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BACTERIOLOGICAL EFFECTS OF GREEN MANURES

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INTRODUCTION.

Since this Bulletin is to be introductory to a series of studies on soil bacteria and green manures which have been undertaken at this Station; it is well that we should give a brief preliminary discussion of these and related subjects.

There is in the surface soil numerous microscopic plants known as soil bacteria. Though they can not be seen with the unaided eye, they are of the greatest importance to the farmer. It is the soil bacteria that prepares the soluble food for the growing crop. They break down the organic matter of the soil and form the nitrates. The humic, carbonic and other acids produced by their growth aid largely in dissolving the minerals of the soil and in making them available as plant food.

The organic material in the soil is the food for the soil bacteria. It is the fuel from which they derive their energy and out of which they manufacture the food for the crops. Organic material must, therefore, be constantly and continuously added to the soil. Its source is the dead remains of plants and animals. Products from animal sources are on the market as blood meal, tankage, and other fertilizers. These products are expensive. The much cheaper source of nitrogen is from the dead plant tissues. These are obtained from crop residues, and green and stable manures. The crop residue is that part of the field's growth that remains after harvest. It consists of the roots, stubble, straw or stalks that remain or that are returned to the field to be turned under along with what weeds and other wild plants that have grown in the field.

By green manure is meant the use of any green crop which is grown and turned under as plant food for a succeeding crop. Legumes are used almost exclusively for this purpose.

The partially decayed remains of all these organic materials occurring in the soil form the humus, which gives to a soil the characteristic dark, rich color, and a lack of which is indicated by the whitish gray soils.

It is not unusual for Mississippi soils to be deficient in humus. With the intense bacterial activity due to an abundance of moisture and heat the humus is rapidly used.

The increase, maintenance and control of humus in the soil is of vital importance to every Mississippi farmer. These subjects will now be considered in some detail.

SOIL BACTERIA.

Bacteria are microscopic one-celled plants. They are too small to be seen with the eye except where many grow together in one place. It takes a high power microscope to see them individually. From 1,000 to 2,500 placed end to end are required to reach one inch. They are spherical or rod shaped; the rods may be either straight or curved.

Their relation and importance to the growing of farm crops have only been understood in recent years. We now know that without their help crops could not long be grown. If it were not for the decay and return to the soil of the dead plants and animals the surface of the earth would soon be clogged with their remains. This constant decay and formation of soluble plant food for the higher plants is continually going on by the tireless energy of these microscopic plants. The most important of these processes is the formation of soluble nitrates from the animal and plant residues. In this formation three distinct steps are recognized, viz.: the formation of ammonia, nitrites, and nitrates.

Kinds of Bacteria.—The formation of the substances named above are known to be brought about by three distinct groups of bacteria; and neither group can do the work of the other. The organisms that form nitrites can not act upon the protein substances of plants and animals; but must take the ammonia products formed by the organisms of fermentation and decay. Neither can these organisms go beyond the formation of nitrites. Nitrates are not formed when pure cultures of the nitrite organisms are added to the sterile ammonia products in the soil; but the addition of the nitrate organisms is necessary and when added will give in a short time the test for nitrates. It is thus seen that the division of labor is carried to a high degree among soil bacteria. Each having its special work to do; and their great efficiency is, no doubt, due to this specialization.

Ammonifying Organisms.—The organisms capable of producing ammonia out of plant and animal residues are called "ammonifying bacteria." There are a number of kinds of ammonifying bacteria. Some are aerobic, others are anaerobic; *i.e.*, some live best with abundance of oxygen as in well tilled fields, others live where oxygen is partially excluded, as in wet swamps and heavy clays. Some are much more efficient in the production of ammonia than others. In Marchal's experiments, the species employed showed marked differences in their ability to produce ammonia out of egg albumen. The following proportions of the protein nitrogen were converted into ammonia in twenty days:

B. mycoides	46 per cent.
B. vulgaris	36 per cent.
B. subtilis	23 per cent.
B. flourescent	16 per cent.

Not only is this variation noted between different species but marked differences have been noted between one strain and another of the same kind of bacteria.

Beside the class of ammonifying organisms mentioned above there is another special class of bacteria, known as urea bacteria, that transform urea and other like products into ammonium carbonate. These organisms play a most important part in connection with stable manure added to the soil. This chemical transformation is brought about by means of an enzyme, known as urease, which is formed by these organisms.

Nitrifying Organisms.—Nitrification as generally understood goes on in two steps by two distinct groups of organisms. The organisms oxidizing ammonia to nitrites, called the nitrite bacteria were named by Winogradski *Nitrosomonas* and *Nitrosococcus*. The organisms oxidizing nitrites to nitrates were named by the same author *Nitrobacter*. The history of the experimental researches that led to the discovery and the isolation of these organisms is intensely interesting; but the scope of this paper does not permit us to give it here.

Nitrogen Fixing Organisms.—Nitrogen fixing organisms are those that have the power of utilizing the free nitrogen of the atmosphere in their life processes. There are two classes of these organisms. One class lives free in the soil and the other beside living free in the soil has the power to enter the roots of the legumes and form nodules on them and within which the bacteria live symbiotically.

