, or numbers of bacteria.

NITRIFICATION OF NITROGENOUS MATERIALS IN SOILS AND ITS RELATION TO OTHER SOIL PROCESSES

Nitrification in soils, as a measure of certain biological and chemical relationships, has been used with varying degrees of success for many years. It is considered by some investigators as a true measure of soil productivity since the conditions most favorable for the nitrifying process are quite similar to those for plant growth. If this be true or partly so, a study of nitrification in these soils should give some information of value in determining the changes taking place in soils due to soil treatment.

Since the beginning of nitrification studies in soils, different methods of procedure and different materials for nitrification have been used. Many of these methods were modifications of existing

Table 4. Rate of Nitrification of Urea and Several Ammonium Salts by One of the Soils Studied

Treatment	Weekly Production of Nitrate Nitrogen							
	Milligrams of NO_3				Per Cent NO_3 Based on Result for Check			
	1st	2nd	3rd	4th	1st	2nd	3rd	4th
$(NH_4)_2SO_4$	2.40	3.36	5.40	5.84	60	112	175	178
NH_4Cl	1.70	2.08	2.08	1.95	42	70	69	60
$(NH_4)_2HPO_4$	2.00	4.19	6.07	10.41	50	141	176	318
$(NH_4)_2C_2O_4$	1.60	2.31	7.50	11.28	40	78	243	344
$NH_4C_2H_3O_2$	5.95	4.09	7.85	10.11	149	138	254	308
$(NH_4)_3C_6H_5O_7$	5.30	4.28	8.74	10.48	133	144	282	320
$(NH_2)_2CO$	6.80	6.82	9.34	11.68	170	230	302	357
Check	4.00	2.97	3.09	3.28	100	100	100	100

methods changed to fit the conditions of the particular investigation. In recent years an effort has been made to use only the most generally accepted methods, so that results may have a more uniform interpretation and a wider use. It is evident that a better picture of the nitrifying capacity of a soil may be obtained by using a standard method of procedure a and several materials containing the nitrifiable nitrogen. The most commonly used method is to add the nitrogenous material to 100 grams of soil in tumblers, make up to optimum moisture, incubate for twenty eight days, and determine the quantity of nitrates formed.

From a survey of the more recent literature on this subject, it is evident that all nitrogenous materials are not equally nitrified and that the nitrogen in some materials may be nitrified rapidly under one set of soil conditions but very slowly under others. To determine the most suitable materials for nitrification studies in the soils, a group of organic and inorganic salts of ammonium and urea were selected for a preliminary trial on one of the soils used in this investigation. The materials were added in sufficient quantities to give fifty milligrams of nitrogen per one-hundred grams of air dry soil. No attempt was made to determine the most suitable quantity of nitrogen, because several investigators have shown that thirty to fifty milligrams of nitrogen per one-hundred grams of soil is well suited for nitrification studies. A sufficient number of soil samples were prepared to permit of determining nitrate production at weekly intervals. The data are given in table 6 in milligrams per one hundred grams of soil and also in per cent based on nitrate production in the untreated soil. The former data were

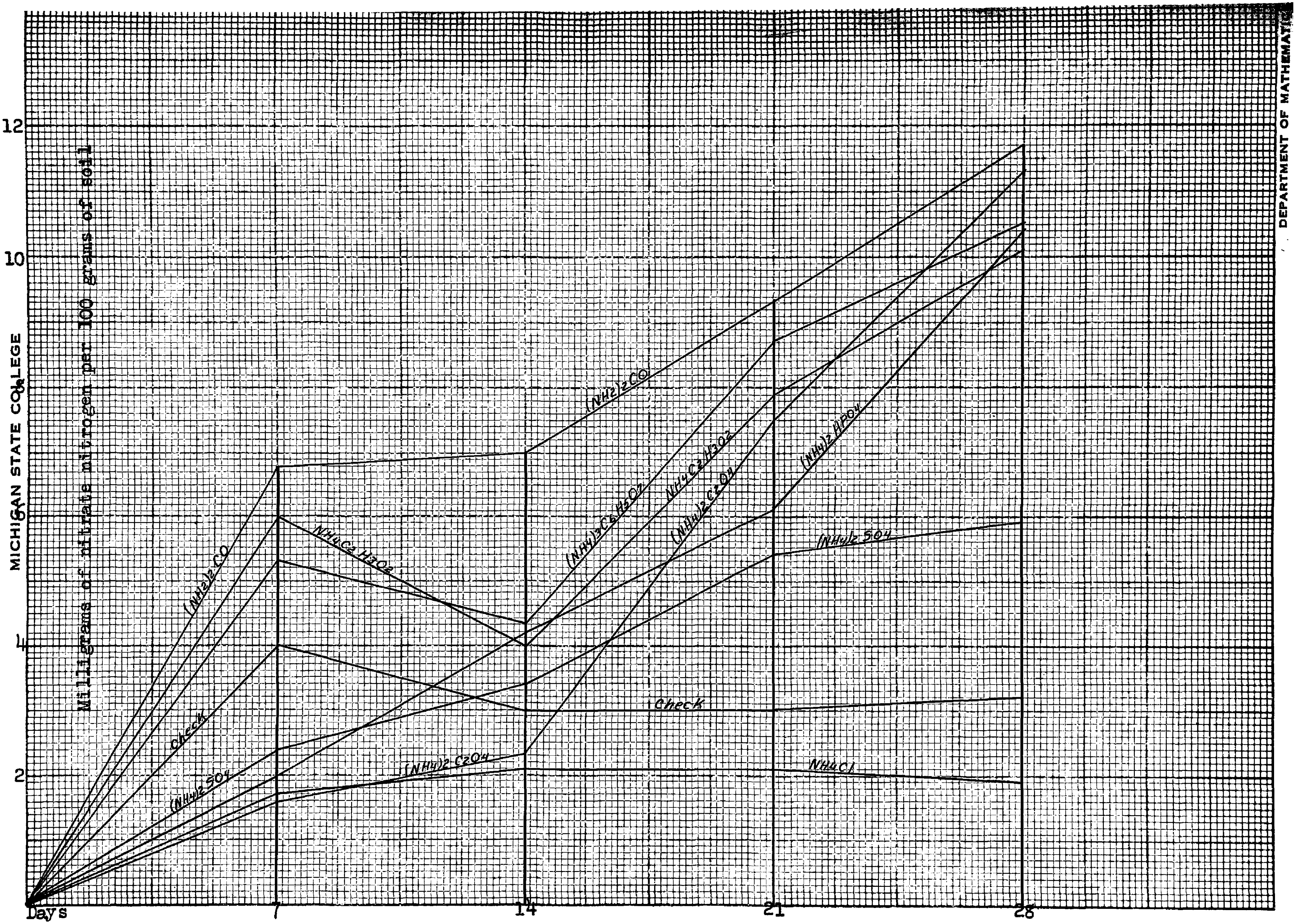

FIG. 3

Comparative Rate of Nitrification of Soil Nitrogen, Urea, and Several Ammonium Salts in One of the Soils Studied used for constnuction of figure III.

Some decided differences are shown in the rate at which this soil nitrified these materials. More nitrates were produced from all the salts except ammonium chloride, than from the soil's own supply of nitrogen. Ammonium sulphate was also a poor source of nitrifiable nitrogen. This decrease in nitrification with the addition of ammonium chloride and the relatively slow nitrification of ammonium sulphate is in agreement with the results of Kulik (15) and Turchin (28) on similar soils. Urea was the most rapidly nitrified of the materials used, followed closely by the organic salts of ammonium. Diammonium phosphate was not nitrified rapidly during the first week, but at the end of twenty days it was well nitrified.

From these results it is evident that ammonium chloride and sulphate would not be as suitable for nitrification studies on these soils as some of the other salts. To study nitrification in these soils, urea, ammonium oxalate, citrate, acetate or phosphate could be used as sufficient quantities of nitrates were produced from them to permit of comparisons of the effects of fertilizer treatments. For the next study, urea and diammonium phosphate were selected, because the former consistently produced the greatest amounts of nitrates and because the latter produced sufficient quantities of nitrates for the purposes of the study and in addition the phosphate factor was eliminated for all plot treatments. To these were added ground alfalfa and cottonseed meal in order to have a greater variety of materials since it is maintained (31) that a better picture of nitrification in soils can be obtained by using several materials and more than one method of procedure. The materials were added to one hundred grams of air dried soil in quan-

Table 5. Nitrate Production from Different Materials in Relation to Field Treatment of Soils

Soil		Mgm. Nitrate Nitrogen per 100 grams of Soil for 28 Days			
No.	Treatment	Alfalfa	Cottonseed Meal	$(NH_4)_2HPO_4$	$(NH_2)CO$
301N	No Treatment	3.27	5.35	2.55	8.40
301S	Lime	4.43	8.45	4.43	10.35
302	LNPK	5.40	10.40	6.60	12.98
305	LPK	6.00	12.50	7.28	14.43
307	LN	5.04	10.20	6.88	11.25
308	LK	4.92	11.40	6.53	11.75
309	LP	4.80	11.30	6.90	11.59
310	L RP. 0	3.65	9.10	5.35	9.28

