NITROGEN FIXATION IN LEGUMINOUS PLANTS. V.

GAINS OF NITROGEN BY MEDICAGO AND TRIFOLIUM IN ACID AND ALKALINE SOIL.

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Introduction.

It was shown in a previous paper (Jensen, 1943) that the reaction of the growth medium (sand or soil) influences the rate of nitrogen fixation in leguminous plants partly through its effect on the ability of the nodule bacteria to infect the plant roots, and partly through its effect on the activity of the nodules subsequently formed. The uptake of nitrogen per unit of dry nodule-substance was as a rule smaller at acid than at alkaline reaction, although the total yields of nitrogen were not always significantly different. The quantities of nitrogen that might have been taken from the medium or excreted into it from the roots were not taken into account. Another series of experiments has therefore been added, in which it has been attempted to give as accurate a balance-sheet of nitrogen as possible by analysis of the seed, the crop, and the growth medium which was represented by natural soil. Compared with pure sand, the use of natural soil has the advantage of showing the influence of reaction on the two separate sources from which leguminous plants normally derive their nitrogen supply under natural conditions—namely, on the process of nitrogen fixation in the root nodules and on the assimilation of combined nitrogen from the soil. Owing to its greater absorptive capacity, the natural soil is also likely to be more favourable for excretion of fixed nitrogen from the legume root systems, a process which under Australian climatic conditions does not normally take place in sand media, as shown by Trumble *et al.* (1937) and Swaby.* On the other hand the method of analysing the soil before and after plant growth is very laborious and has been little used since the earliest investigations on symbiotic nitrogen fixation; experiments of this kind have been described by Albrecht (1920), Brown and Stallings (1921), Bjälfve (1935), and Engel and Roberg (1938), but only in one instance (Fred and Graul, 1916) are the experiments seen to involve modification of the soil reaction, and this was not expressed in terms of hydrogen ion concentration. Several other investigators have determined the amounts of nitrogen left in sand media by leguminous crops, but systematic experiments on the effect of reaction in this respect do not appear to have been made. Ludwig and Allison (1940) found no excretion of nitrogen from pea plants in sand of pH 6·4 to 7·7.

METHODS.

Most experiments were done on lucerne (*medicago sativa*), and subterranean clover (*Trifolium subterraneum*), a few also on annual medics (*M. tribuloides* and *M. orbicularis*) and red clover (*T. pratense*). The culture vessels were mostly small glass jars of 4 in. diameter or wide-necked reagent bottles, holding 500 to 700 gm. of soil, but in one series glazed earthenware pots of 6 in. diameter were used. Admittedly the very small vessels provide conditions that do not come very near to the natural, but it was deemed advisable to accept this disadvantage in exchange for the great reduction in sampling error attached to the nitrogen determinations, which results from working with small portions of soil. The soils were used in a very finely ground condition and mixed with sand passing a 30-mesh sieve. Separate portions of soil and sand were weighed out for each vessel, and the mixtures were moistened to approximately 75% of the water-holding capacity. Only seeds uniform in appearance were sown, after inoculation with effective strains of the corresponding nodule bacteria. After sowing, the soil surface was covered with a mulch of clean, coarse gravel, and the sides of the

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glass vessels were protected from light by a wrapping of thick brown paper. During growth, the plants were kept in a greenhouse and watered with distilled water. At harvesting, the plant tops were cut off immediately above the soil surface, dried and weighed, the roots and the soil were separated as completely as possible, and nitrogen was determined by the Kjeldahl method with selenium as a catalyst. The roots were digested *in toto*, and the tops likewise if the amount of material was small; otherwise they were finely ground and aliquot samples taken. Soil was digested with equal parts of water and sulphuric acid, and with addition of reduced iron for reduction of any nitrate present. At least six replicate determinations were made on 10 to 25 gm. of soil, depending on the nitrogen content; care was taken to weigh out the soil for analysis in a moist condition in order to avoid segregation, and digestion was continued for three hours after the acid had become colourless. N/28 H_2SO_4 and NaOH were used for the titrations, with methyl red plus methylene blue as indicator. The standard error of the mean N-content could in all except a few cases be kept well below 1%. Nitrate and ammonia were determined by the method of Richardson (1938).

