THE INFLUENCE OF PLANT RESIDUES ON NITROGEN FIXATION AND ON LOSSES OF NITRATE IN THE SOIL.

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(With Three Figures in text.)

THE incorporation of plant residues with the soil may give rise to a number of different changes, any one of which tends to dominate the rest according to the varying conditions of the general environment—air and water supply, temperature, the reaction of the soil itself. Whilst, under average conditions the easily decomposable constituents of the plant are speedily resolved by the action of the soil flora, the more resistant structures often persist unchanged for considerable periods, but ultimately lose their identity and as humus become an integral part of the soil. The generally accepted statement that organic matter possesses value on account of its humus forming properties does not express fully the influence of organic residues, and it is quite probable that the primary and intermediate stages of decomposition are equally important from the standpoint of soil fertility

An indication of such effects of plant residues is afforded by two of the Rothamsted plots which, since 1882 and 1885, have been allowed to revert from arable to prairie conditions. As the result of analyses of the soil of these plots, Hall⁽¹⁾ was able to show that during the twenty year period of the observations very considerable accumulations of nitrogen had taken place, and although it was recognised that the conditions were complicated by the presence of leguminous plants in the herbage of one of the plots, by the absorption of free ammonia from the atmosphere, and the possible capillary uplift of nitrates from the permanent soil water it was, at the same time, considered that part of these gains might reasonably be ascribed to the activity of certain nitrogen fixing organisms which have been found in these soils by Ashby⁽²⁾. It was, in fact, considered that the carbohydrates formed by the decomposition of the crop

residues might to some extent subserve the process of nitrogen fixation. This view accords with the conclusions formed by Henry (3), who was able to demonstrate that when leaves are kept in a moist but aerated condition a slight assimilation of atmospheric nitrogen takes place. This was in the first instance disputed, but subsequently confirmed, by Hornberger (4).

The experiments discussed in the following pages were originally undertaken with the object of ascertaining in the first place whether and in what manner these residues become available for the assimilation of atmospheric nitrogen, and how far the conditions in the field are favourable for the operation of the process to any appreciable extent.

Since the discovery of such nitrogen fixing organisms as *Clostridium Pastorianum* by Winogradsky (5) in 1893, and more particularly *Azotobacter chroococcum* by Beijerinck (6) in 1901, a considerable amount of work has been carried out, but chiefly with reference to the cultivation of these organisms under laboratory conditions and with synthetic media. Such work as that of Freudenreich (7), Gerlach and Vogel (8), Remy (9), Löhnis and his scholars (10), Heinze (11), Lipman (12), Haselhoff and Brede-mann (13), Warmbold (14), Stoklasa (15), Schneider (16) and many others sufficed to show not only that these bacteria are widely distributed, but that under certain conditions of temperature, aeration and food supply, an energetic assimilation may be induced.

Much of this work, though valuable from a physiological standpoint, was somewhat lacking in general applicability, and it is mainly due to the investigations of Koch and his collaborators (17) that we possess some conception of the intensity of the process under more natural conditions in the soil itself.

In these experiments the addition of sugar to the soil resulted in gains of 3-10 mgrms. of nitrogen per gram of carbohydrate supplied, but later work showed that when calculated on the amounts of sugar actually oxidised these increments were of a much higher order. The best utilisation of the sugar occurred when dextrose was applied at the rate of 2.0 per cent. of the weight of the soil, or when 0.2 per cent. was added eight times, or 0.5 per cent. five or eight times. A limit to the efficiency of these repeated applications was however soon reached, and the interesting observation was made that when the lower proportions were more frequently used or when the higher doses such as 1.0, 1.5 or 2.0 per cent. were applied five times, a proportionate decrease in activity ensued. Part of this effect was, no doubt, due to the high concentration of sugar in the soil water, but it may also be attributed, with a fair degree of

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probability, to the inimical effect of an ever increasing degradation of the nitrogen compounds already formed by the bacteria and a consequent accumulation of available nitrogen compounds.

Definite fixation was also obtained by the use of saccharose, but the addition of molasses to the soil resulted in a loss during six weeks to the extent of 30 per cent. of the nitrogen originally present. This was attributed, though without any definite experimental evidence, to the liberation of free nitrogen from the easily decomposable nitrogen compounds in the molasses and to the above mentioned suspension of nitrogen fixation in the presence of such bodies.

Filter paper and buckwheat straw were without apparent effects when incorporated with the soil, but mustard caused appreciable losses under similar conditions.