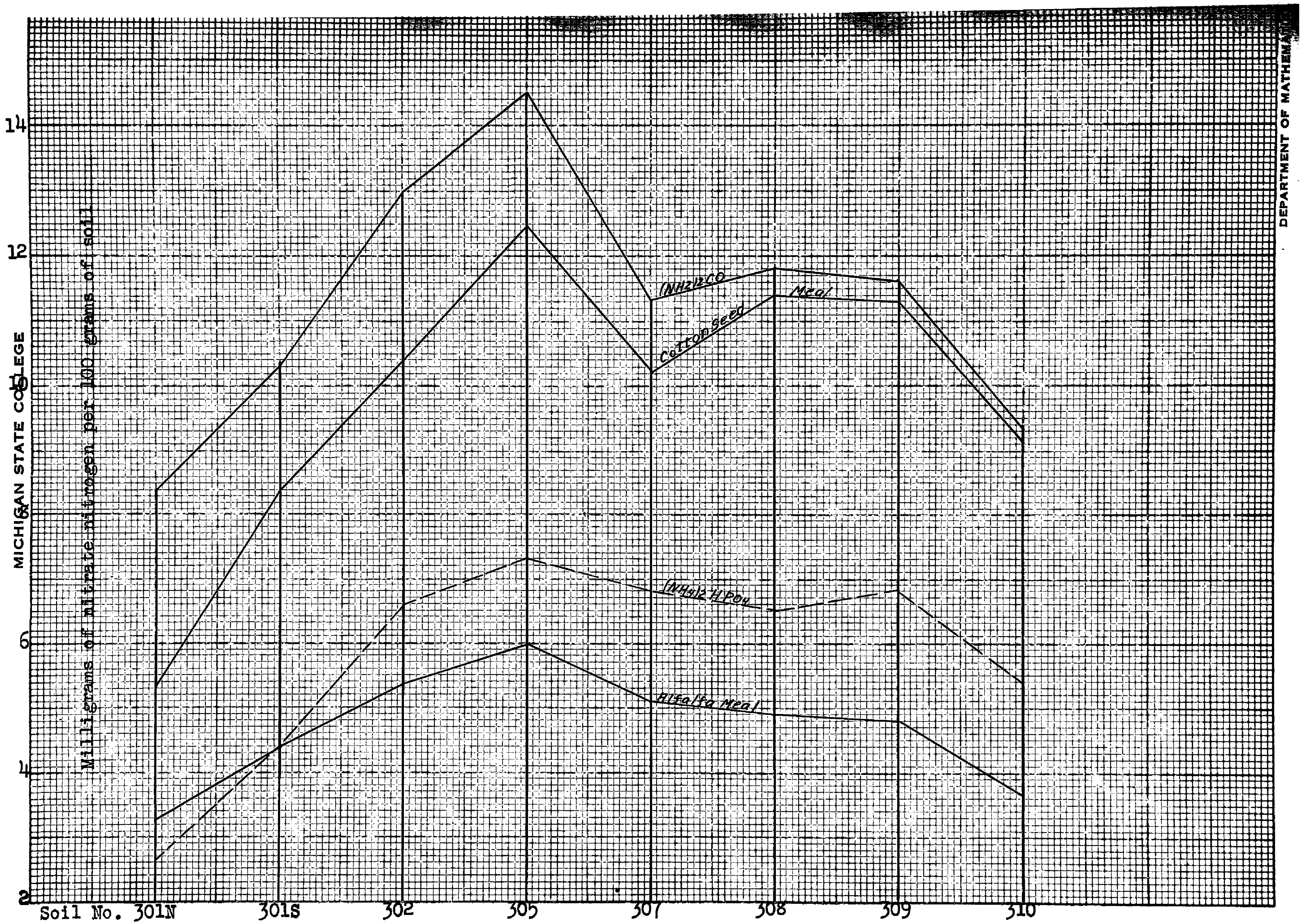

FIG. 4

Nitrate Production from Different Materials in Relation to Field Treatment of Soils tities to give fifty milligrams of nitrogen for urea and diammonium phosphate and one-half of one per cent of the organic plant materials. The usual soil tumbler method was followed throughout the study of nitrification.

Nitrate production in milligrams from the four applied materials is given in table 5 and in figure IV. It is evident from the data that field treatment has changed the ability of these soils to nitrify certain nitrogenous materials. The combination of lime, phosphorus, and potassium in soil 305 has resulted in the greatest nitrification power, followed closely by lime, phosphorus, nitrogen, and potassium in soil 302. Lime plus superphosphate, lime plus potassium, and lime plus nitrogen produced in the order given greater quantities of nitrates, than lime plus rock phosphate or lime alone. Of importance is the relatively low nitrifying capacity of the soil receiving no field treatment, which indicates a raising of the nitrifying capacity, for the materials studied of the other soils through lime and fertilizer treatment.

A close correlation is observed for nitrification of urea and cottonseed meal and for alfalfa meal and diammonium phosphate. The first two materials were nitrified to a greater extent during this period than the latter two. Although there is a difference in rate at which any soil nitrifies these materials, the relationship between soil treatment and nitrification of all of them is similar enough to permit of taking an average of the results for the four materials on a percentage basis for a comparison with other soil factors.

Nitrification of different materials as related to available phosphorus, organic matter, and total nitrogen is shown in table 6 and

Table 6. Nitrification of Nitrogenous Materials in Relation to Organic Matter, Total Nitrogen, and Available Phosphorus Content of Soil Expressed on a Percentage Basis

Soil		Percentage Nitrification							
No.	Field Treatment	Alfalfa	Cottonseed meal	Diammonium Phosphate	Urea	Average for all Treatments	Available Phosphorus	Organic Matter	Total Nitrogen
301N	None	100	100	100	100	100	100	100	100
301S	Lime	135	158	173	123	147	112	104	94
302	LNPK	165	195	259	155	194	144	120	103
305	LPK	183	234	285	172	218	148	121	103
307	LN	155	191	270	134	187	126	106	87
308	LK	150	213	256	140	189	131	107	89
309	LP	147	211	270	138	191	139	101	87
310	L RP. C	112	170	238	111	180	174	100	81

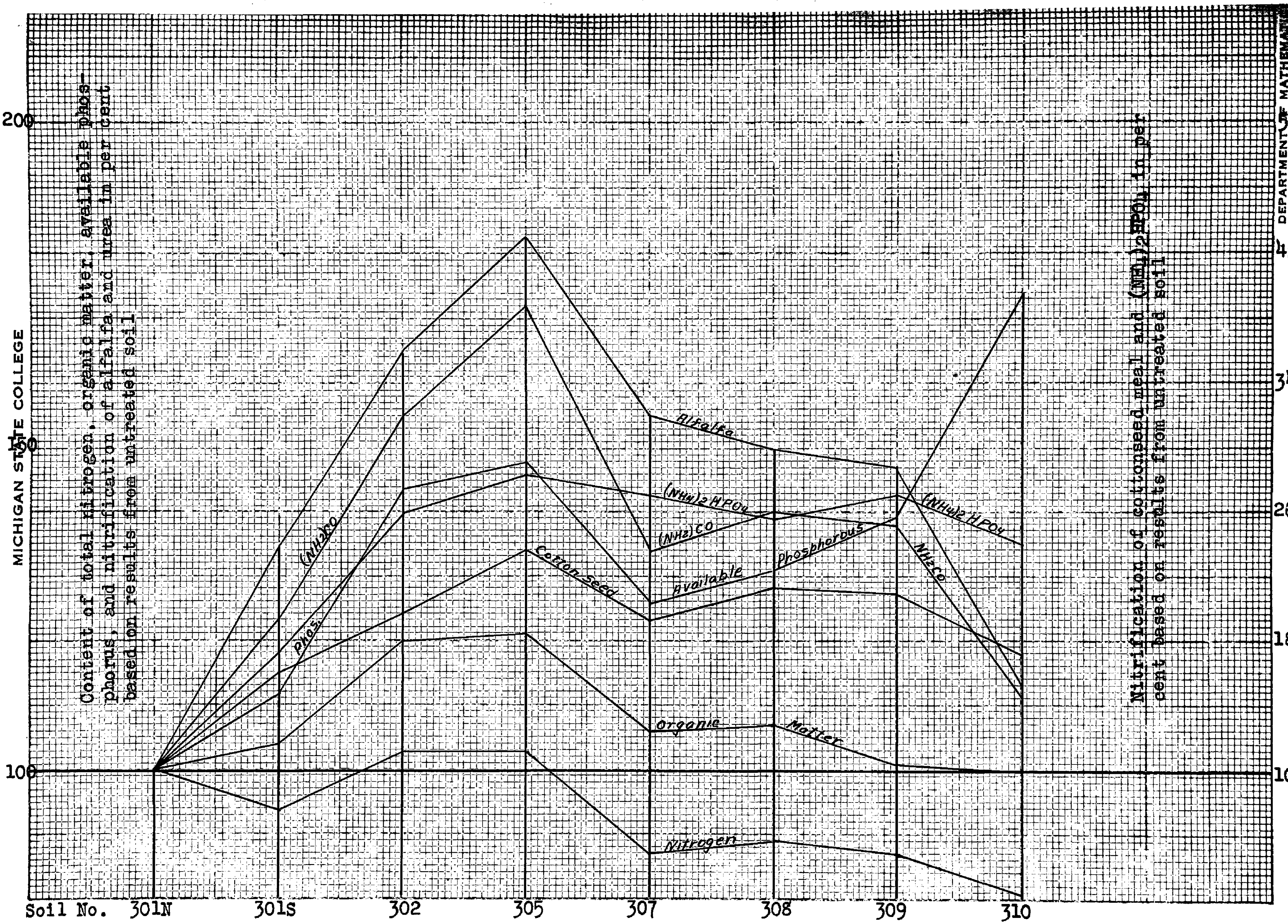

FIG. 5

Relation of Nitrification of Several Substances in Soils Previously Receiving Different Field Treatments to the Soil Content of Total Nitrogen, Organic Matter, and Available Phosphorus figure V on a percentage basis. With the exception of soil 310N which is high in available phosphorus and relatively low in nitrifying capacity for urea and cottonseed meal, there is a close correlation between available phosphorus and nitrate production from these materials for all soils studied. Nitrification of alfalfa meal correlates nicely with available phosphorus, except for soils 309 and 310, which are high in this element and relatively low in nitrate production from this material. Nitrate production from diammonium phosphate shows little correlation with available phosphorus.

