VARIABLE LEVELS OF BIOLOGICAL ACTIVITY IN SANBORN FIELD AFTER FIFTY YEARS OF TREATMENT¹

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Sanborn Field, where the predominant soil treatment has been manure additions and some fertilizers in continuous cropping and different rotations, provides an opportunity to study the properties and the performances of the soil organic matter as an index of the biological activity in relation to soil management practices. After fifty years of treatment the organic matter fraction of the soil might well be expected to have some ear marks of stability in nature and behavior which could be recognized by analytical study.

The hypothesis for undertaking such a study is the belief that organic matter returned to the soil is a greater source of nutrients than commonly considered. Organic matter is readily recognized as the source of energy for the majority of soil chemical changes. As the source of carbon dioxide, we grant it a prominent place, and have been led to believe that in setting free this acidic agent through microbiological struggles, it serves to break the bases, or cations, out of the crystal minerals to be absorbed or held less firmly by the clay complex, and thus changed from the unavailable to usable forms for the plants. Skepticism toward such a belief in the mineral breakdown as the main source of nutrients for plants should arise from the acquaintance with the high ash content of the so-called "humus" or organic fraction taken from the soil. Also, the stability of the colloidal mineral, or clay fraction, under rather drastic chemical treatment in the laboratory, indicates its insignificant change during a single growing season. When organic matter decay reduces soil acidity, isn't such possibly the result more from oxidized alkali or alkaline earth residues left as ash than from basic residues liberated by rock and mineral breakdown under carbonic acid attack? By determining the more exact nature of the carbonaceous and nitrogenous fraction of the soil, and then by learning its rate of breakdown, -- particularly

during the growing season, --it will be possible to learn whether or not this fraction of the soil is the main, or almost sole, contributor of nutrients to the plant during the growing season.

Sanborn Field is of the Putnam silt loam type in the Putnam-Vigo-Clermont association, whose profile consists of a silty surface underlain by an impervious horizon of compact clay. Its climatic equilibrium locates its surface acre nitrogen content at approximately twenty-five hundred to twenty-six hundred pounds. Its exchange capacity amounts to 13-15 M.E. per 100 grams soil, and its degree of base saturation is about fifty per cent.

The cropping systems on Sanborn Field include continuous crops and rotations while the list of soil treatments contains manure, ammonium sulfæte, sodium nitrate, superphosphate, complete fertilizers, and limestone. Some of these treatments have extended through only the last twenty-five years, while some have been regularly followed since the establishment of the field fifty years ago. tempts have been made to learn something not only about the changes in the supply of the organic matter, but also about the nature of the organic matter, particularly the differences in its composition as shown (a) by the carbon and nitrogen contents, (b) by the differences in the extracted humus, (\underline{c}) by the degree of lginification, and (d) by the differences in rates of its nitrification. studies on the nature of the organic matter of this old field were undertaken to learn more about the biochemical activity within a soil as related to its treatment in the hope that such knowledge might contribute to more wise soil management.

Changes in Supply of Organic Matter

Careful analyses of the plots for nitrogen and carbon were made at the end of

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twenty-five years and again after fifty years. Not all of the plots will be cited here. Only a few of them have been selected for citation as they lend themselves for common use through the other phases of this study. Table I gives plots in continuous wheat and continuous timothy to illustrate the extremes in organic matter changes in the field.

Table 1

THE LIGNIN CONTENT OF THE ORGANIC MATTER AFTER FIFTY
YEARS WITH THE CHANGES IN THE ORGANIC MATTER STOCK IN
THE SOILS OF SANBORN FIELD DURING THE LAST TWENTYFIVE YEARS

		Organic Conte	Matter ent %		
Plot No.	Crop and Treatment	After 25 Years	After 50 Years	Change	Lig- nin (%)
2	Wheat-Fertilizer	1.94	1.75	19	39.5
5	Wheat-Manure 6T, 25 yrs. Manure 3T, 25 yrs.	2.62	2.18	44	48.9
29	Wheat-Manure 6T, 25 yrs. Ammonium sulfate, 25 yrs.	2.37	1.84	53	46.1
30	Wheat-Manure 6T, 25 yrs. Sodium nitrate, 25 yrs.	2.77	2.24	33	50.5
23	TimothyNo treat- ment	2.29	2.49	+.20	40.5
22	Timothy-Manure 6T	2.91	3.51	+.60	48.8