From numerous pot experiments with oats, buckwheat and sugar beet it was evident that the nitrogen stored up by bacterial action readily becomes available for plant growth, and that by the treatment of the soil with sugar, its fertility may be increased to a marked degree.

These results have been fully confirmed by Remy(18) who was also able to demonstrate the intimate relationship existing between the supply of magnesia, lime, and mineral manures, and a rich and potentially virulent population of *Azotobacter* in field soils.

The possibility of reproducing on a field scale the conditions obtaining in the above experiments appears at first glance to be somewhat remote, but attention is drawn by Ebbels(19), to the fact that molasses has for a long time been looked upon as a valuable fertiliser for sugar-cane lands in Mauritius. As he was in possession of a large quantity of molasses which could not be disposed of in any other manner a portion of this material was applied to field soil which subsequently was planted with sugar-cane. The soil so treated was found to be not only more productive than that which had not received molasses, but showed five years later a much higher nitrogen content than the control. These results have been corroborated by Boname(20) who obtained considerable increases of crop, especially in those cases where phosphatic manures were applied in addition to molasses. The effect of treatment was greatest with the first crop, but was found to persist up to the third ratoons. Experiments in the Leeward Islands(21) gave, in three cases out of four, increases varying from 1.8 to 7.7 tons of sugar cane per acre, and in this case also a residual effect was observed. Similar work has also been carried out in Java(22).

On the other hand, numerous instances are on record, of which those

described by Harrison and Ward (23) are typical, where the application of saccharine material has been quite ineffective in promoting greater fertility.

In many of these cases it is difficult to account for the absence of any specific effect of the treatment on crop yield, owing to the lack of information as to the precise conditions under which the experiments were carried out. The possibility is not excluded, however, that this ineffectiveness is the resultant of two distinct sets of changes, namely, the beneficial effects which have been discussed above, and the distinctly injurious ones which have been observed by Hiltner (24), von Seelhorst (25), Stützer (26), Bartels (27) and others to result from the admixture of straw, green manures and so forth, with the soil. The tendency towards divergent results has been recognised by Peck (28) who states that harmful effects are likely to attend the application of molasses at frequent intervals to growing sugar cane, but that when applications are made some weeks prior to the introduction of a crop, beneficial results may be obtained.

The experiments described in the following pages afford additional evidence of each of these effects. The incorporation of such substances as sugar, starch, or plant residues with the soil is likely to give rise to two opposite processes, one of which results in a diminution, and the other in an increase of the fertility of the soil. Whether the one preponderates over the other, or an equilibrium is attained, depends largely on the quantity and type of material applied, the prevailing temperature, the interval before the introduction of a crop, and the presence of the specific micro-organisms.

In any case the destructive changes come first into operation but their relative intensity and the period of persistence are increased by low temperatures. High temperatures by permitting of the entrance and subsequent predominance of constructive bacterial changes, tend to limit the extent and persistence of soil losses.

The experiments show that the introduction of a crop immediately after the incorporation of material containing carbohydrates will result in decreased yields. If a long period is allowed to elapse before the crop is sown this decrease may be only slight or entirely absent in spite of low temperatures. If the soil temperature is high and sufficient time is given before a crop is sown, adverse changes are likely to be reduced to a minimum and favourable after-effects may be anticipated.

The autumn application of plant residues, strawy material, etc., may therefore be expected to lead to beneficial changes; spring applications

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of such material in any quantity are inadvisable unless the constituents likely to give rise to adverse changes can be previously eliminated. This possibility forms the subject of a separate inquiry which is proceeding at the present time.

It is apparent that the question possesses something more than purely academic interest, especially in its relation to the present day tendency towards a decreased production of manure and an increased output of straw and similar materials. It is also linked up more or less directly with a number of minor issues such as the utilisation of green manures, the preparation of compost, and of leaf and turf mould. At the same time it is unfortunately a field in which little more than sporadic investigation has yet been attempted, and where consequently practice not infrequently follows a purely arbitrary course.

EXPERIMENTAL.

A. Field Experiments on Nitrogen Fixation.

In order to ascertain the extent to which nitrogen fixation processes come into operation in the field, the halves of two of the Hoos Field plots which had been under continuous barley experiments since 1852, were used for this work. Of these two plots, one (6. I.) had not received any manurial dressing since the above year, while the other (4. O.) had received annually a complete mineral manure without nitrogen. The experiment was continued from 1906 to 1911, an application of one ton of sugar per acre being given each year with the exception of 1907, when potato starch was applied at the same rate. The immediate effect of the application of sugar in 1906 was a practical extinction of the barley crop, but owing to the proximity of the sugar application to the sowing of the crop it was thought that this effect might have been due to the primary decomposition products of the carbohydrate. During the next three years, therefore, a longer interval was allowed to elapse between the time of treatment and the introduction of the crop, but although the injurious effect was not so marked it was nevertheless evident in all the cases where the sugar was applied in spring. Examinations of the soil showed that the treatment had resulted in a very great increase of bacteria, and it was presumed, for the time being, that these were acting prejudicially on the crop either by the decomposition of the soil nitrates with the liberation of free nitrogen, or by the assimilation and retention of available nitrogen compounds.

