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SOME SOIL FACTORS IN NITROGEN FIXATION BY LEGUMES*

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BY MEANS of a plant medium of colloidal clay[3] on which various nutrient cations and anions are adsorbed, it is now possible to put the soil phase of nitrogen fixation by legumes under chemical control as accurate as that possible by the water culture methods for plants, or the agar cultures for isolated bacteria. Rather than establish our ideas about nitrogen fixation by piecing together the information gained by each of these performers in isolation, we can now interpret nitrogen fixation by observing the soil, the plant, and the bacteria, all performing cooperatively under accurate chemical control. Though colloidal clay mixed into quartz sand is not truly a soil, yet its simulation of such is so close as to help us evaluate more exactly the significance, in the process of nitrogen fixation, of (a) the soil texture, (b) the pH, or degree of acidity, (c) the degree of calcium saturation of the clay, (d) the fertility in terms of organic matter content, (e) the phosphorus mobility, (f) the exchangeable magnesium content, and (g) the exchangeable potassium content of the soil.

SOILS OF "HEAVY" TEXTURE GIVE INCREASED NITROGEN FIXATION

It has been observed often that legumes may be growing successfully on a heavy clay soil the degree of acidity of which is such as would cause their failure in most soils. Experiments with colloidal clay media show that increasing amounts of clay at certain degrees of calcium saturation give improved nitrogen fixation and better growth by the legume.[1] When electrodialyzed colloidal clay that was originally at pH 3.6 and was titrated by calcium hydroxide so as to give a range in pH from 4.0 to 6.5 by intervals of 0.5, was added in increasing amounts to a constant quantity of sand, the growth of soybeans showed improvement. Such growth improvement corresponding with more clay, or with a heavier soil texture, occurred for the clays of different acidities when these were pH figures above 4.0. At this

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latter degree of acidity, very little growth occurred. These results point to the clay fraction as a significant soil factor in the process of nitrogen fixation.

The efficiency with which the soybean plants, for example, can withdraw the calcium from the exchangeable supply in the calcium-saturated colloidal clay was determined. Plants given increasing amounts of such clay in sand were analyzed,[4] and their nitrogen fixation was determined as related to the efficiency with which they consumed the exchangeable calcium from the clay when they were restricted to other nutrients supplied only in the seed. The results given in figure 1 point out that even with an ample but

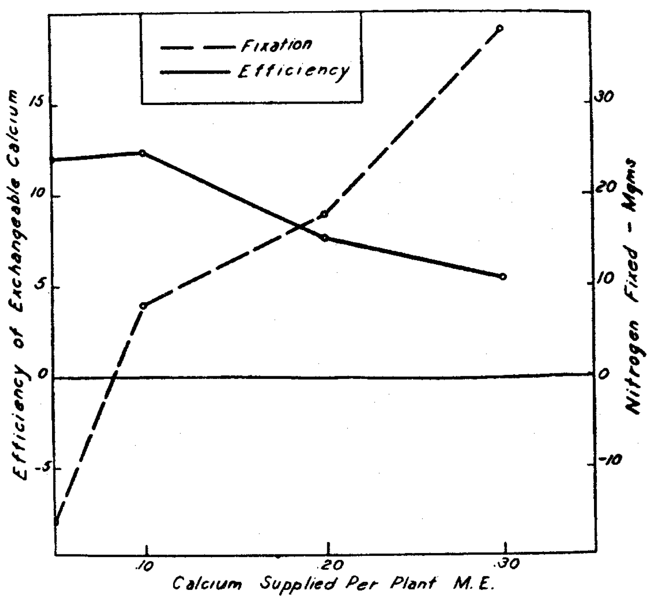

FIG. 1. Nitrogen fixation as related to efficient use of exchangeable calcium with increasing clay content of soil.

constant total supply, the maximum efficiency of such calcium use was about 12.5 per cent. This efficiency was lower as the fixation was higher, and as a result, with the most effective nitrogen fixation only about 6.5 per cent of the exchangeable calcium supply was taken, or served the plant.

For these legumes the exchangeable calcium would not be available *in toto* but only to about one-sixteenth during these 5 to 6 weeks of growth. This suggests that before we can use chemical measures of the exchangeable nutrient supplies in the soil as indexes of fertility, it is necessary to determine the efficiency with which these nutrients in exchangeable form serve the different crops.