Organic matter content and nitrate production from cottonseed meal and urea show a clear correlation, while nitrate production from alfalfa and diammonium phosphate shows less relationship.

Total nitrogen shows a similar relation to nitrate production from the different materials as organic matter content.

CARBON DIOXIDE PRODUCTION FROM CELLULOSE IN RELATION TO SOIL TREATMENT

Biological processes in soils resulting in the liberation of carbon dioxide from organic materials have been studied extensively for many years. It has been claimed by some investigators that a measure of this process furnishes a better index of biological activity in soils than any other method yet devised. In this study we are concerned chiefly with the effect of field treatment on chemical and biological relationships in several soils. A measure of carbon dioxide production from
soil
the decomposable material in these soils may show changes related to soil treatment, if changes of measurable magnitude have taken place.

Table 7. Carbon Dioxide Production from Cellulose with Different Chemicals added in Relation to Soil Treatment, Expressed in Milligrams and Per Cent

Soil		Carbon Dioxide Production in 24 Days										
		Cellulose		$CaCl_2$ + Cellulose		KCl + Cellulose		NA_2HPO_4 + Cellulose		$NANO_3$ + Cellulose		Average For All Treatments
No.	Field Treatment	Mgms.	Per Cent	Mgms.	Per Cent	Mgms.	Per Cent	Mgms.	Per Cent	Mgms.	Per Cent	Per Cent
301N	None	149	100	126	100	130	100	294	100	493	100	100
301S	Lime	169	113	151	120	166	127	231	78	548	111	110
302	LNPK	204	137	213	169	216	166	268	91	619	126	138
305	LPK	223	150	231	183	248	191	286	98	643	130	150
307	LN	171	115	216	171	238	183	263	89	614	124	136
308	LK	171	115	207	164	224	172	250	85	629	127	132
309	LP	158	106	190	151	197	152	243	83	560	114	121
310	L RP. C	122	82	181	144	174	134	214	73	585	118	111

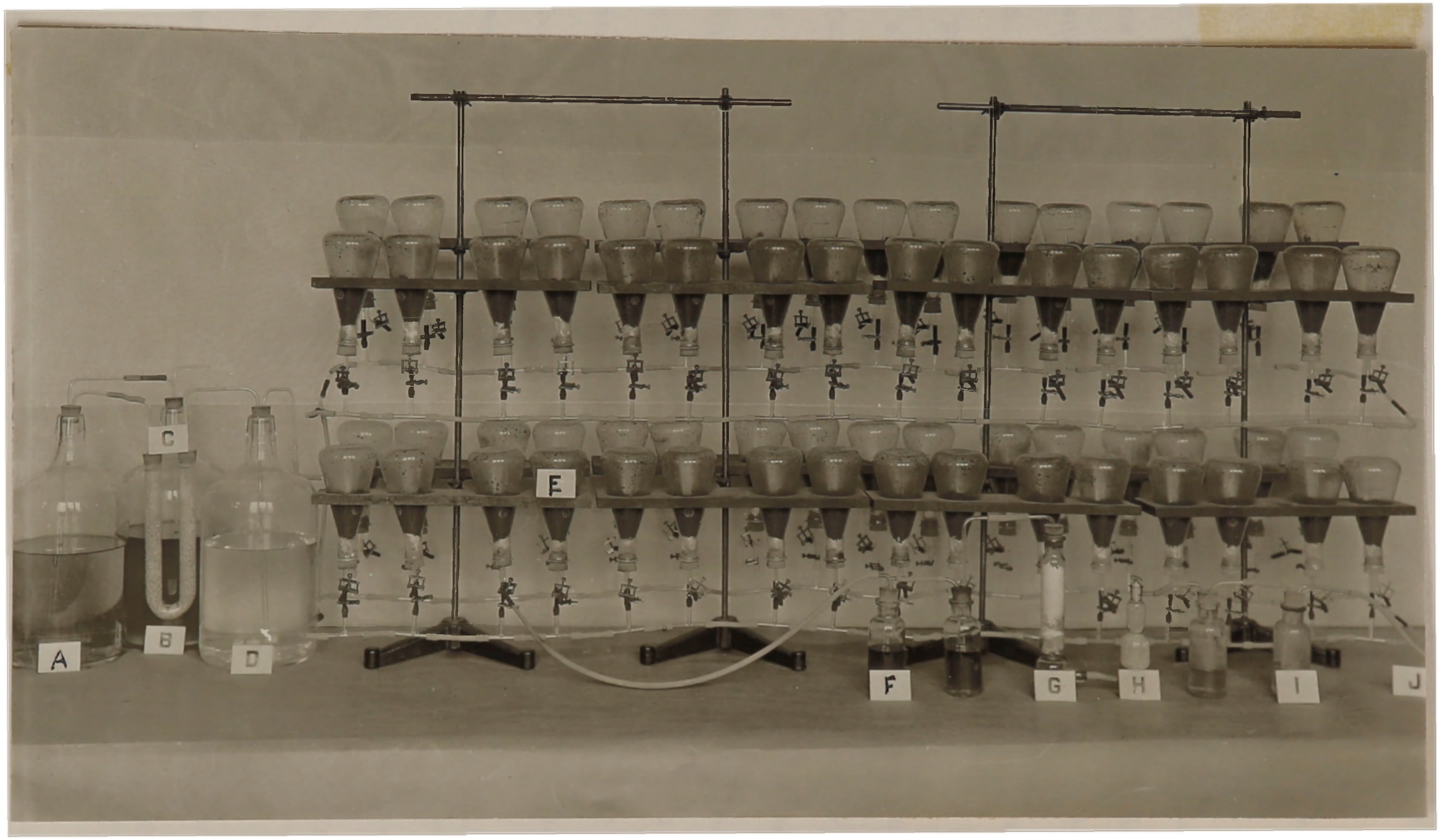

Fig. VI. Apparatus used in carbon dioxide evolution studies.

The usual method of studying carbon dioxide production in soils is to add an organic material and collect the carbon dioxide evolved by absorption in an alkaline solution. Different organic materials have been used as a source of energy for microorganisms in carbon dioxide production studies with varying degrees of success. In this study cellulose was used as the source of energy, because it contains carbon, oxygen, and hydrogen, without any mineral elements, and because other investigations have proved its value in studies of this kind. It has further been shown that certain elements, namely nitrogen and phosphorus, influence the rate of CO_2 production from cellulose and since the soils under study are all low in these elements but show differences due to fertilizer treatment, it was deemed advisable to make some determinations in which phosphorus or nitrogen was added with cellulose to the soils. For additional comparisons, potassium and calcium in the form of chlorides were added with cellulose to some cultures. Instead of using an alkaline solution for absorbing the carbon dioxide, as is usually done, a solid commercial product known as "ascarite" was used.

Suitable apparatus was assembled as shown in figure VI, to make the necessary determinations of carbon dioxide production. This method is similar to the one found entirely satisfactory for this kind of study by Turk (27). In figure VI, letters A, B, and C represent concentrated sodium hydroxide, sulphuric acid, and soda lime, respectively, for purification of the incoming air. D is distilled water for moistening the air to prevent excessive drying of the soils. E is a 300 cc. Erlenmeyer flask containing the treated soil; F is concentrated sulphuric acid for the removal of water vapor; G contains phosphorus pentoxide