Experimental.

Experiment No. 1.—Lucerne and subterranean clover were grown in a soil of pH 5·4 and of moderate nitrogen content (769 p.p.m.), composed of three parts of sand mixed with one part of an acid loam very rich in humus and containing approximately 0·30% nitrogen; 0·05% $CaHPO_4$ and 0·01% KH_2PO_4 were added as fertilizer, and 0·5% $CaCO_3$ was added to produce alkaline reaction. Small glass jars, each holding 600 gm. of dry soil and six plants, were used as culture vessels. Sowing took place on 14th August, 1941; lucerne was harvested after 91 and clover after 85 days. Both plants grew well and did not at any stage show visible differences according to soil reaction. Table 1 shows the nitrogen balance of crop and soil. The nitrogen content of soil is in this as in the following tables expressed on the basis of oven-dry soil.

Table 1.

Nitrogen Fixation by Lucerne and Subterranean Clover in Acid and Alkaline Soil.

	Initial.	Final.			
		$-CaCO_3$.		$+CaCO_3$.	
		a.	*b.*	*a.*	*b.*
1. Lucerne.—pH of soil	5·4	5·3	5·5	7·6	7·6
N in soil, mgm.	451	442	437	436	426
,, ,, seed, mgm.	1·4	—	—	—	—
,, ,, plant tops, mgm.	—	44·9	39·2	59·7	53·6
,, ,, ,, roots, mgm.	—	18·2	12·6	24·8	20·1
,, total, mgm.	452·4	505·1	488·8	520·5	499·7
Net gain of N, mgm.	—	52·7	36·4	68·1	43·7
N taken from soil, mgm.	—	9±3·6	14±4·9	15±4·8	25±5·4
2. Clover.—pH of soil	5·4	5·5	5·5	7·5	7·5
N in soil, mgm.	451	449	437	428	425
,, ,, seed, mgm.	5·4	—	—	—	—
,, ,, plant tops, mgm.	—	69·3	79·1	62·8	66·3
,, ,, ,, roots, mgm.	—	13·1	14·6	11·7	11·1
,, total, mgm.	456·4	531·4	530·7	502·5	502·4
Net gain of N, mgm.	—	75·0	74·3	46·1	46·0
N taken from soil, mgm.	—	(2±3·0)	14±3·9	23±3·1	26±2·7
Production of NO_3—N in soil after removal of plants, p.p.m.—					
After lucerne	—	12·6	11·7	19·8	20·3
After clover	—	12·2	12·6	23·7	21·3

The total nitrogen content of lucerne plants (tops plus roots) is only slightly higher at alkaline reaction, and this is accompanied by a somewhat larger uptake of combined nitrogen from the alkaline soil, so that the net gains of nitrogen at pH 5·3–5·5 and pH 7·6 are not significantly different; unfortunately, the agreement between the duplicate jars is not very good.

The corresponding figures for clover show quite a different picture. The total nitrogen content of the plants is slightly higher at acid reaction, but, as in lucerne, more combined nitrogen has been taken from the alkaline soil; in one of the jars with acid soil the decrease in nitrogen content of the soil is not even significant. As a result, the net gain of nitrogen is, at pH 5·5, more than 50% higher than at pH 7·5. Both plants thus draw more heavily on the soil nitrogen at alkaline reaction. This seems due to the fact that the addition of lime stimulated the mineralization of the humus nitrogen, as shown by a nitrification test on the residues of soil after removal of the plants. Soil samples were adjusted to approximately two-thirds water-holding capacity and incubated for 4 weeks at 30°C. Only traces of ammonia and nitrate were present at the start, and no ammonia accumulated during the incubation, while the production of nitrate was greatly stimulated where lime had been added, but was not significantly different after lucerne and clover (Table 1, bottom).