THE DEGREE OF ACIDITY, OR pH OF THE SOIL, AND NITROGEN FIXATION

The foregoing studies served to emphasize the significance of the degree of soil acidity and the nature of the colloidal clay complex as factors in legume growth and nitrogen fixation. In the first case cited, nitrogen fixation during a 6-week period of growth failed at all pH figures below 5.5, even with increasing amounts of clay in which the total absorbed, or exchangeable, calcium was no greater than 0.2 m.e., or 4 mgm., per plant. Nitrogen fixation occurred, however, and was improved with increasing amounts of clay—and therefore of calcium—at pH figures of 5.5 and higher. With the increasing nitrogen fixation there were larger amounts of calcium in the crop as taken from the clay. These were larger both as the amount of clay increased and as the pH figure increased.

There is an inclination to give emphasis to the relation between the pH figure and the process of nitrogen fixation. The plant growth and this fixation process were influenced more, however, by the amount of clay—and therefore of the calcium offered—than by the pH. In fact, doubling the amount of clay improved the process as much as, or more than, a reduction of 1.0 pH, or by ten times in the hydrogen-ion concentration. Thus, since the nature of the clay is such that an increased degree of acidity, or a lower pH figure, represents less calcium, then, in reality, nitrogen fixation is related to acidity, or pH, only as this represents a decreasing supply of calcium as a plant nutrient. This statement holds for the studies with soybean plants, the isoelectric point of which was at approximately pH 5.5. At degrees of acidity less severe than this, the soil calcium moved into the plant for improved growth and nitrogen fixation. At degrees of acidity more severe than pH 5.5, the plants took no calcium from the soil and fixed no nitrogen. On the contrary, they lost bases, or positive ions, from the seed supply to the clay, and a growth resulted with plants of less calcium content than that originally represented by the seed. Thus, it is true that the pH can be so low that no nitrogen fixation occurs, but the calcium deficiency is the true cause and the pH merely a contemporaneously associated variable. This process cannot be expected unless significant increase in the calcium content of the plant is brought about by the delivery of this element to the plant by the soil.

DEGREE OF CALCIUM SATURATION OF THE CLAY AND NITROGEN FIXATION

The nature of the colloidal clay as a complex which can absorb cations—possibly anions—and exchange them with other ions, suggests that the influence of increasing H-ion concentration on nitrogen fixation by legumes may be an effect of the reciprocal, namely, the decreasing degree of calcium saturation, on this Beidellite mineral. A test was made on the influence of the degree of calcium saturation on nitrogen fixation by soybeans. Saturation degrees of 40, 60, 75, 87.5, and 97 per cent of the capacity of the clay for calcium were used when the accompanying ions were hydrogen, giving clays of varying acidity, or when they were magnesium, potassium, or barium, all giving purely neutral clays. Amounts of the clays were taken to give constant amounts of exchangeable calcium per plant.

All of the visible plant characters demonstrated the importance of the degree of calcium saturation, but there was no significant influence of the accompanying ions when these were the non-nutrients, namely, hydrogen and

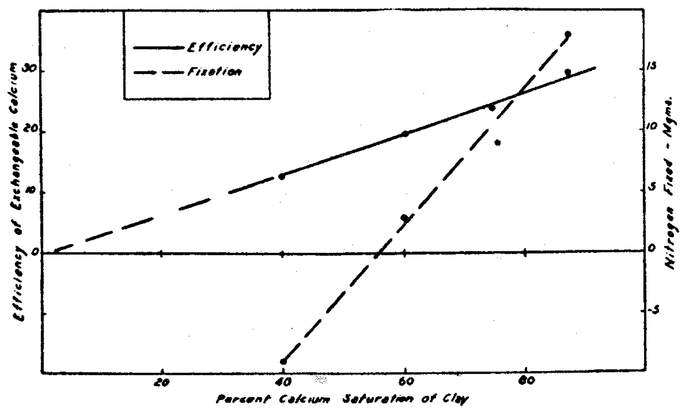

Fig. 2. Nitrogen fixation as related to the efficiency of calcium use at different degrees of calcium saturation of the clay. (H, Ba, Mg, K as reciprocal ions. Mean results of six trials.)

barium. Here again the effectiveness with which the calcium was delivered served as a measure of the plant performance as a nitrogen fixer. Increased degree of saturation gave greater nitrogen fixation, irrespective of the accompanying cation. Along with it went a greater percentage use of the total calcium offered, or a larger supply of exchangeable calcium was delivered to the plant, as shown in figure 2.