Outside of the plots in continuous sods, the depletion of the organic matter is the characteristic not only of the plots cited in the table but for most of the plots. Since no analyses were made of the original soil, the change in the organic matter content, as based on carbon, is determinable for only the last twenty-five years. During this brief period the changes amount to as much as a loss of more than ten to twenty per cent in the original supply with continuous cultivated cropping and a gain of twenty per cent under continuous sod cropping.

Nature of the Organic Matter

The lowest supply of organic matter after fifty years occurs in Plot No. 2 under continuous wheat given heavy additions of

commercial fertilizer. This seems to suggest that the addition of the nitrogen and minerals has supplied a microbiological deficiency, and that fertilizers served thus to deplete the organic matter further here than on any other plot. With over 750 pounds of mixed fertilizers, of which no small part was mineral nitrogen, the carbon supply of the soil has been extensively burned out. That the phosphate and potassium are essential contributions toward increased microbiological activity is testified to by the higher content of organic matter in the continuous wheat plots given ammonium sulfate and sodium nitrate. These are still higher at this time in organic matter than the plot given complete fertilizer. The ammonium sulfate treated wheat plot which is now very acid, is rapidly moving toward the lower level of organic matter found in the plot given complete fertilizer. If the minerals are deficient here the nitrification process may be liberating some from the mineral fraction of the soil.

These facts suggest that organic matter decay in the soils of many of the Sanborn Field plots is a much slower process because of the deficiency in phosphorus and possibly in potassium. The addition of mobile or soluble nitrogen is also instrumental in decomposing the organic matter or carbon as based on the determinations of this latter element. Thus, we may reason that the organic matter in the soils like those on Sanborn Field must be approaching a very stable condition. This stability would seem to rest on its highly carbonaceous nature, or its supply of immobile nitrogen, which does not serve microbiological life for its digestion or decay unless balanced or supplemented by soluble nitrogen and minerals. If this is the nature of the organic matter then its accumulation under the timothy would suggest such an occurrence because of a deficient supply of nitrogen, deficient phosphorus, and deficient minerals, or it may be simply remaining there because these deficiencies prohibit the microorganisms from decomposing or removing it.

$\begin{array}{c} \textbf{Changes in the Nature of Organic Matter with} \\ \hline \textbf{Time} \end{array}$

A study of the carbon-nitrogen ratios of these sample plots also points further to a wide difference in the nature of the organic matter, and, consequently a wide variation in microbiological activities. In Table 2 are presented the carbon-nitrogen ratios along with percentage contents of these elements for a few of the interesting plots.

The shift to a wider carbon-nitrogen ratio as a result of the nitrogen additions indicates a more active form of organic matter

Table 2

TOTAL CARBON AND NITROGEN CONTENTS TOGETHER WITH THEIR RATIOS AT TWENTY-FIVE-YEAR INTERVALS ON SANBORN FIELD

			After 25 Years			After 50 Years			Chang- es in	
Plot No.	Crop and Treatment	Car- bon%	Nitro- gen %	C/N	Car- bon %	Nitro- gen %	C/N	Car- bon	Nitro- gen	
2	WheatFertilizer	1.13	.107	10.5	1.02	.100	10.3	11	07	
5	WheatManure 6T, 25 yrs. Manure 3T, 25 yrs.	1.52	.140	10.8	1.27	.119	10.6	25	21	
29	WheatManure 6T, 25 yrs. Ammonium sulfate, 25 yrs.	1.38	.145	9.5	1.07	.081	13.2	31	64	
30	WheatManure 6T, 25 yrs. Sodium nitrate, 25 yrs.	1.61	.171	9.4	1.30	.094	13.8	31	77	
23	WheatNo treatment	1.33	.141	9.4	1.45	.135	10.7	+.12	06	
22	WheatManure 6T.	1.69	.177	9.5	2.04	.195	10.4	+.35	+.18	