Experiment No. 2.—The experiment was repeated in bigger pots holding 2·5 kgm. of soil, which contained a higher proportion of sand, giving a nitrogen content of 478 p.p.m.; 0·1% superphosphate and 0·01% KCl were added as fertilizer, and 0·2% $CaCO_3$ was added to change the reaction to neutral. The soil was found to be somewhat more

TABLE 2.

Nitrogen Fixation by Lucerne and Subterranean Clover in Acid and Alkaline Soil of Low Nitrogen Content.

	Initial.	Final.			
		$-CaCO_3$		$+CaCO_3$	
		a.	*b.*	*a.*	*b.*
1. Lucerne.—pH of soil	4·9	5·0	5·1	7·1	7·3
N in soil, mgm.	1189	1118	1115	1079	1088
,, ,, seed, mgm.	4·0	—	—	—	—
,, ,, plant tops, mgm.	—	87·3	94·9	177·6	172·5
,, ,, ,, roots, mgm.	—	48·2	62·2	120·6	108·0
,, total, mgm.	1193·0	1253·5	1272·1	1377·2	1368·5
Net gain of N, mgm.	—	60·5	79·1	184·2	175·5
N taken from soil, mgm.	—	71±4·8	74±5·5	110±3·7	101±3·2
2. Clover.—pH of soil	4·9	5·1	5·1	7·2	7·5
N in soil, mgm.	1189	1106	1111	1082	1098
,, ,, seed, mgm.	18·0	—	—	—	—
,, ,, plant tops, mgm.	—	197·5	186·7	192·3	182·1
,, ,, ,, roots, mgm.	—	53·2	51·4	45·6	55 2
,, total, mgm.	1207·0	1356·7	1349·1	1319·9	1335·3
Net gain of N, mgm.	—	149·7	142·1	112·9	128·3
N taken from soil, mgm.	—	83±4·7	78±6·3	107±5·5	91±4·4
3. Wheat.—pH of soil	4·9	5·2		7·2	
N in soil, mgm.	1189	1115		1080	
,, ,, seed, mgm.	6	—		—	
,, ,, plants, mgm.	—	60		71	
,, total, mgm.	1195	1175		1151	
Net loss of N, mgm.	—	20±5·9		44±6·4	

acid than in the previous experiment (pH 4·9). One additional pot with acid and one with neutral soil were sown with wheat to serve as controls on possible non-symbiotic nitrogen fixation. Lucerne was sown on 30th April, clover on 4th May, and wheat on 1st May, 1942; the plants were harvested after 121, 102, and 134 days, respectively. Each pot carried twelve plants of lucerne, six of clover, and eight of wheat. In this experiment the growth of lucerne was definitely better in the neutral soil, while the clover, as before, showed no visible difference. The wheat grew poorly, showed signs of nitrogen starvation, and suffered badly from rust in the neutral soil. When the roots were collected, the nodules on clover appeared similar at both reactions, while those on lucerne in acid soil were fewer and bigger than in neutral soil, and largely of the branched or multiple type, as observed before (Jensen, 1943). Table 2 shows the analytical data.

The lucerne plants in acid soil contain only about half as much nitrogen as at neutral reaction, and the net gain of nitrogen is, at pH 5·0–5·1, only some 40% of what it is at pH 7·1–7·3, although the soil nitrogen is again utilized more strongly at neutral reaction. By comparison with the previous experiment, it appears that the interval from pH 4·9–5·1 to pH 5·4–5·5 represents the range where the acidity begins to exert a marked depressing influence on nitrogen fixation in lucerne. This agrees with the fact that the same range of pH was found critical for nodule formation on lucerne in agar culture (Jensen, 1943), and that Olsen (1925) observed a big increase in yield of lucerne when the soil pH was raised from 5 to 6. Olsen's results, however, were obtained from soil of high humus content and supplied with nitrate, so that we can form no idea of the actual gains of nitrogen.