A higher efficiency in the use of the calcium was shown in these trials than in the preceding cases. This efficiency was related to the degree of saturation as illustrated by a straight line in figure 2. This line demonstrated a mean ratio of 0.3215 for the degree of calcium saturation to the percentage of utilization, or a tangent of an angle of 17.8°, for the increased efficiency by calcium with increasing degree of clay saturation by it. It shows forcibly that calcium from the same original total supply is delivered into the plants to a much larger extent when it is on a nearly saturated clay than when on one only partly saturated. As an illustration, the 200 mgm. of calcium distributed on enough clay to saturate it to but 40 per cent, delivered only

21.4 mgm. to the plants. When this same total amount was put on less clay so as to saturate it to 97 per cent, the delivery to the plant amounted to 58.2 mgm., or 2.7 times as much.

As the degree of calcium saturation of the clay becomes lower, more total exchangeable calcium must be present in the soil to be equally as effective as that at the higher degrees of saturation. More exchangeable ions can be provided by supplying more clay. As an illustration, let us suppose that the situation in a silt loam with 15 per cent clay is the same as that for 87.5 per cent calcium saturation in these trials where 29.3 per cent of the total exchangeable calcium moved into the plant. Then let us calculate how much clay equivalent would be required to deliver the same amount of effective calcium, and what quantities of exchangeable calcium would be represented in each of the different degrees of calcium saturation and percentage efficiency

TABLE 1

PERCENTAGES OF CLAY REQUIRED IN A SOIL AT DIFFERENT DEGREES OF SATURATION TO DELIVER AS CONSTANT AN AMOUNT OF EFFECTIVE CALCIUM AS WAS FOUND FOR 87.5 PER CENT SATURATION

Degree of saturation	Exchangeable calcium		Effective calcium	Clay required	Clay in soils
	Per cent used	Pounds per acre	Pounds per acre	Pounds per acre	
Per cent					*Per cent*
87.5	29.3	3,412	999.8	300.000	15.00
75	23.8	4,200	999.8	430.769	21.53
60	19.7	5,075	999.8	650.641	32.53
40	12.3	8,128	999.8	1,563.076	78.15

cited. Such data are given in Table 1 for the Beidellite clay of which the total exchange capacity was taken as 65 m.e. per 100 gm. of clay.

Since the effectiveness of exchangeable calcium decreases decidedly as the percentage saturation decreases, the amount of clay demanded to provide constant amounts of effective calcium, according to these studies, rises so rapidly that a silt loam at 87.5 per cent calcium saturation is as effective in providing calcium as is a clay soil with 78 per cent clay at 40 per cent saturation. These figures for efficiency of calcium at different degrees may be far from the actual truth in the field, but they serve, nevertheless, to emphasize the fact that calcium delivery to the plant is much more efficient at the higher degrees of calcium saturation of the clay.

These results showed that growth of the plants occurred at all these degrees of saturation by calcium, but nitrogen fixation became significant only at the higher degrees. Thus, plants may be growing but failing to fix nitrogen because of a deficient degree of saturation rather than because of a deficiency in the total supply of this element in exchangeable form. The results suggest that nutrient additions to the soil might well be applied so as to saturate a limited soil volume, with consequently more efficient use by the plants than when applied through a greater soil volume with correspondingly lower degree of soil saturation. This suggests drilling rather than mixing fertilizers with the soil.

ORGANIC MATTER CONTENT OF THE SOIL AND NITROGEN FIXATION BY LEGUMES

Since it is now recognized that the mineral clay complex of the soil unites chemically with residues from organic matter decomposition,[5] an attempt was