in the soil as a result of nitrogen fertilization. Even though the total supply in this soil was decidedly lowered as measured in terms of either carbon or nitrogen, yet this lower supply with its wider carbon-nitrogen ratio suggests less stable or more active compounds. These changes which occurred in these nitrogen treated plots within twentyfive years suggest that the stage of activity was very low already twenty-five years ago, when the carbon-nitrogen ratio was very narrow, and that more activity is now prevalent in the material of wider ratio. On the wheat plot, given complete fertilizer, the nature of the organic matter changed little during twenty-five years as indicated by the stability of the carbon-nitrogen ratio, or its similarity in the two intervals. Manure

additions, likewise, maintained a stable carbon-nitrogen ratio, in the face of a declining total supply of organic matter. Under timothy without manure, the total carbon increased while the total nitrogen decreased, thus, giving a wider ratio. Where manure was applied on the timothy soil, the carbon-nitrogen ratio widened at the same time while the total organic matter increased. This suggests a tendency to hoard carbon. In four of the plots, whether manured or not, the carbonnitrogen ratios were narrow but similar. They were much wider after twenty-five years where nitrogen was applied, even though the total organic matter supply was decidedly lowered during that same interval.

Table 3

ANALYSES OF EXTRACTED HUMUS FROM SOME SANBORN FIELD PLOTS

		Soil	Humus Composition							
Crop and Treatment		Content %	Nitrogen %	Carbon %	C/N	Calcium %	Phosphorus %	Silica %		
Corn	Manure No manure	3.280 3.218	7.40 3.45	14.15 8.76	1.91 2.54	2.24 2.12	.710 .448	6.08 3.34		
Timothy	Manure No manure	4.712 3.314	7.09 5.52	14.11 16.51	1.99 2.99	1.71	.648 .842	5.44 2.82		
Rotation	Manure No manure	3.958 3.322	5.34 4.99	18.09 17.07	3.38 3.42	2.74 1.56	.724 .808	5.70 - 2.16		

Nature and Quantity of the Extracted Humus

As a partial attempt to differentiate the organic matter on the different long continued plots, humus extracts were made of six of them, and analyzed for their contents in calcium, phosphorus and silica in addition to their carbon and nitrogen. These data are presented in Table 3.

The total quantity of humus was lowest in the cultivated soil, followed closely by the soil in the rotation and that in timothy both where no manure was used. They take this same order, but at a higher figure, where manure was added.

The nitrogen content of the humus varied from 3.45 to 7.40 per cent, with the high figures under corn with manure, and sod given the same treatment. The lowest nitrogen figure occurred under corn that had no treatment. Under rotation, the manure addition gave the least variation in nitrogen content of this extract. These rotated plots and the sod plot without manure fluctuated closely around the nitrogen content of five per cent customarily considered as the average for humus.

The carbon content of the humus fluctuates from the high figure under the rotated soils to the low one under the corn without manure. In each case, the extract from unmanured soil is lower in carbon than from the manured soil. The carbon-nitrogen ratio is also wider for unmanured than for the manured soil, and points to relatively more nitrogen in this humus form as a consequence of manure additions. Manure nitrogen then must be retained, in part at least, in the more stable humus fraction of the soil.

The mineral contents of the humus are also widely different with the different soil treatments. Except for the timothy soil where the two analyses are the same, the calcium contents are higher for the soils with manure than for those with no manure. That the lowest calcium content should be in the untreated soil with rotation may not be causally related to the fact that this is the only soil in the group attempting to grow a legume. The silica content was higher in every case of manure treatment, agreeing in general in this respect with the calcium content. The phosphorus showed the reverse in the case of the timothy and the rotation, but paralleled the calcium and silica with reference to manure for the continuous corn. The lowest phosphorus content in the humus extract occurred under corn given no manure. Manuring seems to have increased the calcium and silica contents in the extracted humus and raises the question whether the extra

silica means merely more silica as ash from manure decay, or a contribution from the mineral fraction to the organic fraction by more interaction between the colloidal organic matter and the colloidal mineral matter of the soil.