A second factor which demanded consideration was the low soil temperature prevailing at the time of these earlier applications, and since other observations had shown the great differences in nitrogen fixation which occur at various temperatures up to 30°, it was decided to make the next application of sugar with a high prevailing soil temperature, that is, in early autumn.

This change was fully justified by the yields of the 1910 crop, an appreciable increase being shown by the plots which had been treated with sugar and had received an application of phosphatic and potassic manures. A second autumnal application was therefore made in 1910, and the 1911 results were of a still more promising order. (Table I and Fig. 1.)

TABLE I.

The Influence of Carbohydrates on the Fertility of Field Soils.

Hoos Field Experiments: Crop Barley: Produce per acre.

Year	Time of application	Carbo- hydrate supplied, 1 ton per acre	Mean soil temperature during month following application	Plot 6. I. (Unmanured 1852-1911)				Plot 4. O. (complete minerals, no nitrogen), 1852-1911			
				Grain, lbs.	Straw, lbs.	Total pro- duce, lbs.	Rela- tive yields	Grain, lbs.	Straw, lbs.	Total pro- duce, lbs.	Rela- tive yields
1906	March 6th	None		866	631	1497	100	1209	1276	2485	100
		Sugar	4° C	—	—	—	—*	—	—	—	—*
1907	Jan. 17th	None		534	1070	1604	100	858	2720	3578	100
		Starch	-2° C.	400	792	1192	74	809	2440	3249	91
1908	Feb. 4th	None		560	508	1068	100	812	1008	1820	100
		Sugar	5° C.	301	347	648	61	538	866	1404	77
1909	Feb. 19th	None		836	693	1529	100	1289	1859	3148	100
		Sugar	0° C.	542	810	1352	88	897	1364	2261	71
1910	Sept. 27th, 1909	None		625	1008	1633	100	850	1232	2082	100
		Sugar	11° C.	689	868	1557	95	1215	1287	2502	120
1911	Sept. 22nd, 1910	None		297	671	968	100	372	872	1244	100
		Sugar	12° C.	303	722	1025	105	677	1238	1915	154

* Crop failed completely.

Two interesting points arise from these results. In the first place, it may be noted that although the total yields of plot 4. O. are distinctly increased as a result of treatment with sugar, this is due to a great extent to the effect of the treatment on the yield of grain rather than on that of the straw, the mean relative yields of the former being 155 per cent. as against 120 for the latter.

Secondly, it is noteworthy that in both 1910 and 1911 the application of sugar to plot 6. I., which did not receive mineral manures, was quite ineffective in bringing about any crop response. Experience has shown

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that although barley grown on this soil responds readily to an application of phosphatic manures alone, it nevertheless does so to an equal or greater extent when a simple nitrogenous dressing is given. It is possible therefore that the growth of the crop on 6. I. was not limited primarily by the lack of phosphates, but by the absence of any appreciable fixation of nitrogen. This is perfectly compatible with other work, and amongst others Koch has shown the great importance of an adequate supply of available phosphates for the assimilation of nitrogen by free living organisms, as is shown by the following table.

	Soil alone	Soil + 0.1 % superphosphate	Soil + 0.6 % basic slag	Soil + 1.0 % basic slag
After 4 weeks	11.2 mgrm.	19.2 mgrm.	16.7 mgrm.	16.9 mgrm.
After 7 weeks	25.5 „	33.8 „	27.0 „	28.2 „

Finally, it should be recognised that the soil of plot 6. I., which failed to give any definite return on treatment with carbohydrates, had been persistently starved for more than fifty years, and it must not be concluded that equally unfavourable results would be obtained with any normal soil.

The experiments carried out by Boname, to which reference has already been made, indicate the expediency of ensuring an adequate supply of phosphates if an economic utilisation of the carbonaceous material is to be expected. The same also applies of course to basic compounds where the soil is naturally deficient in these constituents (29).