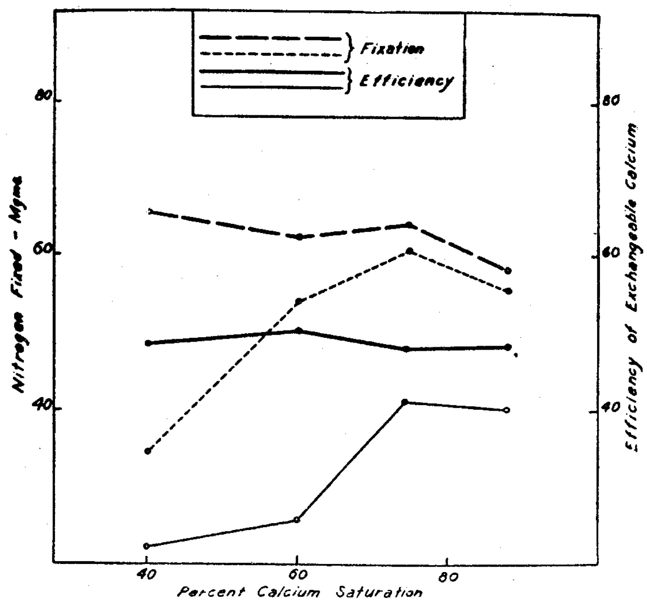

FIG. 3. Nitrogen fixation as related to the degree of calcium saturation when reciprocally accompanied by methylene blue or hydrogen. (Heavy line—methylene blue; light line—hydrogen.)

made to simulate a clay having part of its saturation capacity taken by calcium and part by organic matter. This was brought about by giving the clay variable degrees of calcium saturation as previously cited, while the remainder of its capacity was given methylene blue, an organic compound of great molecular size and weight. Soybeans were grown on this medium, delivering the same total exchangeable calcium as that in the preceding test, and their nitrogen fixation was noted. They were compared with a series using hydrogen as the ion accompanying the calcium, with the results given graphically in Figure 3.

The most noticeable result was the fact that the efficiency of calcium use by the legume was not related to the degree of saturation. Rather, the efficiency was almost a constant (48.5-51.0 per cent, mean 49.4 per cent) for all degrees of saturation. Here with the larger organic cation accompanying the calcium, the efficiency of calcium use was more closely related to the total supply of exchangeable calcium than was the case when less strongly adsorbed ions, H, Ba, Mg, or K, accompanied the calcium. Such results suggest that, probably, when a soil is liberally supplied with partly decomposed organic matter, this may exercise beneficial effects on nitrogen fixation indirectly. These may be exercised through the replacement of the hydrogen on the clay by a very large organic molecule, the high adsorption energy of which holds it so firmly that only calcium and other more mobile cations move from the clay into the plants. Such a view may help in the understanding of why certain degrees of acidity in soils of decidedly higher organic matter content are less disturbing to nitrogen fixation by legumes than are these same degrees of acidity in soils almost devoid of organic matter.

PHOSPHORUS MOBILIZATION AND NITROGEN FIXATION

The phosphate cation may be adsorbed by the colloidal clay, hence the clay medium lends itself to the study of this anion, as well as to cations, as a factor in nitrogen fixation by legumes. The adsorption of the phosphorus is influenced by the adsorbed calcium. Studies of the effect of phosphorus on nitrogen fixation by the colloidal clay medium for soybeans show that the mobility of the phosphorus at low levels and its activity in plant growth and nitrogen fixation are linked with the calcium behavior.

Variation in the phosphorus levels of the colloidal clay were without effect on the nitrogen fixation at low levels of calcium. Unless significant amounts of the latter element were present, the plants failed to metabolize into their tissues even the phosphorus present in the seed. Unless the entire phosphorus content of the seed moved into the plants, the latter failed to fix nitrogen during a growth period of 6 weeks. When ample calcium was applied, the quantity of phosphorus in the seed was enough to give significant nitrogen fixation during this period. Four trials were made with increasing amounts of calcium per plant to note the mobilization of the seed phosphorus and its possible relation to the nitrogen fixation. The mean results of the seed phosphorus metabolized in the plants as related to the calcium content of the crop and to the nitrogen fixation activities are shown graphically in Figure 4.

It is interesting to note that with a mean total of 49 mgm. of phosphorus in the seed at the outset, much of this was returned by the plants to the soil unless 0.2 or more m.e. of calcium per plant was present. Further, significant nitrogen fixation occurred only when the total seed phosphorus was moved into the plant and when none was returned to the soil. Thus, there was a close relation between the phosphorus supplied to the plant and nitro-