The clover has given the same gross yield of nitrogen at both reactions, and the larger uptake of nitrogen from the neutral soil results, as in the previous experiment, in a somewhat (roughly 20%) higher net gain of nitrogen at pH 4·9–5·1.

The pots with wheat give no indication of any non-symbiotic nitrogen fixation; on the contrary there is a small but significant net loss of nitrogen. The cause of this loss seems uncertain, but might possibly consist in the occurrence of denitrification before the plants had reached sufficient development to use up the NO_3–N present at the start (5·8 p.p.m., or 14·5 mgm. per pot) and produced during the early stages of the experiment (cf., Engel and Roberg (1938), who in unplanted soil in a pot experiment observed a small loss of nitrogen, which they ascribed to denitrification). The question whether such a loss of nitrogen may also have occurred in the soil under the leguminous plants, and whether this may not be the real cause of the stronger decrease in nitrogen content of neutral or alkaline soil, is of course impossible to answer on the basis of the available data. It would seem that experiments involving the use of isotropic nitrogen as a tracer-element would be needed to supply the final answer.

Experiment No. 3.—This included the two annual medics and red clover. The plants were grown in a mixture of soil and sand similar to Exp. No. 1, with addition of 0·1% superphosphate and 0·005% KH_2PO_4, besides 0·4% $CaCO_3$ to give alkaline reaction. The nitrogen content was 744 p.p.m., but the reaction was found more acid than in the first experiment (initial pH 4·6). The culture vessels were wide-necked reagent bottles of 500 c.c. capacity, each holding 4 plants and 540 gm. of soil. *Medicago tribuloides* was sown on 23rd February, *M. orbicularis* on 24th February, clover on 20th February, 1942, and the plants were harvested after 73, 86, and 115 days, respectively. The two medics, but particularly *M. tribuloides*, grew poorly in the acid soil, where the red clover on the other hand showed better growth than in the alkaline.

The analytical data in Table 3 show that the two medics, in spite of their scanty growth, had fixed certain amounts of nitrogen in this strongly acid soil, but the net gains of nitrogen at the final pH of 5·0–5·2 reach only one-fifth to one-fourth of the gains at pH 7·1–7·4. As in the previous experiments, more nitrogen is taken from the alkaline soil. The red clover shows a surprising result: the total nitrogen content of the plants is about twice, and the net gain nearly three times, as high at pH 4·9 as at pH 7·3–7·4. Although the result suggests a definite preference of red clover for acid soil reaction, one must necessarily hesitate to accept this as indicating anything like a general rule.

TABLE 3.

Nitrogen Fixation by Annual Medics and Red Clover in Acid and Alkaline Soil.

	Initial.	Final.			
		$-CaCO_3$		$+CaCO_3$	
		a.	*b.*	*a.*	*b.*
1. *Medicago tribuloides*.—pH of soil	4·6	5·0	5·2	7·2	7·2
N in soil, mgm.	398	390	392	386	384
,, ,, seed, mgm.	1·6	—	—	—	—
,, ,, plant tops, mgm.	—	13·1	13·0	34·0	36·4
,, ,, ,, roots, mgm.	—	3·0	1·7	5·4	6·0
,, total, mgm.	399·6	406·1	406·7	425·4	426·4
Net gain of N, mgm.	—	6·5	7·1	25·8	26·8
N taken from soil, mgm.	—	8±2·0	6±2·2	12±2·2	14±1·9
2. *Medicago orbicularis*.—pH of soil	4·6	5·1	5·0	7·3	7·4
N in soil, mgm.	398	386	392	385	379
,, ,, seed, mgm.	1·2	—	—	—	—
,, ,, plant tops, mgm.	—	26·7	14·2	64·6	60·9
,, ,, ,, roots, mgm.	—	3·6	1·3	12·0	13·6
,, total, mgm.	399·2	416·3	407·5	461·6	453·5
Net gain of N, mgm.	—	17·1	8·2	62·4	54·3
N taken from soil, mgm.	—	12±5·0	(6±3·7)	13±3·6	19±3·5
3. *Trifolium pratense*.—pH of soil	4·6	4·9	4·9	7·3	7·4
N in soil, mgm.	398	392	386	387	382
,, ,, seed, mgm.	1·0	—	—	—	—
,, ,, plant tops, mgm.	—	49·2	34·0	23·0	23·4
,, ,, ,, roots, mgm.	—	12·7	11·3	4·8	5·4
,, total, mgm.	399·0	453·9	431·3	414·8	410·8
Net gain of N, mgm.	—	54·9	32·3	15·8	11·8
N taken from soil, mgm.	—	(6±3·0)	12±3·5	11±3·9	16±3·0