B. The Utilisation of Plant Residues for Nitrogen Fixation.

Laboratory Experiments. The organic materials returned to field soils are, in the main, the leaves of root crops, the stubble of cereal crops, green manures, and the constituents of farmyard manure. In nature, a similar position is taken by the remains of annual plants and the leaves of perennials. A number of preliminary laboratory experiments were therefore carried out to ascertain whether these substances could be used for the assimilation of nitrogen.

The chief disadvantages inherent in such laboratory work are that, in order to limit experimental error in the determination of nitrogen, the amount of soil must be kept as low as practicable, whilst in order to get appreciable quantities of nitrogen assimilated, the source of energy must be present in relatively high proportions. Hence in the case of soluble compounds the concentration of the solution soon passes the optimum for the process; in the case of plant residues, the decomposition is liable to be strictly localised and the acids initially set up by bacterial

or fungal action are only tardily removed by any base that may be present.

The gains of nitrogen shown below, although not large, are fairly satisfactory in view of the restricted conditions under which they were obtained. The substances to be tested were previously dried and finely ground and then added in quantities of 0.5 to 1.0 grm. to 10 grm. of fine air-dry soil. These portions were placed in Erlenmeyer flasks and incubated at 30° C. after sufficient water had been added to make the mixture nicely saturated.

The addition of straw at the rate of 5 per cent. gave the following results.

	Total nitrogen			
	At beginning	After 30 days	After 44 days	After 240 days
0.5 grm. straw + 10 grm. soil	15.92 mgrm.	17.50 mgrm.	17.71 mgrm.	18.23 mgrm.
Gain (calculated per grm. straw)	—	3.16 "	3.58 "	4.62 "

Finely ground wheat stubble and elm leaves added at the rate of 10 per cent. gave relatively lower gains.

	Total nitrogen		
	At beginning	After 15 days	After 50 days
1 grm. wheat stubble + 10 grms. soil	19.21 mgrm.	20.09 mgrm.	20.82 mgrm.
Gain	—	0.88 "	1.61 "
1 grm. elm leaves + 10 grms. soil ...	33.89 "	38.18 "	37.37 "
Gain	—	4.29 "	3.48 "

The use of sand and of calcium carbonate, in conjunction with soil, gives better results, possibly on account of better aeration and a more efficient neutralisation of acid products.

	Total nitrogen	
	At beginning	After 69 days
1 grm. straw + 5 grms. sand + 5 grms. soil + 1 grm. CaCO ₃	15.30 mgrm.	21.57 mgrm.
Gain	—	6.27 "

In comparison with sugar, these plant materials appear to become available for nitrogen fixation much less rapidly, but the final gains agree well with those frequently obtained when sugar is subjected to the action of a mixed bacterial flora.

Pot Experiments. With the object of obtaining further information as to the value of plant residues and sugar for the fixation of nitrogen, a number of pot experiments have been carried out, the effect of the applications being gauged in the first instance by plant growth, and at the close of the experiments by nitrogen determinations. With sugar, starch, and other non-nitrogenous materials, demonstration of their use

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for nitrogen assimilation is quite simple, but in the case of plant residues some of the effects of the treatment may be due to the nitrogenous compounds contained in them apart from any nitrogen assimilated by the action of soil organisms.

Since it is impossible to make any accurate allowance for the nitrogen originally contained in the residues and which becomes slowly available for plant growth, the experiment resolves itself into a comparison between the effects of a mixed bacterial flora without nitrogen fixing organisms, and such a flora with the latter organisms present. It becomes necessary, therefore, to start with a sterile medium and for this purpose sand, and not soil, must be chosen.

The work was carried out in sixteen glazed earthenware pots, each containing 3,500 grms. of clean sand to which 2.0 per cent. of calcium carbonate, 0.1 gm. potassium phosphate, and 0.04 gm. magnesium sulphate had been added. To each of twelve of the lots of sand an addition of 5.0 grms. of finely ground hay was made, and after thorough admixture each portion was transferred to a large narrow necked bottle with a cotton wool plug. All the bottles were then heated to 95° for one hour, and after the sand had cooled down the bottles were divided into four lots and treated as follows:

- I. Sand alone. Heated and then reinoculated.
- II. Sand with hay dust. Heated but not inoculated.
- III. Sand and hay dust. Heated and then inoculated with putrefactive organisms.
- IV. Sand and hay dust. Heated and then inoculated with putrefactive organisms and *Azotobacter*.

Providing secondary effects did not come into play, the difference between sets I and II might be attributed to the nitrogen compounds liberated from the hay by heat: that between sets II and III would be due to the putrefactive organisms added; any advantage derived from the presence of *Azotobacter* would be expressed by the difference between sets III and IV.