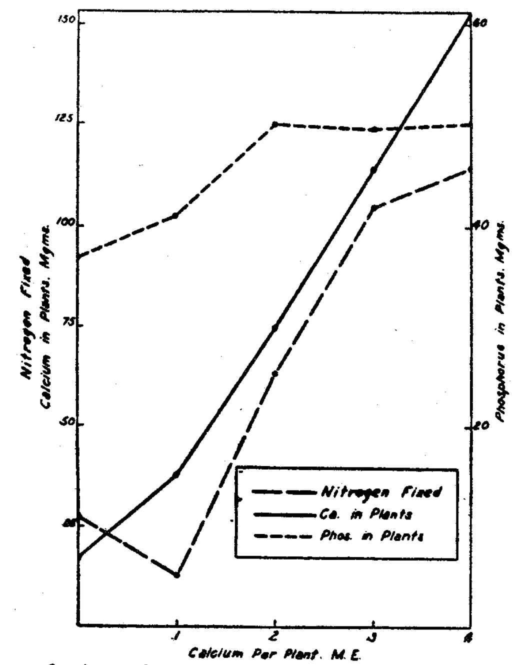

FIG. 4. Nitrogen fixation and seed phosphorus metabolized into soybean plants as related to calcium level provided.

gen fixation. These two, in turn, were related to the amount of calcium supplied.

Chemical studies of legumes in the field[2] point to the increased mobilization of phosphorus from the soil into the crop by the calcium treatments. This increase amounted to as much as trebling the phosphorus taken from the soil. It held true for corn, a nonlegume, as well as for lespedeza, a legume.

Thus, if increased phosphorus is required for improved nitrogen fixation, and this increased phosphorus in the plant is impossible without a significant supply of calcium, then the latter improves nitrogen fixation, in part at least, through its mobilization of phosphorus into the plant.

It seems, then, that the three elements nitrogen, phosphorus, and calcium are closely connected with nitrogen fixation by legumes. The first two might readily be related to nitrogen fixation, since both occur as constituents of proteins. That calcium should be associated with protein production not so much as a constituent of the final product but as an agency in its production is not a common belief. Recent relation of this element in the contraction bands of muscular tissue[6] and its persistent correlation with nitrogen fixation in these studies, suggest that this element may play a role in the production, renewal, or other metabolic phases of protein.

EXCHANGEABLE MAGNESIUM AND NITROGEN FIXATION

When a variable supply of magnesium was used in connection with a constant level of calcium on colloidal clay to determine the significance of mag-

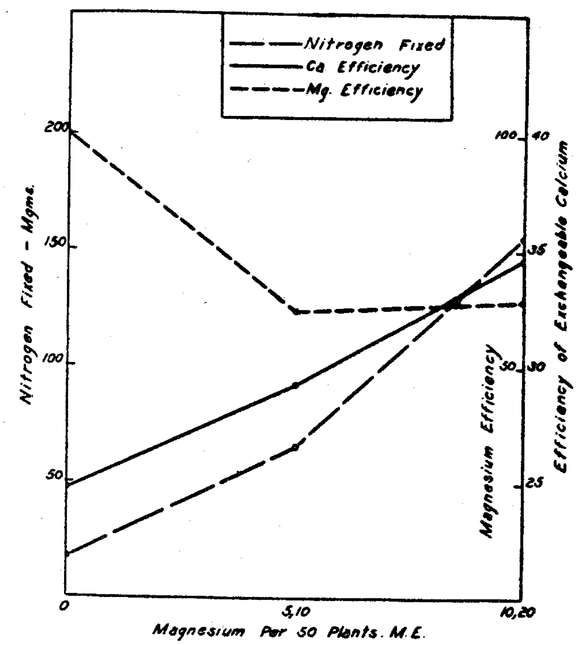

FIG. 5. Nitrogen fixation as influenced by magnesium effects on calcium efficiency.

nesium in nitrogen fixation, this element showed a decided influence on the growth and nitrogen fixation by soybeans, as illustrated graphically in Figure 5. That this influence is not directly related to the amounts of magnesium taken by the plants is indicated by the fact that the efficiency with which the magnesium was used is not related to the nitrogen fixation. The magnesium increase, however, gave an increased efficiency in the absorption, by the plants, of calcium from the constant supply of this element at two different levels. This increased efficiency of calcium paralleled the increased nitrogen fixation. The increased total calcium consumption agreed with the increase in plant growth. Such relationships were not evident between the efficiency with which the magnesium was taken and the nitrogen fixation, or between this latter process and the total or percentage of magnesium in the crop. It would seem more logical, then, to believe that magnesium was instrumental in making the calcium effective in the process of nitrogen fixation, rather than that magnesium is directly active in this performance.