This experiment also included jars sown with *Medicago minima* which made only a slow, and in the acid soil an extremely poor, growth. Nitrogen in the soil was not determined, but analysis of plants 20 weeks old showed the following contents of nitrogen:

	$-CaCO_3$		$+CaCO_3$	
	a.	*b.*	*a.*	*b.*
Final pH of soil	5·1	5·1	7·4	7·4
N in tops, mgm.	8·9	5·1	44·6	43·2
,, ,, roots, mgm.	0·6	0·5	5·4	6·9

M. minima thus seems even more sensitive to acidity than the two other annual medics, a property which was also suggested by tests on nodule formation in agar culture (Jensen, 1943).

Experiment No. 4.—Lucerne and subterranean clover were grown, at two different times, in soil of extremely low nitrogen content (122 p.p.m.), consisting of a fine sandy loam very poor in humus and mixed with sand in the ratio of two parts of soil to five parts of sand; 0·2% $CaCO_3$ was added to give neutral reaction. Lucerne was sown on 25th June, 1942, in 4 in. glass jars holding 6 plants and 700 gm. of soil with addition of 0·1% superphosphate, and harvested after 90 days, when the plants in the acid soil appeared considerably smaller than those in the neutral soil, and carried fewer and bigger root nodules. Clover was grown in somewhat smaller jars holding only two plants and 450 gm. of soil with 0·02% KH_2PO_4 and 0·02% $CaCl_2$. The plants were sown on 12th April, 1943, and harvested after 144 days; the root nodules were collected, weighed and analysed separately. There were no visible differences in the general appearance of

the plants or the type of nodulation in acid and neutral soil. Table 4 shows the analytical data.

TABLE 4.

Nitrogen Fixation by Lucerne and Subterranean Clover in Acid and Neutral Soil of Very Low Nitrogen Content.

	Initial.	Final. $-CaCO_3$ *a.*	Final. $-CaCO_3$ *b.*	Final. $+CaCO_3$ *a.*	Final. $+CaCO_3$ *b.*
1. Lucerne.—pH of soil	5·1	5·2	5·4	7·2	7·1
N in soil, mgm.	86·0	84·2	84·7	83·4	85·3
,, ,, seed, mgm.	1·4	—	—	—	—
,, ,, plant tops, mgm.	—	25·1	24·0	44·3	42·0
,, ,, ,, roots, mgm.	—	10·0	9·5	20·3	23·1
,, total, mgm.	87·4	119·3	118·2	148·0	150·4
Net gain of N, mgm.	—	31·9	30·8	60·6	63·0
N taken from soil, mgm.	—	1·8±0·4	1·3±0·4	2·6±0·4	(0·7±0·7)
2. Clover.—pH of soil	5·0	4·7	4·8	7·0	7·1
N in soil, mgm.	54·4	59·4	64·8	58·0	61·2
,, ,, seed, mgm.	2·2	—	—	—	—
,, ,, plant tops, mgm.	—	72·3	64·9	70·1	82·6
,, ,, ,, roots, mgm.	—	9·6	9·9	8·0	12·1
,, ,, ,, nodules, mgm.	—	9·3	7·9	3·3	3·1
,, total, mgm.	56·6	150·6	147·5	139·4	159·0
Net gain of N, mgm.	—	94·0	90·9	82·8	102·4
Gain of N to soil, mgm.	—	5·0±0·8	10·4±1·1	3·6±0·7	6·8±0·7
Dry weight of tops, gm.	—	2·317	1·921	2·149	2·221
,, ,, ,, roots, gm.	—	0·342	0·342	0·279	0·427
,, ,, ,, nodules, gm.	—	0·135	0·123	0·053	0·041
Net gain of N per gm. of dry nodule-substance	—	696	749	1562	2497

