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# THE ROLE OF NITROGEN FIXING BACTERIA IN SOIL FERTILITY

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Nitrogen is among the elements used in large quantities in plant growth. This is shown by the following figures giving the amount of nitrogen in various farm crops. The figures include the nitrogen in the beet tops, wheat straw and corn stalks.

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| Sugar beets (15 tons) | 75 lbs. | Alfalfa (3 tons) | 150 lbs. |
| Wheat (50 bu.) | 50 lbs. | Red clover (3 tons) | 120 lbs. |
| Corn (50 bu.) | 75 lbs. | | |

When the store of nitrogen in the soil from which plants must draw their supply is examined it is found to be quite limited in ordinary cultivated soils. In 12 Colorado soils examined in this laboratory the one lowest in nitrogen contained 4000 pounds per acre foot and the highest 8000 pounds per acre foot. It can thus be seen that the amount of nitrogen in a soil at any given time, even if it were all available, would be capable of producing only a limited number of crops. For permenent fertility the soil nitrogen must be constantly replenished. This replenishment may take place in various ways. The farmer himself may add nitrogen to the soil in the form of barnyard or green manure or other organic materials grown on the farm. When this is done there is no increase in the nitrogen of the soil since that in the crop returned is never greater and usually is smaller than that removed. The only exception to this is found when properly inoculated leguminous crops are grown and this will be discussed more fully below. Where feed is purchased and fed to livestock and the resulting manure added to the soil there may be more nitrogen returned than the crop has removed. In this case, however, the soil of the farm on which the feed was grown is being robbed. Neither of the above cases represents an actual increase of nitrogen to our soils; there is only a return of part of that which has been removed by crops. For increasing the nitrogen of our soils as a whole we must depend upon the fixation of nitrogen from the air and adding it to the soil. This fixation may take place chemically as is done in fertilizer factories or it may take place biologically as by certain of the soil micro-organisms. It is the purpose of this paper to attempt to evaluate for the farmer the part which the nitrogen-fixing soil bacteria may play in maintaining the nitrogen fertility of his soil.

The actual element nitrogen is a gas and makes up 78 percent of the air around us. Most living organisms can not use this atmospheric nitrogen but must have it in a form already combined with other elements. Thus animals secure their nitrogen in the protein form and are dependent upon plants for food. Plants must also have combined nitrogen but are able to use inorganic forms such as calcium or sodium nitrate. It is principally from these compounds in the soil that they secure their nitrogen. The soil bacteria also require nitrogen for their cells and can utilize these forms but a few of them have also the power of utilizing atmospheric nitrogen under certain conditions. These bacteria we call "nitrogen fixing" bacteria. By this bacterial "nitrogen fixation" we mean the combining of the gaseous nitrogen of the air into protein of the bacterial cells. When these cells die their nitrogen remains in the soil and there is thus an actual addition of nitrogen equivalent to that contained in the bacteria.

It will be well to give a short description of the soil bacteria which have the power of nitrogen fixation. There are two general groups of these organisms and they differ greatly in their way of life. The first of these groups we call the "free living" or "non-symbiotic" forms. Bacteria of this group may live in the soil and fix nitrogen without the help of higher plants. Bacteria of the other group can fix nitrogen only if they are living within the nodules which they produce on the roots of leguminous plants. Alfalfa and peas are examples of these plants and pea nodules are shown in the illustration. These bacteria are called "symbiotic" nitrogen fixers, or more simply "nodule" bacteria.