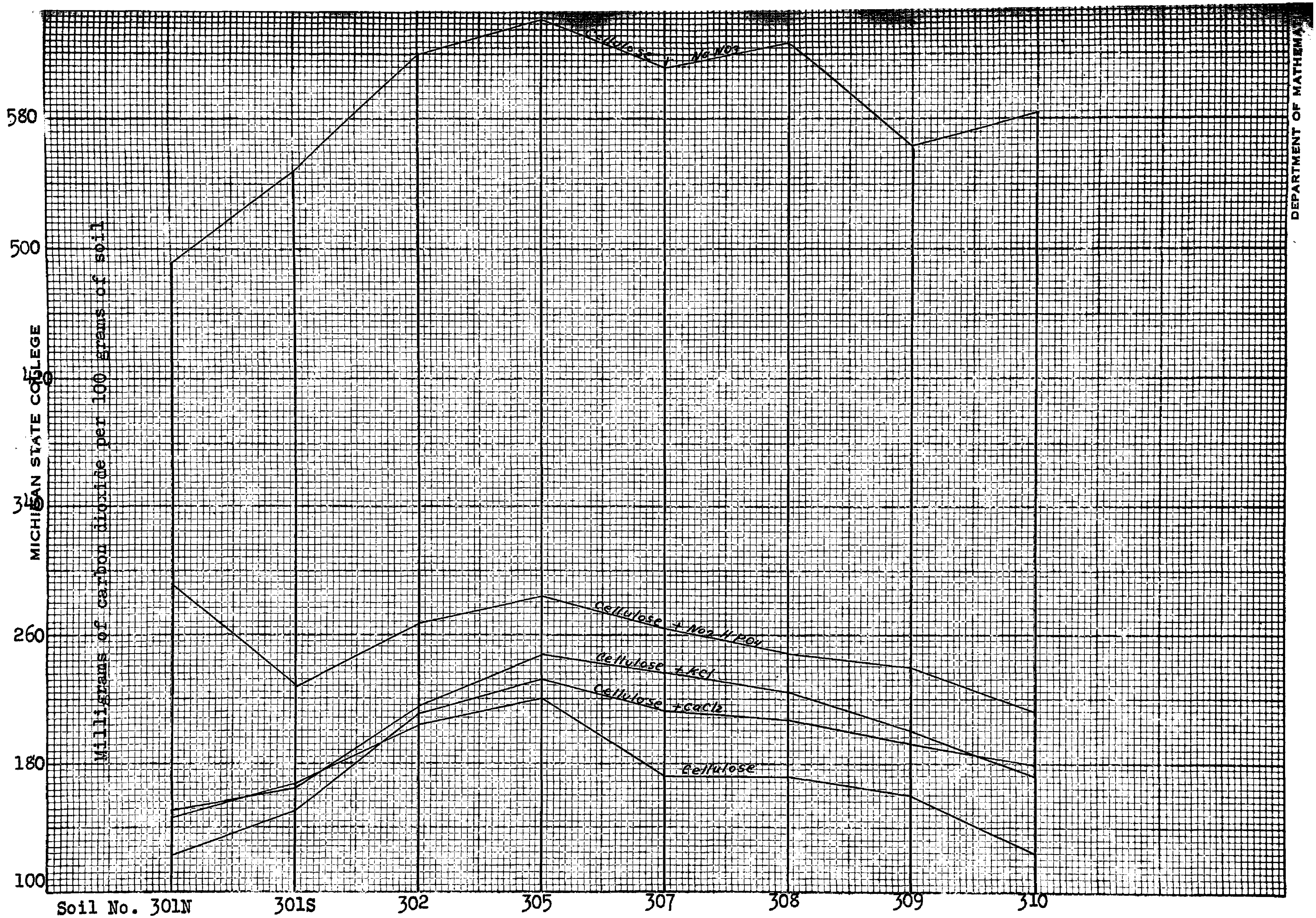

FIG. 7

Influence of Various Chemicals on the Production of Carbon Dioxide from Cellulose in Soils Previously Receiving Different Field Treatments for further dessication of the air; H contains the "ascarite" for absorbing the CO_2 produced; and I contains concentrated sodium hydroxide to prevent back flow of carbon dioxide from the suction pump J. Sufficient air was drawn through the system by suction from the water pump to draw all the carbon dioxide produced in the soil into the "ascarite" tube, where it was absorbed for weighing.

Results of carbon dioxide production determinations in milligrams per hundred grams of soil and in per cent based on results from the untreated soil are given in table 7. A comparison between the amounts of carbon dioxide produced from cellulose in these soils with additions of chemicals is shown graphically in figure VII in milligrams of CO_2 per 100 grams of soil, produced in 24 days.

The addition of nitrate nitrogen gave a much greater production of carbon dioxide from cellulose in all soils than any of the other treatments. These results substantiate conclusions cited in the literature that nitrogen plays a very important part in cellulose decomposition as measured by CO_2 production. They bear out the statement made in an earlier part of this paper that these soils being extremely low in total nitrogen would be expected to respond to applications of this element.

Additions of available phosphorus increased the production of carbon dioxide from cellulose much more than did additions of calcium or potassium chloride. This stimulation of carbon dioxide production from cellulose by phosphorus additions follows from the fact that these soils are relatively low in this element and that available phosphorus supply has been shown to influence the rate of cellulose decomposition as measured by CO_2 production.

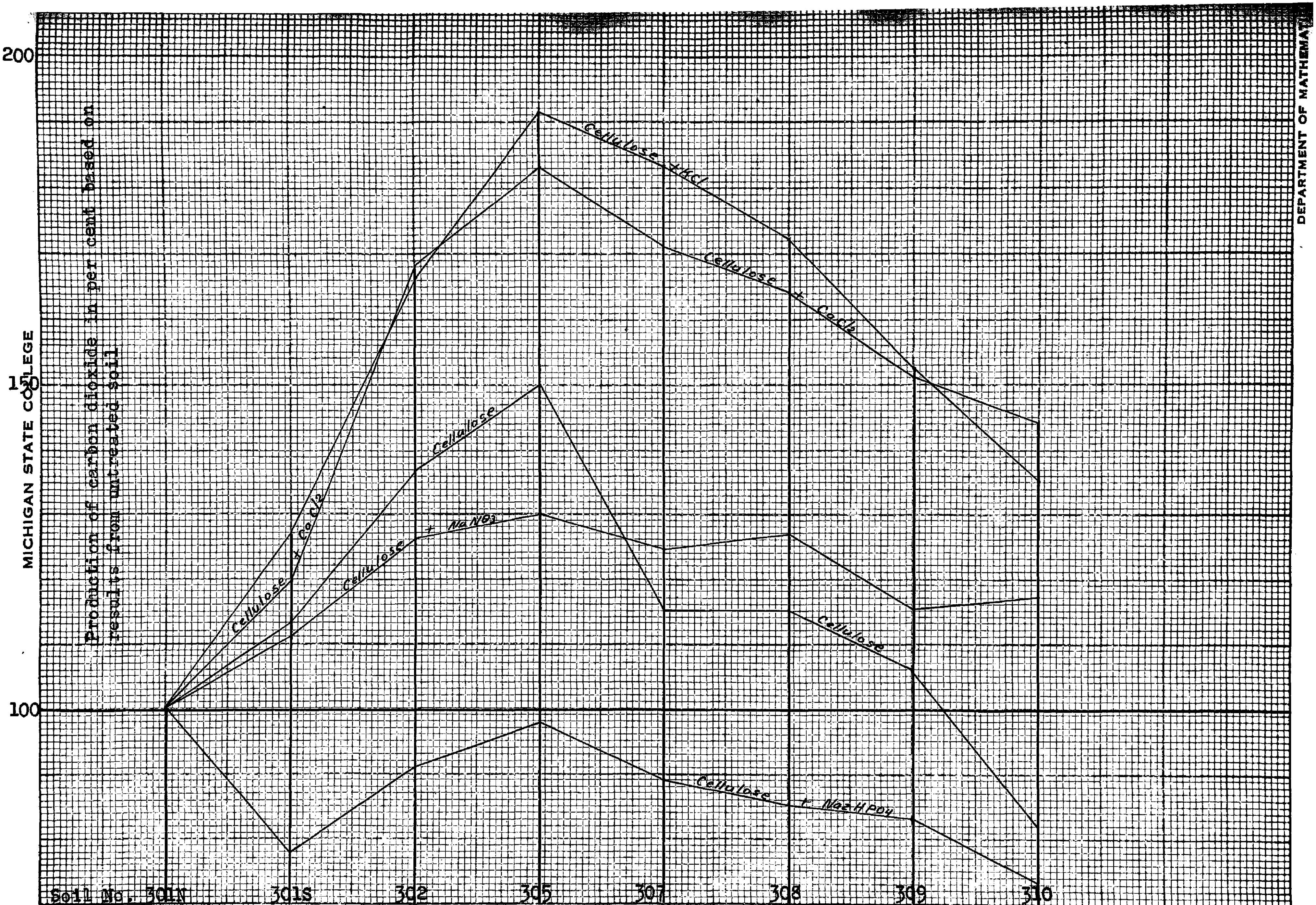

FIG. 8

Effect of Previous Soil Treatment of Production of Carbon Dioxide from Cellulose Alone and from Cellulose Plus Various Chemicals

Additions of calcium and potassium chlorides increased to a limited extend the production of carbon dioxide from cellulose over that from the soils receiving cellulose alone. Potassium chloride seemed to be more effective in this respect than calcium chloride. This would indicate a more important part played by potassium in this process than calcium as the quantities of chlorides added in both cases were equal.

When the results of carbon dioxide production from cellulose with certain chemicals added is calculated in per cent based on the untreated soil as shown in table 7 and figure VIII, the effect of soil treatment on this process may be demonstrated. The results show a marked increase in the soil's ability to produce CO_2 from cellulose for all fertilizer treatments as compared to the untreated soil. Lime alone increased the production of CO_2 from cellulose over the untreated soil, but not to the extent that combinations of lime, nitrogen, phosphorus, and potassium did.