The putrefactive organisms referred to above were obtained by transference of the colonies occurring on six nutrient agar plates which had been inoculated with a suspension of garden soil, and to this mixed culture a few drops of a sub-culture of nitrifying organisms in mineral salt solution were added. The term "putrefactive" is employed here in a very broad sense, the main object being to secure a mixed and effective bacterial flora not containing *Azotobacter*. One cubic centimetre of this suspension was applied to the sand of sets I, III and IV, but set IV

received in addition a similar quantity of a suspension of a pure culture of *Azotobacter chroococcum*. The bottles were stored in the laboratory at 15–20°, with occasional shaking, for six weeks, and their contents were then returned to the corresponding vegetation pots. From this time no special precautions against infection were observed excepting

Fig. 1. Effect of carbohydrates on the yield of barley. Hoos Field experiments, 1907–1911. Relative yields.

that any seeds sown in the pots were previously treated with dilute mercuric chloride solution and only sterilised water was used for maintaining the water content of the sand.

Barley was grown as a first crop, five seeds being sown in each pot on

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May 4th, 1911. Although growth in the pots of the same sets was very similar, that of the different sets varied greatly. During the first few weeks after sowing those plants in the control pots (sand alone) were distinctly superior to any of the others, there being little to choose between the rest. Towards the end of June, however, the barley in the sand inoculated with *Azotobacter* began to improve rapidly and at the end of three months appeared to be the best crop; this was subsequently

Fig. 2. Pot experiments on the utilisation of hay dust for nitrogen fixation.
Production of dry matter by first crop (Barley).

confirmed by actual weighings of the dry crop. The next plants to improve were those in sand to which putrefactive organisms had been added, but those in sand (with hay) without introduced organisms remained comparatively small and weakly during the whole vegetation period.

A second crop of rye was then sown in all the pots and here again the pots with *Azotobacter* possessed the most vigorous vegetation; in

TABLE II. *Utilisation of Hay for Nitrogen Fixation.*

Pots and treatment	Sand		First crop (Barley)			Second crop (Rye)			Total dry matter in first and second crops (grms.)	Total nitrogen in first and second crops (mgms.)	Total nitrogen in crops and sand (mgms.)
	Final nitrogen		Total dry matter (grms.)	Nitrogen in dry matter (per cent.)	Total nitrogen in dry matter (mgms.)	Total dry matter (grms.)	Nitrogen in dry matter (per cent.)	Total nitrogen in dry matter (mgms.)	Total dry matter in first and second crops (grms.)	Total nitrogen in first and second crops (mgms.)	Total nitrogen in crops and sand (mgms.)
	Per cent.	Total mgms.									
			0.0028	400	7.34	0.6685	49.06	2.53	0.8386	21.20	9.87
Sand alone	...	...	8.56	0.8605	73.65	11.38	0.4572	54.07	19.94	127	798
Sand + hay + putrefactive organisms + Azotobacter	...	...	6.82	0.6953	47.42	7.07	0.4115	29.09	13.89	76	619
Sand + hay + putrefactive organisms	...	...	4.65	0.5679	26.41	5.37	0.5483	29.44	10.02	56	584
Sand + hay (sterile)	...	...									

TABLE III. *Utilisation of Sugar for Nitrogen Fixation (Sand Series).*

Pots and treatment	Sand			First crop (Barley)			Second crop (Rye)			Total dry matter in first and second crops (grms.)	Total dry matter in first and second crops (grms.)	Total nitrogen in first and second crops (mgms.)		
	Per cent.	Final nitrogen	Total mgms.	Total dry matter (grms.)	Nitrogen in dry matter (per cent.)	Total nitrogen in dry matter (mgms.)	Total dry matter (grms.)	Nitrogen in dry matter (per cent.)	Total nitrogen in dry matter (mgms.)					
Sand alone	...	...	...	0.0028	400	7.34	0.6685	49.06	2.53	0.8386	21.20	9.87	70	470
Sand + sugar + Azotobacter	...	...	...	0.0056	799	7.48	0.7347	54.95	4.61	0.8424	38.83	12.09	94	893
Sand + sugar (sterile)	...	...	...	0.0035	500	2.01	0.5590	11.23	2.38	0.9470	22.53	4.39	34	534

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contradistinction to the first crop this difference was evident from the earliest stages. The results of these experiments are briefly given in Tables II and V, and the dry matter production is diagrammatically represented in Figs. 2 and 3.