Because of the differences in the bacteria of the two groups we will consider them separately and we shall take first the free living forms. There are two types of bacteria in this group but only one of them is considered to be important in ordinary cultivated soils. This organism was discovered in Holland in 1901 by a Dutch bacteriologist, Dr. M. Beijerinck, who named it Azotobacter. It has since been found to occur in almost all soils which are not too acid. A very large number of Colorado soils have been examined and Azotobacter has been found in almost all of them. For several reasons, however, its presence in a soil does not necessarily mean that it is actively fixing nitrogen. This organism can use other forms of nitrogen and if nitrate is present it apparently prefers this to using the atmospheric form. When the organism does this no nitrogen is fixed. Again, for proper growth and nitrogen fixation the Azotobacter must have available a proper form of organic matter such as sugars or organic acids. Only very small quantities of these are found in soils and Azotobacter must compete with other soil bacteria for their use. That this competition is very intense is shown by the relative number of the bacteria present. Thus, it was found that in an ounce of fairly fertile soil there were about one and one-half billion bacteria other than Azotobacter and only about 1500 Azotobacter cells. In other words, the Azotobacter cells had to compete with one million times as many other bacteria for the organic food which they needed before they could fix any nitrogen. Considerations such as these have led soil microbiologists to doubt if under ordinary soil conditions Azotobacter is capable of adding very much nitrogen to the soil. It would seem that a simple chemical analysis might settle the problem. This method does not work well on field soils because other factors also are changing the nitrogen content and it is not possible to separate accurately the effect produced by Azotobacter. Such chemical studies as have been made mainly agree with the deduction made above, namely, that Azotobacter does not add sufficient nitrogen to cultivated soils to offset that removed by crops. By adding certain forms of organic matter to soil the number of Azotobacter can be greatly increased and perhaps also the amount of nitrogen fixed. However, because of the high cost of the particular forms of organic matter required this can not be done economically. The farmer consequently can not depend upon the free living nitrogen fixing bacteria to keep up the nitrogen part of his soil fertility.

Turning to the symbiotic nitrogen fixing bacteria we find first that they are divided into groups or strains depending upon the particular leguminous plants on which they will form nodules. Thus one strain will inoculate alfalfa and sweet clover, another peas, vetch, etc., another beans, and still another the clovers, as red, alsike, mammoth and white. Secondly, we find that organisms of these various strains are not as universally distributed in soils as is Azotobacter but that they tend to occur only where their host plant has been grown. Where a plant, as for example alfalfa, has long been grown its corresponding bacteria are certain to be found. On the other hand, when a new leguminous plant of a different inoculation group, such as the soybean, is introduced, then it is necessary to introduce the proper bacteria also. If this is not done nodules will not be formed and no nitrogen will be fixed. The proper bacteria may be introduced in various ways. A common method is to mix the seed immediately before sowing with a small quantity of soil from a field in which root nodules on plants of the same kind have been found. A larger quantity of soil containing the bacteria may be spread over the field before it is seeded. A very simple method is to inoculate the seed with a pure culture of the proper organism. Such cultures may be purchased from various commercial concerns or from a number of state agricultural experiment stations producing them.

The inoculated leguminous plants can also utilize the soil nitrate as well as atmospheric nitrogen. The amount of nitrogen taken from the air then depends somewhat upon the amount of avilable nitrogen in the soil. In poor soils with a low nitrogen supplying power leguminous plants may secure almost all their nitrogen from the air while in very fertile soils they may take only a small portion from the air. An old rule is to the effect that probably under many conditions the amount of nitrogen in the tops of alfalfa and clover plants represents that taken from the air while that in the roots may be considered as representing that coming from the soil. In plants such as beans and soybeans having a small root system perhaps not more than half of the nitrogen in the tops could be considered as coming from the air. This rule is undoubtedly not strictly true but it is probably applicable to many cases. It will serve, at any rate, to emphasize an important point which must be observed if a soil is to benefit from the growing of leguminous crops. That is, if the tops are removed from the field and no part of them returned there is no gain of nitrogen to the soil. As a matter of fact, there may be and probably often is an actual loss of nitrogen to the soil. It is clear then that if growing leguminous crops is to permanently benefit the soil a portion of them must be either plowed under as green manure or they must be returned as manure from livestock to which they have been fed. Such a use of leguminous crops is basic in the conservation of soil nitrogen and organic matter.