That this seems the proper conclusion is indicated by the fact that when magnesium supplemented the calcium, the efficiency of the latter was increased decidedly. The concentration of calcium in the crop was also lower as this element was more effective in nitrogen fixation. Use of atmospheric nitrogen occurred at a calcium concentration in the plant tissue of 0.45 per cent, a concentration lower than those in previous trials, which were commonly 0.50 to 0.55 per cent. At this lower concentration within the plants, in this case using magnesium, the phosphorus and the potassium from the seed were completely metabolized into the plant at the same total amount of calcium per plant required for this in previous trials giving less total growth. Thus,

TABLE 2

LOWERED NITROGEN CONCENTRATION IN SOYBEANS* AS RELATED TO APPARENT POTASSIUM SUBSTITUTION FOR CALCIUM

Exchangeable cations			Crop weight	Nitrogen		Magnesium		Calcium		Phosphorus		Potassium		K/Ca	Calcium efficiency
Mg	Ca	K													
m.e.	m.e.	m.e.	gm.	per cent	mgm.	per cent	mgm.	per cent	mgm.	per cent	mgm.	per cent	mgm.	per cent	per cent
5	10	0	14.207	2.86	407	.36	52	.74	105	.25	39	1.01	150	1.36	49.5
5	10	5	14.592	2.55	372	.36	54	.32	46	.18	26	1.90	285	5.93	21.2
5	10	10	17.807	2.19	390	.30	55	.27	48	.14	25	2.15	384	7.96	22.1

* Seed content in mgm.: N = 364, Mg = 16.7, Ca = 12.2, P = 39.4, K = 171.

the supplementing of the calcium allotment by magnesium increased the effectiveness of calcium in nitrogen fixation by these legumes. We may, therefore, consider the exchangeable supply of magnesium in the soil as a factor indirectly significant in nitrogen fixation.

VARIABLE POTASSIUM SUPPLIES AND NITROGEN FIXATION

When soybeans were grown on colloidal clay with such constant amounts of calcium and magnesium as were previously found effective, but which were supplemented by variable amounts of exchangeable potassium, an increase in growth followed the potassium increments. The nitrogen fixation within the plant decreased, however, with larger amounts of exchangeable potassium. The percentage and the total amount of calcium also decreased decidedly, while the percentage and the total of potassium increased equally noticeably. The data, as given in Table 2 for these elements, show the failure of the plants to use the calcium, whereas their use of the potassium is a distinct feature. These relations and the decreasing concentration of nitrogen are given graphically in Figure 6.

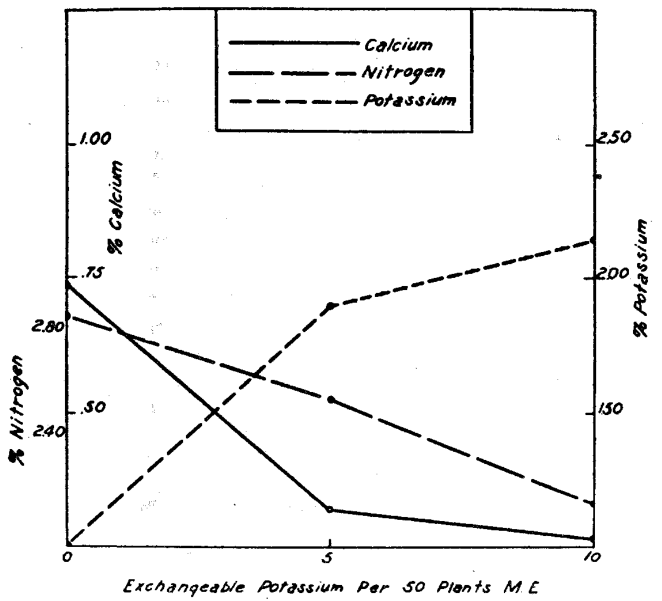

Fig. 6. Reciprocal relation of calcium and potassium concentrations within soybean plants associated with declining nitrogen concentration as the result of increasing potassium provided.

Since the clays were all completely saturated, the introduction of the increasing amounts of potassium along with the constant amounts of calcium and magnesium, lessened the degree of calcium saturation from 66, to 50 and 40 per cent, respectively. That this decreasing saturation is not the sole reason for calcium reduction in the crop is indicated by the use of the calcium at a higher percentage efficiency in these trials than was the case in the previous saturation studies in the absence of magnesium and potassium. It suggests, rather, that the calcium was replaced by potassium. When the calcium concentration is represented by potassium equivalents and these are added to the potassium units as concentration within the plants, the results give almost a constant equivalent for the concentration of these two mineral elements for the three treatments. This suggests a calcium replacement by the potassium. With this replacement came a lowered nitrogen concentration and a lower nitrogen fixing activity.