The production of CO_2 from cellulose was greatest in soil 305, which had received lime, potassium, and phosphorus in the field. Soil 302 followed 305 in CO_2 production from cellulose alone, while combinations of lime plus nitrogen, lime plus potassium, and lime plus phosphorus were little if any better than lime alone in increasing the production of CO_2 from cellulose in these soils.

Carbon dioxide production from cellulose with additions of calcium chloride and of potassium chlorides was greatest in soils 305, receiving lime, potassium, and phosphorus, followed by soils 307, receiving lime and nitrogen in the field treatments. All of the field treatments increased CO_2 production from cellulose with additions of

Table 8. Carbon Dioxide Production in Soils in Relation to Available Phosphorus and Total Nitrogen Based on the Untreated Soil in Per Cent

Soil		Carbon Dioxide Production							
No.	Field Treatment	Cellulose	$CaCl_2$ + Cellulose	KCl + Cellulose	Na_2HPO_4 + Cellulose	$NaNO_3$ + Cellulose	Average For All Treatments	Available Phosphorus	Total Nitrogen
301N	None	100	100	100	100	100	100	100	100
301S	Lime	113	120	127	78	111	110	112	94
302	LNPK	137	169	166	91	126	138	144	103
305	LPK	150	183	191	98	130	150	148	103
307	LN	115	171	183	89	124	136	126	87
308	LK	115	164	172	85	127	132	131	89
309	LP	106	151	152	83	114	121	139	87
310	L RP. C	82	144	134	73	118	111	174	81

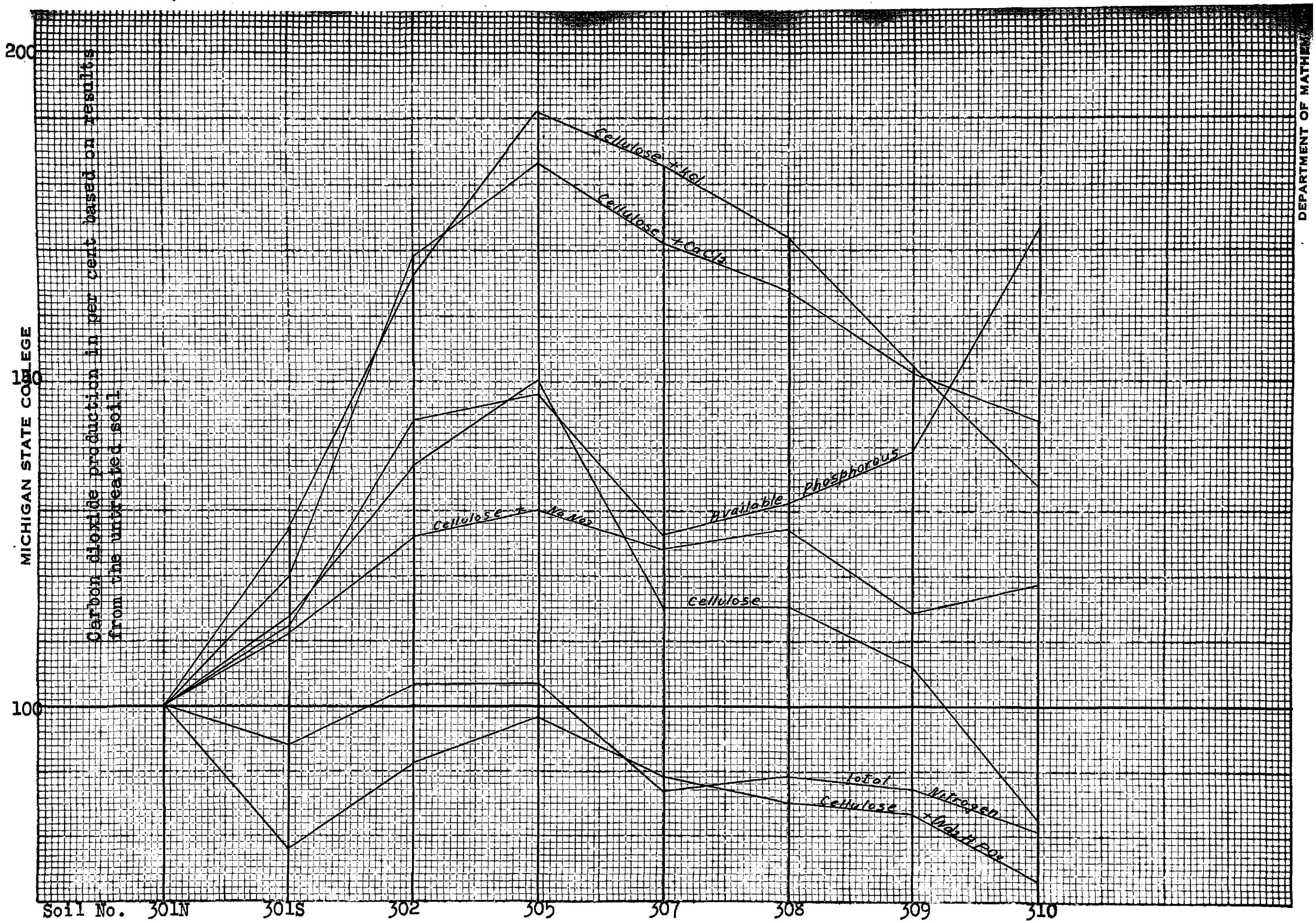

FIG. 9

Relation between Total Nitrogen and Available Phosphorus Content of Soils Previously Receiving Various Field Treatments and Carbon Dioxide Production from Cellulose Alone and from Cellulose Plus Chemicals potassium chloride or calcium chlorides over the untreated soil, but the increases were greatest for combinations of all the fertilizing elements.

Where additions of phosphorus were made with cellulose, CO_2 production was greater for the untreated soil than for soil receiving field treatments. Lime in combination with phosphorus, nitrogen, and potassium give larger amounts of CO_2 than lime in combination with only single elements.

From these results it is evident that fertilizer treatments have changed the ability of these soils to produce CO_2 from cellulose, with and without additions of chemicals.

It has already been shown in table 7 and figure VIII that nitrogen and phosphorus play a very important part in cellulose decomposition in these soils, so that a comparison of the soils supply of these elements with this process may show further relationships. Cellulose decomposition with additions of fertilizing elements in relation to the suppły of nitrogen and phosphorus in the soil are given in table 8 and figure IX. It may be said that where total nitrogen is low and phosphate has been added, the amount of CO_2 produced is determined by the relative amounts of nitrogen present because phosphorus has been eliminated as a factor in the process. Likewise additions of nitrogen, to eliminate this element as a factor in CO_2 production should leave phosphate as the limiting factor and a correlation between available phosphorus and CO_2 evolution from cellulose would be found. This condition exists for all the soils except 309 and 310N, which are unusually high in available phosphorus and are not so active in CO_2

production. This whole relation points to the greater importance of nitrogen than available phosphorus in cellulose decomposition. A better correlation exists between the total nitrogen content of these soils and carbon dioxide production from cellulose with phosphorus added, than carbon dioxide production from cellulose with nitrogen added.

The relation between carbon dioxide production from cellulose alone, total nitrogen, and available phosphorus in the soil further emphasize the importance of these elements, especially nitrogen, in this process. CO_2 production from cellulose with potassium and calcium chloride added show a close relation to total soil nitrogen for all soils.

INTERRELATIONS BETWEEN NITRIFICATION, CARBON DIOXIDE PRODUCTION, BACTERIAL NUMBERS, CROP YIELDS, AND AVAILABLE NUTRIENTS

The previous studies showed that fertilizer treatments of several field soils produced some chemical and biological differences in these soils. The specific changes and relationships have been discussed, and it is of interest at this time to make a comparison of all the relationships, in order to secure a better picture of the conditions as a whole in these soils. In table 9 and figure X all of the results are presented, and expressed in per cent using the data from soil 301N, which received no fertilizer treatment, as a basis.

Soil 305, treated in the field with lime, phosphorus, and potassium, showed the greatest nitrifying power, carbon dioxide production, number of bacteria, and quantity of organic matter and ranked second in the production of crops. Soil 302, treated in the field with lime, nitrogen, phosphorus, and potassium, produced the largest yields of crops, but ranked second to soil 302 in organic matter, nitrifying capacity,