The extracted humus reflects the soil treatments and follows trends in amounts and in composition as one might surmise their influence. The widest spreads occur between manure and no manure additions under continuous corn, for all the tested properties of the humus. The differences between these two soil treatments were less significant under timothy sod and even less so under rotation. Manure gave the humus with the highest content of nitrogen, with the narrower carbon-nitrogen ratio, and the larger total amount of humus in the soil. It also gave the higher calcium content, and silica content to this extracted fraction. It suggested, however, the reverse relation for phosphorus.

Humus as a Possible Supply of Minerals for the Crop

If we were to assume that this humus is the sole supply of soil nitrogen to the growing plants and that, during the liberation of this nitrogen, the humus is broken down to liberate also its calcium and phosphorus, it is interesting to calculate the crop possibility in terms of calcium and phosphate so released. Taking the continuous corn plot without manure, as an illustration, of which the soil contained 3.2% humus, there would be 64,000 pounds of humus per acre, carrying 3.45% of 2208 pounds of nitrogen. This plot has been producing twenty bushels of corn per acre as an average requiring thirty pounds of nitrogen as an estimated figure. The release of this amount of nitrogen would break down 1.36% of the total humus in the soil. If at the same time, the calcium and phosphorus in this corresponding amount of humus were liberated, these amounts would be 18.4 pounds and 3.91 pounds, respectively, or the calcium needed for a corn yield of fifty bushels, and the phosphorus for twenty-five bushels, both of which are yields above that being produced per plot. If this is a correct assumption, then the organic matter or humus could supply not only the nitrogen, but the calcium and phosphorus needs of the crop as well.

Degree of Lignification of the Organic Matter

As an attempt at further differentiation of the nature of the organic matter in relation to soil treatment, tests were made of the degree of lignification according to the method suggested by Waksman. By the treatment

of the soil with seventy-two per cent sulfuric acid it is assumed that only those carbonaceous compounds approaching lignin remain unhydrolyzed and insoluble. The percentage of the organic matter found by this method to be in the lignin form is given in Table 1, which gives also the changes in total organic matter as based on the carbon.

There are no excessively wide differences in these lignin figures. The lowest percentage of this inactive fraction in the organic matter occurred where complete fertilizer has been applied heavily on continuous wheat. The highest percentage occurred where the sodium nitrate was used and has apparently been responsible for an organic matter that is highly lignified. Manure addition seems to have had an effect similar to that of nitrate additions.

These few determinations suggest that nitrogen additions alone, or manure with its high nitrogen content tend to give an organic matter of high lignin percentage and of a stable or less inactive nature, while under phosphate-potash fertilizers the organic matter remaining is much lower in this decay resistant fraction.

<u>Variable Nitrification Activities as Related</u> to Soil Treatments

Laboratory studies were undertaken to evaluate the nitrate accumulating capacities by these soils under different long continued cropping and soil treatments as a measure of the influence of these on the microbial behavior, within the soils. The samples were brought into the laboratory in late February, handled at optimum moisture into jelly tumblers, with treatments including lime and organic matter, and tested for their accumulated nitrate content at forthnightly intervals during ten to twelve weeks. Twenty-eight plots were included and the data assembled to include the effects of different cropping systems and rotations, and then for the different soil treatments including (\underline{a}) manure, (\underline{b}) superphosphate, (\underline{c}) fertilizer, (\underline{d}) ammonium sulfate, (e) sodium nitrate, and (f)limestone.

The nitrifying activity under all the soils with continuous crops was relatively low. The low level for corn, oats, wheat, and timothy or for all of these crops rather than a differentiation amongst them is the significant fact about the separate crop influence on this microbial soil process.

The rotations were not, however, without their influence. The nitrate accumulations were regularly higher for the three-year rotation, followed by the four-year and

then in order by the six-year rotation. Except for the six-year rotation, they were higher in nitrate activity than the highest of the continuous crops, namely, timothy. Since clover occurred only once in each of these rotations, it suggests that the greater number of recurrences of this crop in the shortening of the rotation may be the reason for their taking their particular order in levels of nitrate accumulating activity.