POSSIBLE CALCIUM-POTASSIUM RATIO IN SOIL DEVELOPMENT SUGGESTED BY NITROGEN FIXATION STUDIES

As a theoretical consideration, it may be of interest to note the indication in these results that with a narrow potassium-calcium ratio, the concentration of nitrogen in the soybeans was high, or, as a microbial ration in the form of green manure, these soybeans have also a narrow carbon-nitrogen ratio. With a widening of the potassium-calcium ratio, the nitrogen concentration decreased decidedly, and such plants would represent a bacterial ration with a wider carbon-nitrogen ratio. On soils with the narrow potassium-calcium ratio, green manures would fix more nitrogen to be added to the soil, and this in turn would hold more carbon to build up the soil organic matter. This raises the question whether we might not expect soils in partly developed, or relatively immature, condition with large amounts of exchangeable calcium, and therefore a narrow ratio of potassium-calcium in exchangeable form, to encourage nitrogen fixing plant growths in order to build up the soil organic matter? Conversely then, as the exchangeable potassium-calcium ratio becomes wider, as is true in more highly developed or leached soils, would it be unreasonable to expect the plants to be of a less nitrogenous and a more carbonaceous composition? Such plants are less likely to build up an organic matter reserve in the soil because of the absence of a liberal nitrogen supply where no nitrogen fixation can occur. Chernozem soils, then, with a liberal calcium supply, or possibly a narrow potassium-calcium ratio, probably owe their dark color to their efficiency as nitrogen fixers and carbon retainers. Podzolization, on the contrary, represents calcium removal and consequent reduction in the total calcium to a low level and a wider potassium-calcium ratio to foster nonleguminous plants, even to those of distinctly woody nature. Such reasoning may not be amiss as applied to an ecological series of plants, when one considers that within a single kind of plant, as the soybean, the shift in the potassium-calcium ratio can change the plant from a proteinaceous to a carbonaceous producer.

SUMMARY

By means of studies using electrodialyzed colloidal clay, partly and completely saturated by different nutrient ions, the role of the soil factors in nitrogen fixation by legumes may be more clearly understood. Such studies lead to the following beliefs:

As the soil texture becomes heavier, legumes may be grown more successfully because of the larger supply of exchangeable bases, particularly calcium, offered by the increased clay content, than is at their disposal in soils of lighter texture, or with less clay of the same mineral nature.

The degree of acidity is not of direct significance in nitrogen fixation; but rather its more common reciprocal, the degree of calcium saturation, plays the significant role.

Nitrogen fixation by legumes is closely related to the amount of exchangeable calcium which the crop can take from the soil. The amount so taken is not necessarily related to the total supply: it may be only a small and variable portion of this supply.

The efficiency with which the exchangeable calcium is taken is related to the degree of calcium saturation. The efficiency increases decidedly as the degree of saturation increases. No nitrogen fixation occurs in Beidellite clay, unless the calcium saturation exceeds 50 per cent and the clay carries only adsorbed inorganic ions.

When the larger organic cations are adsorbed on colloidal clay, the accompanying inorganic cations are more effectively used by the plants. This suggests a possible explanation for effective nitrogen fixation by legumes on soils of higher organic matter content, even under significant degrees of acidity.

Calcium plays a possible role in the assimilation into the growing plant of the seed phosphorus, which may even be lost to the soil by soybeans, for example, at low calcium levels. No nitrogen fixation is possible when such loss occurs.

Exchangeable magnesium is of significance in nitrogen fixation possibly indirectly through its influence in increasing the effectiveness of calcium.

Exchangeable potassium of the soil plays a significant role in nitrogen fixation, but as larger amounts are taken in relation to the amount of calcium, the nitrogen fixation in relation to plant growth by legumes is reduced. The potassium may replace the calcium, thus resulting in a widening of the potassium-calcium ratio to the point where possibly the legume does not exercise its nitrogen-fixing ability.

The role of such a potassium-calcium ratio may possibly be helpful in understanding the relation of the organic matter levels in different soils in relation to their degree of calcium depletion, or to their stage in soil development.

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