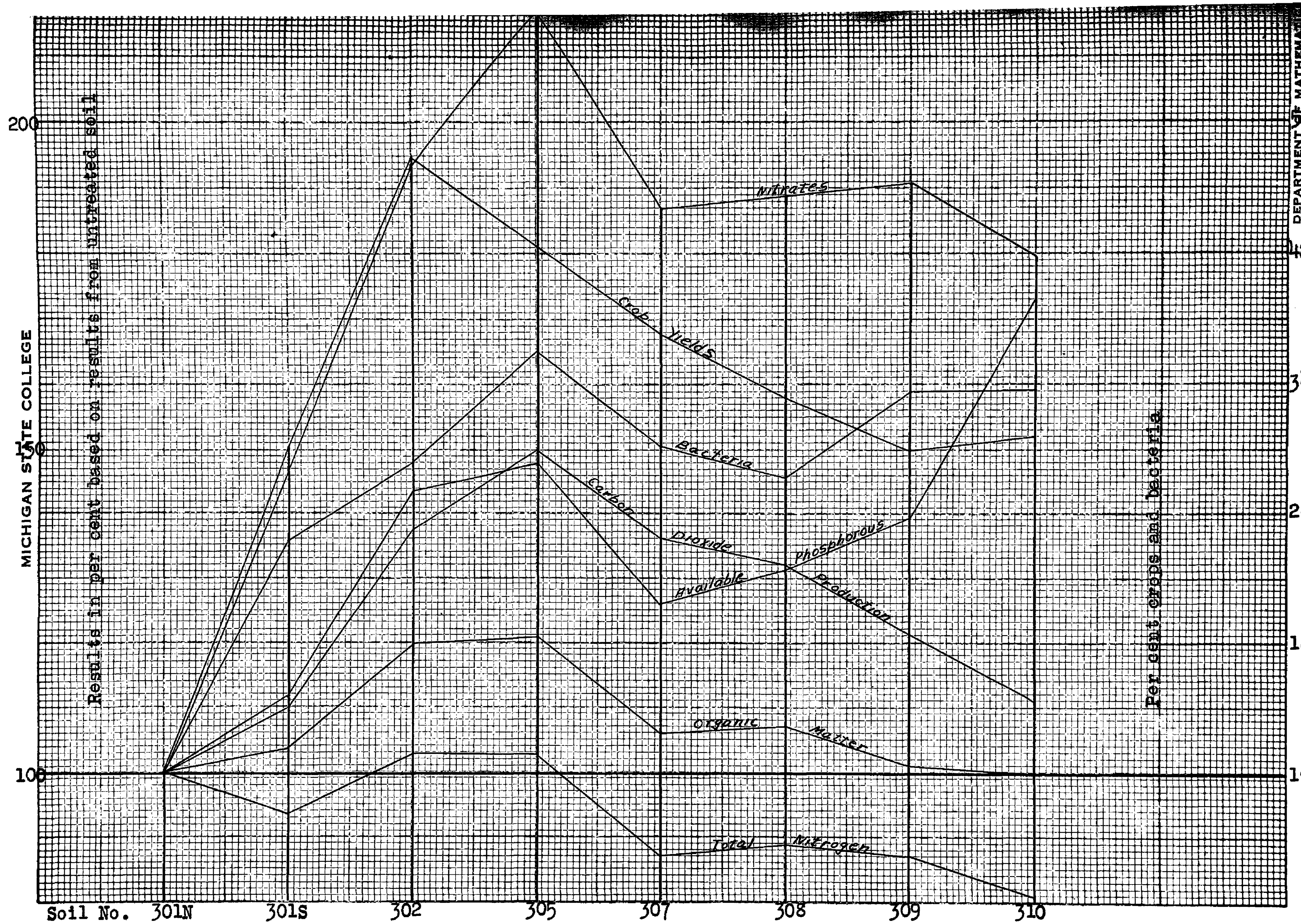

FIG. 10

Interrelations Between Nitrate Production; Carbon Dioxide Production; Content of Available Phosphorus, Organic Matter, Total Nitrogen; Bacterial Numbers; Crop Yields; in Soils Receiving Different Field Treatments carbon dioxide production, and equalled it in total nitrogen content. The soil treatments in their order of efficiency for maintaining organic content of the soil were: LPK, LNPK, LK, LN, L, LP, with LRPC and no treatment on a par.

Available phosphorus was proportionately higher in those soils receiving this element in the field than those receiving lime or combinations of lime and other fertilizing elements. This condition was especially pronounced in soil 310, which received large amounts of rock phosphate. From these results it is evident that the supply of available phosphorus can be increased in these soils by additions of certain forms of this element, especially is this true where large amounts are applied and relatively small amounts are removed by the crops.

With the exception of available phosphorus and bacterial numbers, field applications of lime plus superphosphate, and lime plus rock phosphate and gypsum, showed the smallest effect on all other factors of any treatment except lime alone.

The results as a whole indicate a greater effect on all factors studied, from the application of combinations of fertilizer elements with lime than from applications of the elements singly with lime. Furthermore, no single element in combination with lime is more effective in all respects than some other element with lime. These data clearly indicate that the soils studied are in need of all three fertilizing elements. The need for potassium and phosphorus is evidently greater than for nitrogen since the effect of the LPK treatment is equal to or greater than the effect of the LNPK in all respects except crop yield.

Table 9. Effect of Field Treatment of Soils on Crop Yields and on the Various Chemical and Biological Conditions Studied

Soil		Data in Terms of Per Cent Based on Results from Untreated Soil						
No.	Field Treatment	Crop Yields	Organic Matter	Available Phosphorus	Colonies of Bacteria	Nitrate Nitrogen	Carbon Dioxide	Total Nitrogen
301N	None	100	100	100	100	100	100	100
301S	Lime	298	104	112	245	147	110	94
302	LNPK	478	120	144	293	194	138	103
305	LPK	423	121	148	358	218	150	103
307	LN	370	106	126	305	187	136	87
308	LK	331	107	131	285	189	132	89
309	LP	290	101	139	336	191	121	87
310	L RP. C	309	100	174	338	180	111	81

GREENHOUSE STUDIES

To determine further the effect of soil treatment on chemical composition and biological processes in the soil, crops of sweet clover, rye, and soy beans were grown on the soils in pot cultures in the greenhouse. The crops were cut after a 12-weeks growth period, air dried, and weights determined. The harvested material was then ground and returned to a portion of the soil on which it grew for carbon dioxide production study. It was the object of this study to determine if soil differences found in the previous work on these soils could be measured by crop yields in the greenhouse and if carbon dioxide production when the crops grown were returned to the soils was comparable to results from previous carbon dioxide production studies.

Duplicate two-gallon pots of the different soils were used. The moisture content was maintained by weighing the pots at frequent intervals and bringing up to weight with distilled water. The crop yields in grams and in per cent based on the yield from the untreated soil are given in table 10. The data in per cent are also presented graphically in figure XI

The results show that soil 302, treated in the field with lime, nitrogen, phosphorus, and potassium, produced the largest yields of all three crops and that soil 305, treated in the field with lime, potassium, and phosphorus ranked second in yield of plant materials. These results are in accord with those obtained under field conditions. Field treatments of lime plus nitrogen, and of lime plus potassium, gave approximately the same yields of legume plant material as lime alone, but the yields were not so large as for soils 302, 305, and those receiving lime with phosphorus. The soil receiving lime alone treatment gave larger yields of all

Table 10. Crop Yields from Greenhouse Pot Studies Expressed as Grown of Air-Dry Plant Tissue and in Per Cent Based on Results from Untreated Soil

Soil		Sweet Clover		Rye		Soy Beans		Average
No.	Field Treatment	Grams	Per Cent	Grams	Per Cent	Grams	Per Cent	Per Cent
301N	None	1.00	100	15.15	100	6.14	100	100
301S	Lime	4.36	436	16.45	108	9.55	155	233
302	LNPK	6.61	661	22.35	148	10.74	175	328
305	LPK	4.99	499	20.10	133	10.45	170	267
307	LN	4.44	444	19.55	129	9.95	162	245
308	LK	4.37	437	17.80	118	9.70	158	238
309	LP	4.79	479	18.95	125	10.32	168	257
310	L RP. C	4.41	441	16.20	107	10.22	166	236