The long continued soil treatments manifest their influences markedly on nitrate accumulating activities in the soil. The manured soils were especially responsive with nitrate production when limed in the laboratory, pointing out that these soils are not nitrifying their stock of organic matter rapidly because of lime deficiency. That this effect by lime is not so much a matter of acidity as that of supplying calcium, is demonstrated by the influence of the superphosphate treatment. Soils, receiving superphosphate for twenty-five years, given nitrifiable organic materials in the test were almost as active in nitrate production as when given both lime and organic matter. Where the latter combined treatments produced 9.7 mgms. of nitrate, the former single addition gave 8.08 as the increase during six weeks of incubation. The higher general nitrate level by this phosphated soil when given something to nitrify was the distinguishing feature.

The effects by fertilizers were similar to those by phosphate as might be expected, since the latter is their main constituent.

In the plots treated with ammonium sulfate and sodium nitrate, the former has lowered the activity in the soil as a nitrifier. Lime additions were decidedly effective on nitrate production where ammonium sulfate had been used, though it was not without significant effect on soils treated with sodium nitrate, suggesting again the need of the soil for calcium. Where the field treatment of limestone had been used, the soil was at a lower nitrate level than where such had not been used. This was particularly noticeable by the failure of the soil to respond to liming in the laboratory in contrast to the marked response by the unlimed soils when given lime. However, the limed soil given something to nitrify was almost on a par in nitrate accumulation with the unlimed soil given both lime and green manure as laboratory treatments.

The general levels of biological activities as demonstrated by nitrate accumulation in these soils can be measured from the data in Table 4, giving the average nitrate

NITRATE NITROGEN LEVELS IN SOILS UNDER DIFFERENT LABORATORY TREATMENTS AS INFLUENCED BY THE PAST HISTORY OF CROPPING AND SOIL TREATMENTS. (SOILS FROM SANBORN FIELD)

		(Pounds Nitrogen per Acre)						
Cropping History and Field Treatment		No Treatment	Limestone	Organic Matter	Lime and Organic Matter	Mean of All Treatment	Increase over Lowest Item	
	Corn	25	83	55	122	71		
. Continuous	Oats	30	26	121	190	92	21	
Crops	Wheat	59	102	52	172	96	25	
	Timothy	48	104	110	183	111	40	
	Six-year	34	52	74	176	84	••••	
Rotation	Four-year	56 .	75	109	208	112	28	
	Three-year	44	97	. 182	219	135	51	
	None	40	61	92	179	93	••••	
	Manure	49	93	120	196	114	21	
Soil	Phosphate	50	106	184	282	155	62	
Treatments	Fertilizers	43	94	149	222	127	34	
	Ammonium Sulfate	60	113	150	193	129	36	
	Sodium Nitrate	62	87	174	216	134	41	
	Lime	36	74	223	267	150	57	

contents during ten weeks of laboratory study and particularly the mean of all treatments.

Summary_

From these various observations of the organic matter in the soils under different treatments on Sanborn Field it is evident that there are wide differences in the biological activities now after fifty years. Where the organic matter remains at a relatively high level it has failed to be consumed by the microorganisms very probably because of its deficiencies as a bacterial ration. Some of the deficiencies suggested are (a) nitrogen,

- (b) calcium, (c) phosphates, and possibly
- (d) potassium in the mobile forms to serve in

the nutrition of the microorganisms. Where these have been added as soil treatments, the total supply of organic matter has been reduced. The increased crop returns, running parallel with organic matter reduction in the soil, suggest strongly that where the organic matter fraction of the soil is not breaking down, it is being retained at modest levels because these mineral elements are too deficient in the soil for the microbiological processes. They are then consequently deficient in the soil for the plants' activity on a high level. Such conditions suggest that when these deficient nutrient items are delivered by the soil to the plant, they may come in the main from the more slow decay of the more stable organic matter.