As in Experiment No. 2, the nitrogen content of lucerne at pH 5·1–5·4 is very nearly one-half of that at pH 7·1–7·2, but almost all of it represents net gain at both reactions, because the uptake of nitrogen from the soil is very small, and in one of the jars not even significant, although the soil originally contained 4·2 p.p.m., or 2·9 mgm. per jar, of $(NO_3 + NH_4)N$. The yield of nitrogen by subterranean clover is the same at acid and neutral reaction, and the nitrogen content of the soil has actually increased during the growth of the plants. It is difficult to say whether this increase is due to active excretion of fixed nitrogen, to decayed nodules and root fragments, or to both sources. The second cause might seem probable in view of the long growth period and the fact that many authors (Bjälfve, 1935; Trumble *et al.*, 1937; Nowotnowna, 1937; Bond, 1938, 1941; Bond and Boyes, 1939; Scholz, 1939; Wyss and Wilson, 1941) have after growth of leguminous plants in sand medium observed small increases in the nitrogen content of the sand; these increases were ascribed to detached fragments of the root systems, which are virtually impossible to separate from the medium. On the other hand the increases in soil nitrogen observed in the present instance are of an order of one-third to one-half of all the nitrogen in roots plus nodules, which seems rather more than one would expect from this source, particularly as the root systems looked healthy and showed no signs of decay. We can thus hardly go further than to say that in this single experiment it is possible but by no means certain that a small proportion, not exceeding 10%, of the fixed nitrogen has been excreted from the roots. The fact that no significant decrease in the nitrogen content of the soil took place in

one of the lucerne jars as well as in a few previous cases (Tables 1 and 3), although the medium originally contained certain amounts of nitrate which had disappeared at harvesting, might well be due to small root fragments being left behind in the soil.

The weight of the clover nodules, both absolutely and as percentage of total dry weight, is considerably smaller in neutral soil, as also observed before (Jensen, 1943); this is due to a smaller average size of individual nodules, since the numbers of nodules per plant were not significantly different in acid and alkaline soil. The efficiency of the nodules, expressed as net gain of nitrogen per unit weight of nodule substance, is seen to be two to three times higher at pH 7·0–7·1 than at pH 4·7–4·8.

The following percentages of nitrogen in dry matter of plant tops were found in the different experiments:

		Acid Soils. (pH 4·7–5·5)	Alkaline Soils. (pH 7·0–7·7)
Medicago sativa	(Exp. 1)	3·39–3·44	3·32–3·59
,, ,,	(,, 2)	3·28–3·28	3·64–3·77
,, ,,	(,, 4)	3·50–3·70	4·10–4·24
M. tribuloides	(,, 3)	2·19–3·36	3·32–3·39
M. orbicularis	(,, 3)	3·51–3·64	3·67–3·76
M. minima	(,, 3)	2·92–3·07	3·20–3·22
Trifolium subterraneum	(,, 1)	3·28–3·38	3·28–3·28
,, ,,	(,, 2)	3·21–3·21	3·17–3·17
,, ,,	(,, 4)	3·12–3·38	3·26–3·77
T. pratense	(,, 3)	3·11–3·43	3·67–3·80

The nitrogen content is thus upon the whole somewhat lower at acid reaction, but this effect is neither constant nor very marked, such as found by Virtanen (1928) in peas and clovers grown in artificially acidified sand.

Discussion.