As the result of treatment with putrefactive organisms alone, no definite increase in the nitrogen of the sand and crop is evident, but where *Azotobacter* was additionally present the nitrogen content of the sand was distinctly increased. After deducting the amount of nitrogen supplied in the hay (148 mgrm.) there still remains a surplus of about 120 mgrm.

Similarly, the amount of nitrogen in the first and second crops was increased from 49 mgrm. to 73 mgrm. and from 21 to 56 mgrm. respectively. Taking all the gains into account one finds that by the utilisation of the hay dust as a source of energy, a nett increase of 180 mgrm. occurred. When bacteria were not added but must have been eventually introduced by air infection the crop nitrogen was reduced from 49 to 26 mgrm. in the first crop, and only rose by 8 mgrm. in the following rye crop.

On the whole one must conclude either that the nitrogen supplied in the hay dust was extremely badly utilised or that some degeneration process resulting in the withdrawal or loss of nitrogen took place during the initial period of growth. Since the pots with *Azotobacter* also passed through this stage of depression it is probable that a more accurate gauge of the intensity of assimilation would be obtained by a comparison between the set with *Azotobacter* and that without any bacteria added.

If the fact be taken into consideration that such gains occurred within six to seven months from the time of treatment, the degree of utilisation may be regarded as fairly satisfactory.

Utilisation of Sugar for Nitrogen Fixation.

In order to allow of some comparison of the value of plant residues and that of some directly available source of energy, two further sets of pot experiments were carried out in which sugar was applied to the sand at the rate of 0.2 per cent. The general preliminary treatment was the same as in the preceding sets—potassium phosphate and calcium carbonate being supplied. After being sterilised, the sand belonging to one set of pots was maintained in a sterile condition, but the other portion was inoculated with a pure culture of *Azotobacter*. Later treatment, sowing, harvesting, etc. was the same as with the hay dust sets of pots.

The initial depressing effect of the treatment with sugar on subsequent growth was even more pronounced than with hay dust, and by

the time the first crop was removed the plants with sugar and Azotobacter had merely succeeded in equalling those in the control pots, but the crop in sand and sugar which had been kept free from infection up to the time of sowing was only one-third of the control. The yield of

Fig. 3. Pot experiments on the utilisation of hay dust for nitrogen fixation.
Production of dry matter by second crop (Rye).

rye, as a second crop, was distinctly better with Azotobacter, but by far the largest proportion of nitrogen assimilated was found to remain in the sand. It is, however, reasonable to suppose that this is due largely to the fact that putrefactive organisms were not supplied to these sugar

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pots, and in their absence the breakdown of the nitrogen compounds stored up in the *Azotobacter* cells would therefore proceed but slowly.

As a result of inoculation with *Azotobacter* the nitrogen in the two crops was increased from 34 mgrm. (without inoculation) to 94 mgrm. with *Azotobacter*: the total nitrogen in sand and crop showed still greater differences, being 460 mgrm. in the control pots as against 534 and 893 mgrm. in the treated pots. (Table III.)

Further experiments consisted in the application of sugar to soil in pots similar to those used in the above experiments. Since *Azotobacter* is normally present in these soils it was not necessary to add this organism nor was it considered desirable to introduce other factors by subjecting the soil to heat prior to the application of the sugar, as was done in the case of the sand cultures. One set of pots was retained as control, and of the two sets treated with sugar, one was stored at a temperature of 5–10° C. whilst the other was stored at between 15–30° C.

The influence of temperature on nitrogen fixation has been noted in various laboratory experiments and the general result was obtained that when the temperature falls to the region of 5° fixation comes to a standstill, whilst it is most active between 18–31°. This is well borne out by these pot experiments, the plants of both the first and second crops grown in soils receiving sugar and incubated at 30° being not only heavier but also richer in nitrogen than those in the other two sets. The general results suffice to show the importance of supplying the source of energy when the soil temperature is favourable for the growth of *Azotobacter* and thus confirm those obtained in the field experiments discussed in the first part of the paper. (Table IV.)