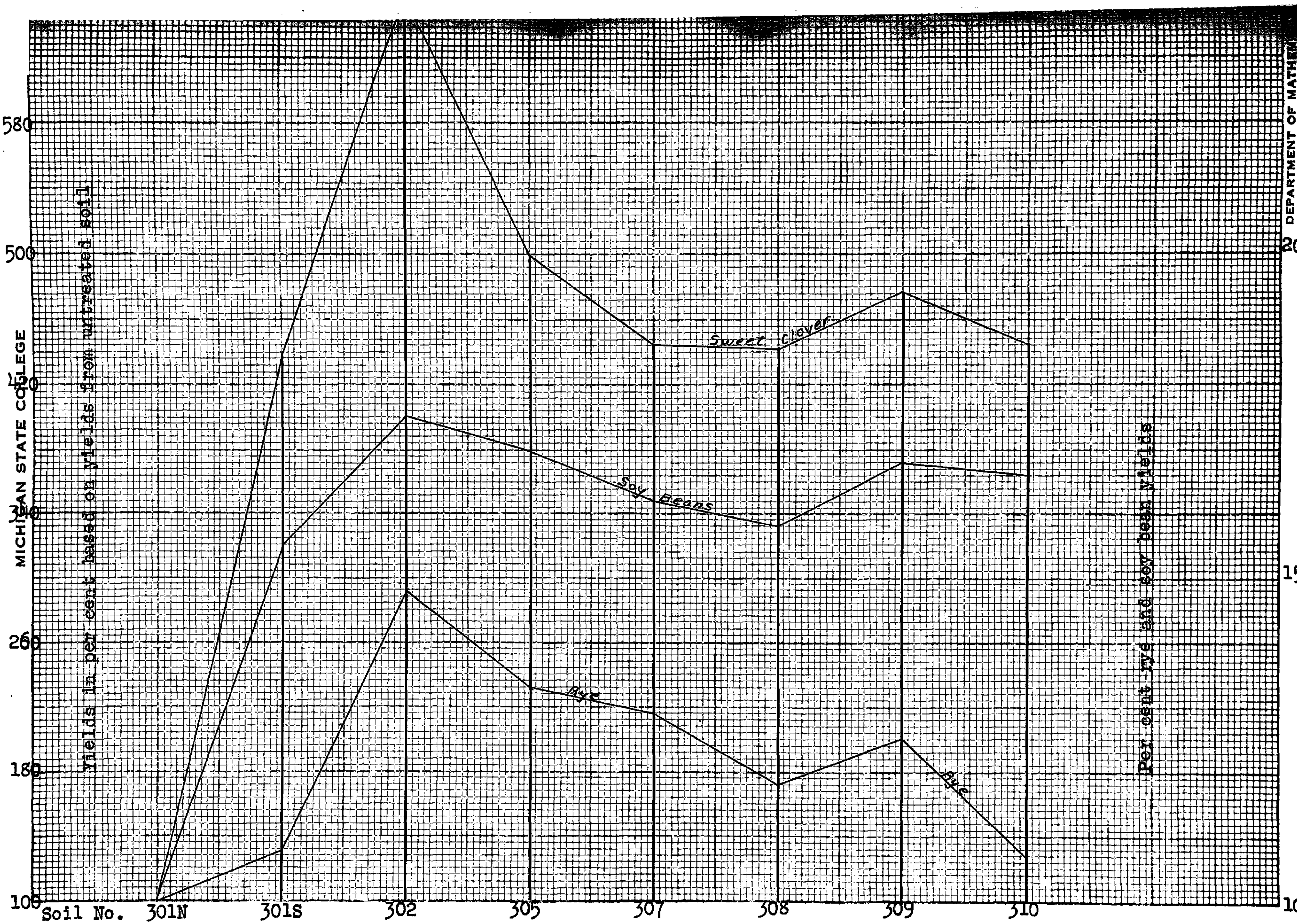

FIG. 11

Effect of Previous Field Treatment of Soils on the Yield of Crops in the Green House crops in the greenhouse than the untreated soil, but not so large as soils receiving any combination of fertilizer with lime.

The unusually low yields of all crops on the untreated soil 301N is of special interest as they indicate the low fertility in the soil where no fertilizer treatments have been made. Apparently field cropping of this soil has impoverished it to such an extent that available supplies of nutrients are inadequate to meet the needs of crops grown in the greenhouse. This decrease in ability to produce crops is especially marked for the legumes, where considerable difficulty was experienced in securing measurable yields of plant material. Undoubtedly this condition was due primarily to a deficiency of lime.

A definite relation was found to exist between fertilizer treatment of the soils and residual effects on crop production in greenhouse pot tests. In general the variations in yields with different soil treatments was similar for all three crops. This relationship was especially noted for the sweet clover and soy bean crops.

CARBON DIOXIDE PRODUCTION IN SOIL AFTER ADDITION OF ONE PER CENT PLANT MATERIAL PREVIOUSLY GROWN ON THE SOIL

Samples of soil from the pots used in the greenhouse tests were placed in the aspirating flasks used in the previous experiments for determining carbon dioxide production. The procedure described for the previous study of carbon dioxide production was followed. The results obtained both in milligrams and in per cent based on results from the untreated soil are shown in table 11. Figure XII presents the percentage data graphically.

Table 11. Carbon Dioxide Produced from Soils to which Had Been Added Plant Tissue Previously Grown on Them

Soil		Sweet Clover		Rye		Soy Beans		Average
No.	Field Treatment	Mgms.	Per Cent	Mgms.	Per Cent	Mgms.	Per Cent	Per Cent
301N	None	287	100	225	100	252	100	100
301S	Lime	296	103	200	89	262	99	99
302	LNPK	343	120	238	106	283	113	113
305	LPK	323	113	224	100	285	109	109
307	LN	320	111	216	96	282	106	106
308	LK	325	113	200	89	280	104	104
309	LP	309	108	211	94	289	105	105
310	L RP. C	295	103	201	89	286	102	102

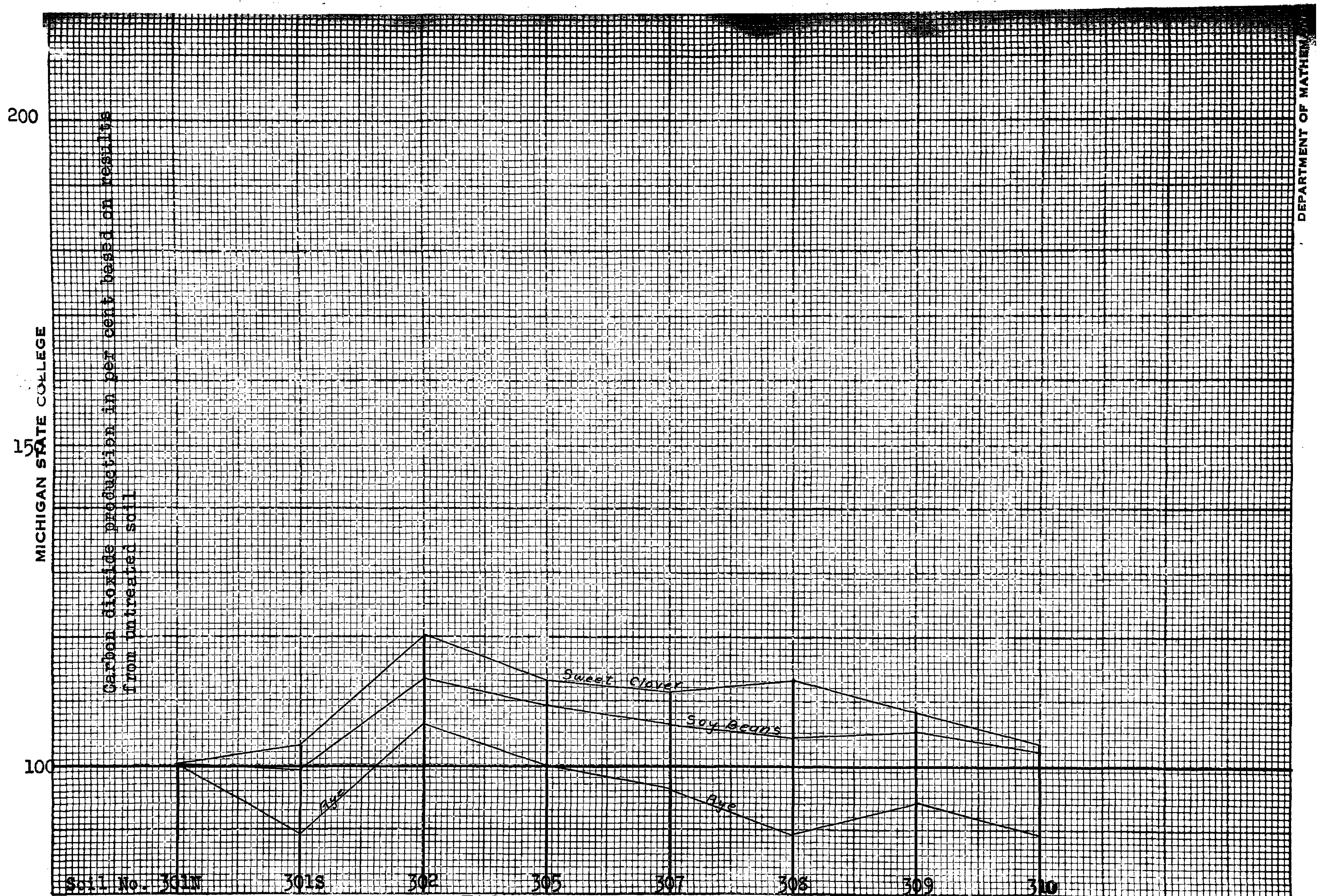

FIG.12

Carbon Dioxide Production in Soils, Previously Receiving Different Field Treatments, from Plant Material Grown on Them

The data show sweet clover to be more readily decomposed than soy beans or rye. These results are in line with the results of other workers, using the same type of material in different soil. The explanation of this relationship has been thoroughly discussed in papers dealing directly on the subject, and hence, will not be repeated.

All soils receiving lime alone or lime plus fertilizer in the field produced more carbon dioxide from sweet clover than did the untreated soil. Likewise all treated soils with the exception of that receiving lime alone produced more carbon dioxide from soy beans than did the untreated soil. On the other hand, only soils 302 and 305 produced as much carbon dioxide from rye as did the untreated soil.

The soil receiving complete fertilizer in addition to lime produced more carbon dioxide from all three materials than did any other soil. This soil also produced the largest crops under both field and greenhouse tests.

A previous study showed marked differences in the power of these soils to produce carbon dioxide from cellulose. Only slight differences, however, were found in the capacity of the soils to produce carbon dioxide from plant material grown on them. It is evident, therefore, that addition of plant tissue, which contains all the nutritive elements, largely eliminates the differences in these soils, arising from previous treatment to produce carbon dioxide.