A striking feature of the preceding experimental results is the remarkable difference shown to exist between *Medicago* and *Trifolium* in respect of tolerance towards acid soil reaction. When adequate supply of calcium and phosphate is provided, the net gain of nitrogen by subterranean clover in soil as acid as pH 5 does not seem to be improved by liming to pH 7·0–7·5, or the gain may actually decrease owing to stronger utilization of soil nitrogen. Lucerne also continues to fix nitrogen at pH 5 and less, but the gain is strongly increased by liming, although the results in Table 1 suggest that considerable latitude exists and that at least a moderately acid reaction is not harmful.

The commonly held view that symbiotic nitrogen fixation proceeds most vigorously at approximately neutral soil reaction thus seems to hold fairly well for *Medicago*, but not for *Trifolium*. Such a difference was also observed in earlier experiments by Fred and Graul (1916), who grew leguminous plants in two acid soils (pH not determined), with and without inoculation of the seed with nodule bacteria and with addition of calcium carbonate to half and full saturation of the lime requirement as determined by the method of Veitch (titration with lime water). Lucerne and red clover showed in the inoculated series the following net gains of nitrogen in mgm. per pot:

Plant.	Lime Addition.	Soil No. 1. (0·198% N).	Soil No. 2. (0·09% N).
Lucerne	None.	2,293	497
,, ...	Half dose.	1,929	2,792
,, ...	Full dose.	2,127	2,081
Clover	None.	2,306	3,916
,, ...	Half dose.	2,514	4,217
,, ...	Full dose.	2,179	4,213

It is further seen that in Experiments Nos. 1–3, which represent soils of normal although rather low nitrogen content, the final quantity of nitrogen in soil plus plant roots is frequently less than the initial nitrogen content of soil plus seed, as also found by Fred and Graul (1916), but in Experiment No. 4 the nitrogen in the root systems alone represents a considerable gain to the soil originally very poor in nitrogen. This also agrees with the results of Bjälfve (1935) who found that a pea crop assimilated

large amounts of combined nitrogen from a fertile loam soil with 0·132% nitrogen, whereas a vetch crop increased the nitrogen content of a sand soil comparable to the one used in the present Experiment No. 4 (N-content originally 81·0 p.p.m., after vetches 101·5 p.p.m.). Soils of such low nitrogen content are not likely to be met with in normal agricultural practice, but such cases might be represented by land denuded through soil erosion. If a stand of legumes were established under such conditions, we might expect considerable benefits to the nitrogen content of the exhausted soil even if all top material were removed by cropping or grazing, a rule which does not necessarily apply to soils with a more normal nitrogen supply.

Summary.

Species of *Medicago* and *Trifolium*, chiefly lucerne and subterranean clover, were grown under greenhouse conditions in soils of pH 4·6 to 5·4, in which the reaction was altered to neutral or faintly alkaline by addition of calcium carbonate.

Determination of nitrogen in seed and crop, and in soil before and after plant growth, showed a marked difference between the two genera. Lucerne fixed at pH 7·0–7·3 roughly twice as much nitrogen as at pH 4·9–5·2; in one experiment there was no significant difference in gains of nitrogen at pH 5·3–5·5 and pH 7·6. *Medicago tribuloides* and *M. orbicularis* were strongly inhibited at acid reaction, but could still fix small amounts of nitrogen at pH 4·6–5·1. Subterranean clover on the other hand fixed the same amount of nitrogen in acid and neutral to alkaline soil, or the net gain was actually smaller in the latter case, when the plants derived a higher proportion of their nitrogen from the soil. Nitrogen fixation in red clover seemed in a single test to be actually favoured by acid soil reaction.

The uptake of combined nitrogen from soil of moderate nitrogen content (0·048–0·077%) was increased by the addition of lime which stimulated the production of nitrate from the soil humus. Only one experiment with subterranean clover in soil of extremely low nitrogen content (0·012%) showed some evidence, but not convincingly, that some combined nitrogen had been excreted by the root systems.

A control experiment with soil carrying wheat plants showed no evidence of non-symbiotic nitrogen fixation, but on the contrary a small net loss of nitrogen.

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