TABLE IV. *Utilisation of Sugar for Nitrogen Fixation (Soil Series).*

Pots and treatment	First crop (Barley)			Second crop (Rye)			Total dry matter in first and second crops (grms.)	Total nitrogen in first and second crops (mgms.)
	Total dry matter (grms.)	Nitrogen in dry matter (per cent.)	Total nitrogen in dry matter (mgms.)	Total dry matter (grms.)	Nitrogen in dry matter (per cent.)	Total nitrogen in dry matter (mgms.)		
Soil alone	7.74	1.233	95.43	13.18	1.226	161.58	20.92	257
Soil + sugar (high temperature)	13.85	1.443	199.85	12.67	1.957	247.9	26.52	448
Soil + sugar (low temperature)	11.20	1.168	130.81	8.19	1.549	126.8	19.39	258

The rapidity with which the assimilated nitrogen becomes available for plant growth appears to differ with the medium employed. In the case of sand treated with sugar (or hay dust) little effect was apparent in the first crop but was marked in the second crop; with soil, the first crop was benefited most and the second crop was slightly below the control,

although it possessed a much higher nitrogen content. This is possibly due in the first place to the numerical superiority of *Azotobacter* already present in the soil and may also be connected with the effect exerted by traces of humates and of iron compounds on nitrogen fixation noted by other workers.

The gains of nitrogen per gram of substance supplied are given in the following table, from which it is seen that the returns from the use of sugar are somewhat higher than those obtained by the addition of hay dust. On the other hand, it must be recognised that the whole of the carbonaceous compounds in the latter material would probably not be completely oxidised within the course of the experiments. The actual return is consequently greater than would appear from the data given.

TABLE V.
Utilisation of Hay and Sugar for Nitrogen Fixation.

Treatment	Total nitrogen in sand and crop, mgrms.	Gain or loss of nitrogen as compared with control, mgrms.	Nitrogen assimilated per grm. of hay or sugar supplied, mgrms.
Sand alone	470	—	—
Sand + hay (sterilised)	584	114* = - 34	—
Sand + hay + putrefactive organisms	619	149* = + 1	—
Sand + hay + putrefactive organisms + <i>Azotobacter</i>	798	328* = + 180	9.0
Sand + sugar (sterilised)	534	64	2.3
Sand + sugar + <i>Azotobacter</i>	893	423	15.1

* Less 148 mgrms. supplied in hay.

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Nitrates.*

During the course of the work described in the preceding pages, a number of instances occurred in which the possible operation of nitrogen fixation processes was effectively masked by the entrance of changes which were adverse to plant growth. This was evident in the earlier stages of the pot experiments but attained greater prominence in the field work when starch or sugar was applied to the plots in spring, and especially when a minimum interval elapsed between the application and the sowing of the crop. This is seen in the following summary of the yields of plot 4. O.

Relative yields (control = 100)	Time of application				
	March 6th	Feb. 19th	Feb. 4th	Jan. 17th	Sept. (mean)
	nil	71	77	91	137

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Quantitative bacteriological analyses of these soils(30) showed that treatment with starch or sugar leads to very marked increases of the bacterial flora and that under field conditions these increases may persist for upwards of 8-9 months. Further work in the laboratory confirmed these results and also indicated that this increased growth occurs at the expense of the easily available nitrogen compounds of the soil. When equal quantities of sugar are supplied to two portions of soil, the one stored at 10° and the other at 30°, the former not only retains the power for denitrification for a longer period, but also tends to have a higher bacterial content—possibly due in part to the absence of competition by nitrogen fixing organisms at this temperature. This is illustrated in the following table. The denitrification power was tested by incubating 25 grms. of the respective soils with 50 c.c. of 0.2 per cent. solution of sodium nitrate for 48 hours at 30°.

Treatment	Soil alone	Soil+ 0.2 per cent. sugar 10° C.	Soil+ 0.2 per cent. sugar 30° C.	Soil+1.0 per cent. sugar 30° C.
Bacteria (millions per gm. soil) 3 weeks after treatment	12.5	161.6	24.8	61.5
Nitrate reduction (expressed as mgrm. nitrogen) 3 weeks after treatment ...	nil	10.3	8.2	14.6
Ammonia and nitrate (pts per million dry soil) 3 months after treatment ...	30.4	19.2	28.9	7.2

Thus, under the most favourable conditions for its destruction the presence of 0.2 per cent. sugar confers the power of denitrification on soils, stored at 30°, for more than three weeks, and only after a period of three months does the nitrate content of the soil so treated approach that of the untreated.

The addition of sugar induced similar changes in two other soils, one of which possessed a low, and the other a high, initial nitrate content. Treatment resulted in the production of high bacterial numbers and these were accompanied by a marked reduction in the amount of soil nitrates (equal to 46 parts per million in the case of soil II). Notwithstanding these pronounced changes, the accumulation of nitrates again occurred in less than eight weeks at laboratory temperature and the differences in the total ammonia and nitrate content of the untreated and treated at the end of the experiment were in each case less than the losses which were originally occasioned by the addition of sugar.