A comparison of nitrification, carbon dioxide production, yields of crops, total nitrogen, and greenhouse studies is made in table 12 and figure XIII. Crop yields in the field and greenhouse, and carbon dioxide production from plant material show a close correlation for all soils except soil 309, which gave relatively higher yields in the greenhouse and

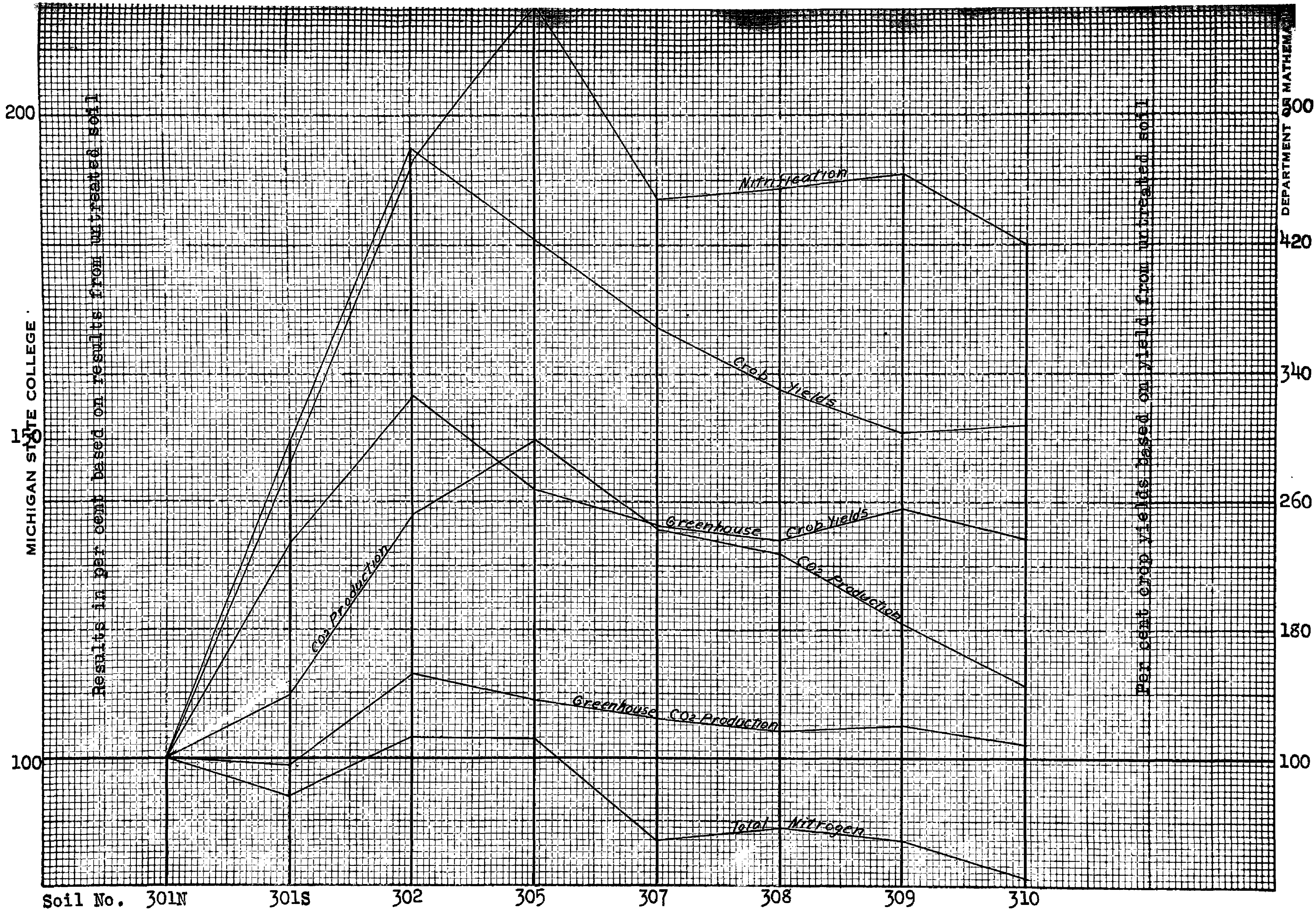

FIG. 13

Comparison of Total Nitrogen Content, Field Crop Yields, Green House Crop Yields, Nitrification, and Production of Carbon Dioxide from Both Cellulose and Plant Tissue Grown on the Soils, in Soils Receiving Different Field Treatments in carbon dioxide produced from crops grown on it.

Nitrification, carbon dioxide production, both from cellulose and plant tissue, and greenhouse crop yields show a very close correlation in all soils but number 302, which gave relatively higher values in the greenhouse studies.

In general it may be said that variations in crop producing power, resulting from previous soil treatment, correlate quite well with results of the studies of biological activities.

SUMMARY AND CONCLUSIONS

A study was made of changes in some of the more important chemical and biological conditions in a Fox sand loam soil, resulting from fertilizer and lime applications over a period of several years. Particular emphasis was placed on crop production in relation to chemical and biological conditions in the soil and to field treatment.

The results permit of the following conclusions:

1. Although the supply of available phosphorus is low in all the soils studied, significant differences in content of this element were found. In general those soils receiving phosphorus as a fertilizer in the field, contained the largest amount of this element. Applications of rock phosphate plus lime and superphosphate plus lime caused greater increase in the supply of this element in the soil than any other field treatment.

2. Organic matter and total nitrogen content were found to be parallel in all but soil 301N, which was relatively high in total nitrogen and low in organic matter. Combinations of lime, nitrogen,

Table 12. Correlations Between the Results Obtained in Greenhouse and Laboratory Studies

Soil		Data in Terms of Per Cent Based on Results from Untreated Soil					
No.	Field Treatment	Greenhouse Crops	Field Crops	CO_2 from Greenhouse Crops	CO_2 from Cellulose	Nitrifying Capacity	Total Nitrogen
301N	None	100	100	100	100	100	100
301S	Lime	233	298	99	110	147	94
302	LNPK	328	478	113	138	194	103
305	LPK	267	423	109	150	218	103
307	LN	245	370	106	136	187	87
308	LK	238	331	104	132	189	89
309	LP	257	290	105	121	191	87
310	L RP. C	236	309	102	111	180	81

phosphorus, and potassium have more favorably influenced the content of organic matter and nitrogen than any of these materials alone, or other combinations of them. A close relationship was found between organic matter content, total nitrogen and crop production in all but soil 301N.

3. All combinations of lime and fertilizers have influenced the reaction of these soils to a small but similar extent and have had a more favorable effect than lime alone.

4. Lime with combinations of nitrogen, phosphorus, and potassium have increased the yields of all crops to a greater degree than lime plus single fertilizing elements or lime alone.

Results of bacterial and fungi counts on these soils in relation to field treatment, available nutrients, and crop yields give rise to the following conclusions:

1. Numbers of bacteria were increased in all soils by the application of lime alone and lime with fertilizing elements. This increase was greatest in the soils receiving phosphates with lime and phosphates in combination with potassium and lime.

2. All field treatments increased the number of fungi developing on agar plates, especially in soils receiving lime with potassium and lime with superphosphate. While the increase in numbers of fungi just mentioned were very pronounced, other fertilizer treatments indicate little if any relation between numbers and field treatment.

3. Numbers of bacteria in the soil correlate with available nutrients and crop production, while numbers of fungi showed this relationship only to a small extent.

Nitrification studies on these soils in relation to crop production, available nutrients, and field treatment allow the following conclusions to be drawn:

1. Nitrogenous materials leaving an acid residue were shown to be undesirable for nitrification studies in these soils, whereas urea, diammonium phosphate, organic salts of ammonium, and organic plant materials were found entirely satisfactory.

2. Nitrification of all the materials selected for this study showed the same relationship to fertilizer treatment. Nitrification was found greatest for all materials in the soils receiving combinations of lime, nitrogen, phosphorus, and potassium, while combinations of lime and the elements singly were found less effective in increasing the nitrifying power of these soils. Lime alone and lime with rock phosphate were least effective in increasing nitrification.

3. A close correlation was found in these soils between nitrifying power, organic matter content, numbers of bacteria, and available phosphorus supply, except for soil 310, which was comparatively high in available phosphorus and numbers of bacteria.

4. Crop production and nitrifying power of the soils correlate, except for soil 302, which produced the largest crop yields, but was not as effective in nitrate production as soil 305. While the nitrates produced in soils receiving lime plus superphosphate, and lime plus rock phosphate were lower than in soils receiving other fertilizer treatments, they were comparatively higher than crop yields on the same soils.

The production of carbon dioxide from cellulose with addition of different salts in these soils, as affected by field treatment and in relation to crop production and available nutrients show certain relationships:

1. A method was developed and suitable apparatus assembled for determining carbon dioxide production in these soils.

2. Additions of nitrogen and phosphorus increased carbon dioxide production from cellulose in all the soils. This increase was found greatest where the element added had previously been found most deficient in the soil.

3. Soils receiving field treatment of lime, nitrogen, phosphorus, and potassium gave greater quantities of carbon dioxide from cellulose than soils receiving these elements singly with lime or lime alone.

4. An excellent correlation of CO_2 production from all additions plus cellulose and crop yields was found for all soils except soil 302. Nitrogen in combination with lime and other elements appears to have exerted a greater influence on crop yields than on carbon dioxide production.

5. A close correlation between nitrifying power and CO_2 production was demonstrated for all soils.

Yields of crops in pots in the greenhouse and carbon dioxide production from plant material grown on the soils permit the following conclusions:

1. Yields of all crops were increased by previous fertilizer treatment in the field. The crop yields correlate closely with yields of crops secured in the field.

2. Although carbon dioxide production from crops grown and returned to the soil were greater for those soils receiving fertilization in the field, the differences due to treatment were small.

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