	Bacteria (millions per grm. of soil)					Nitrogen as ammonia and nitrate (pts per million of soil)				
	At start	After 23 days	After 48 days	After 114 days		At start	After 23 days	After 48 days	After 114 days	
Soil I										
Soil alone	12.9	10.3	21.0	—		17	30	28	30	
Soil + sugar	12.9	47.0	51.0	—		17	13	13	20	

	Bacteria (millions per grm. of soil)					Nitrogen as ammonia and nitrate (pts per million of soil)				
	At start	After 6 days	After 62 days	After 101 days	After 215 days	At start	After 6 days	After 62 days	After 101 days	After 215 days
Soil II										
Soil alone	8.2	13.2	6.9	10.6	12.4	55	51	71	85	98
Soil + sugar	8.2	129.2	96.6	32.3	25.9	55	5	24	37	71

The addition of hay dust to the soil has a like effect. Two soils were tested, the first being an untreated soil, and the second, a tolued soil which had been stored in the laboratory for some months prior to treatment with hay dust. The results are summarised below:

	Bacteria (millions per grm. of dry soil)			Nitrogen as ammonia and nitrate (pts per million of soil)		
	At start	After 7 days	After 74 days	At start	After 7 days	After 74 days
Soil I: soil alone	7	4	12	27	25	27
soil + 0.5 % hay dust	7	94	62	27	13	12
Soil II: soil alone	31	41	38	43	67	58
soil + 0.5 % hay dust	31	175	136	43	23	20

It cannot be assumed from these data, however, that the soil actually loses nitrogen to the extent represented by the disappearance of nitrates after treatment. Many organisms are known to have the capacity of assimilating nitrogen in the form of nitrate and of elaborating it into protein; even under the conditions most favourable for true denitrification (that is, the liberation of gaseous nitrogen) upwards of 25-30 per cent. of the total nitric nitrogen can frequently be recovered as bacterial protein. Nitrate disappearance does not therefore provide a true index of actual soil losses, and it is in fact reasonable to suppose that under conditions where the soil is otherwise likely to lose the greater part of its soluble nitrogen compounds by excessive leaching, such a retention of nitrogen in the organic form may be more or less of an asset.

Finally, sufficient evidence has been advanced to establish a close relation between the results obtained by the addition of hay dust and sugar to laboratory and field soils, and those obtained by Wagner⁽³¹⁾, Maercker⁽³²⁾ and others when simultaneous applications of nitrate of soda and farmyard manure, straw, etc., were made to the soil. Although the conditions which prevailed in their experiments were decidedly abnormal

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and the results have for this reason been widely discounted, it still appears to be fairly evident from the field experiments described above that soil nitrates may be definitely and adversely affected when appreciable quantities of sugar and starch, and therefore of straw, are applied to field soils in spring. The experiments cited by Warington (33), in which it was shown that an annual application of straw at the rate of 2000 lbs. per acre to some of the Rothamsted plots did not result in any definite decrease of crop, cannot be regarded as indicating the complete absence of any adverse changes, but rather that under certain conditions an equilibrium may obtain between destructive and constructive processes.

SUMMARY.

The foregoing experiments give definite evidence, corroborative of the work of Koch, Remy and others, that the nitrogen content of sand or soil may be appreciably increased by the activity of *Azotobacter* when some suitable source of energy is supplied. For this purpose sugars such as dextrose and saccharose are suitable, but distinct gains have also been obtained by the use of plant residues. In laboratory experiments an increment of upwards of 6 mgrm. of nitrogen per gram of plant residues occurred, but in pot experiments gains of 9 mgrm. per gram of substance were obtained.

It is also shown that on the field scale, and in spite of the entrance of complicating factors, definite increases of crop (equal to 20–54 per cent.) resulted from the application of carbonaceous compounds (sugar) when the soil conditions were favourable. Since the difference between the action of sugar and plant residues is largely one of degree and not of type, it is reasonable to suppose that such substances as stubble, leaves, and other complex organic materials may also serve to contribute indirectly to the reserves of soil nitrogen.

The general soil conditions making for the successful operation of nitrogen fixation processes are, in addition to the supply of some source of energy, a suitable temperature, the presence of phosphates and a supply of basic material such as calcium carbonate. Even under the most favourable circumstances for nitrogen fixation, there occurs a period during which adverse processes come into play, and it is not advisable that a crop be introduced before these have run to completion.

Under unfavourable conditions and particularly during periods of low temperature, these adverse changes may persist without any subsequent entrance of soil gains.

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(Received February 15th, 1918.)