Non-Symbiotic Bacteria.—The non-symbiotic organisms are both aerobic and anaerobic. The aerobic or azotobacter group are the most important and are known to have the power to add considerable amounts of nitrogen to the culture media in flasks in which they are grown. Just what agricultural importance these organisms have is but little known. It is known, however, that some abandoned plots, not growing legumes, at the Rothamsted Experiment Station have shown an average annual gain of 25 pounds of nitrogen per acre, and that these organisms are abundant in this soil. Such gains have been noted in the United States at the New Jersey Experiment Station, where horse manure was added to a poor sandy soil. It is believed that these organisms are responsible for this increase in nitrogen; but what part they play in assisting crop production has not been conclusively shown by vegetative tests.

The Root Tubercle Organism.—The symbiotic nitrogen fixing organisms that produce root tubercles play an important part in agriculture. Their power, in association with legumes, to utilize the free nitrogen of the atmosphere was first shown by the experiments of Heilriegel and Wilfarth in 1886. These organisms in the soil and in culture media are thin rods; in the root nodules they take on various branched shapes. This branching is more or less characteristic for the legume in which they are found. The thin rods in the soil under proper conditions come in contact with the root hairs and penetrate these; then by means of filaments they grow toward the interior of the root branch. This stimulus causes the proliferation of the plant tissues of the root and along with the multiplication of the bacteria a tubercle is formed. There is no regularity in the arrangement of the tubercles on the roots, since the bacteria may enter them at different places and times.

Virulence.—It is known that the plant offers more or less resistance to the entrance of bacteria into their roots. The bacteria must be sufficiently vigorous to overcome this resistance. The young legume in soils finding only small quantities of combined nitrogen soon turns yellow from "nitrogen hunger." Thus weakened the nodule bacteria more easily penetrates the root hairs. On the other hand, some bacteria have more power than others to enter the roots of the legume. Under favorable conditions this virulence may be much increased also their power of fixing nitrogen.

From these considerations it appears, theoretically, at least, that light top dressings of nitrate on new fields of alfalfa will benefit the crop by excluding all but the most vigorous bacteria through the increasing of the resistance of the young alfalfa plants to such an extent as to exclude the non-virulent root tubercle bacteria.

HUMUS AND ITS USE IN A SOIL.

Humus consists of the partially decayed organic remains of plants and animals. It gives the dark color to a soil. To understand the function of humus in a soil it is necessary to bear in mind the relation of a soil to the crop. In the first place, the soil furnishes a home for the growing plant. A place to anchor it; it must be of such a nature that the roots can readily enter, grow, and push themselves easily through it. It must be moist and firm, but not soggy, hard nor compact; shifting sand can not well answer the purpose, neither can a clay hard pan nor a wet boggy marsh. It must contain the proper amount and kind of plant food, moisture and soil atmosphere. It must be a suitable home for soil bacteria, for here the bacteria manufacture the available plant foods for the growing crop. Here is made the soluble nitrates and the organic acids and humates which liberate and make available the phosphates, potash and other mineral plant foods.

The humus in many ways helps a soil to better perform these functions. It increases the water holding capacity, improves the tilth, prevents extremes in temperature, makes a home for the soil bacteria and is a store house for plant food.

Increase of Humus in a Soil.—Organic matter is most cheaply added through crop residues, and stable and green manures. No stalks, straw, leaves or other plant material should be burned; all these go to form humus and when turned under and properly managed can readily be digested by all our ordinary soils. It has been shown that the burning of cotton stalks to get rid of boll-weevil does not materially lessen the numbers of this pest for the next year; but destroys a large amount of organic material so much needed for good crop production. The cotton stalks, corn stalks, and other coarse material that would interfere with cultivation can be easily handled by cutting them in short lengths with a stalk cutter or disk-harrow before being turned.

With our lack of farm stock in Mississippi stable manure with crop residues are not sufficient to keep up the humus requirements of our soils. It is, therefore, necessary to grow special green manure crops for this purpose.

NITRIFICATION.

The vast practical importance of nitrification is evident when it is known that crops take practically all their nitrogen in the form of nitrates. Though it is known that plants can utilize ammonia as a source of nitrogen; they do not usually do this since the ammonia compounds are so readily changed to the nitrite and the nitrite to the nitrate forms. It is thus known that the nitrate nitrogen which is so necessary to all growing crops is manufactured by soil organisms from the dead remains of plants and animals. The proper understanding of the nature of these organisms, their living conditions and life processes will aid the farmer in many ways to regulate the preparation, and the cultivation of the soil for his crops, as well as the time and manner of planting.

He must keep in mind that just as soon as the soil is broken in preparation for the crop, these organisms are busy preparing plant food for the young crop that is soon to occupy the ground. He must remember, also, that the soil is a living active thing; not the dead, lifeless clods that they seem. Though they can not be seen by the eye, the bacteria are there and must not be forgotten. Should the soil be left in large dry clods, the activity of the soil bacteria are much hindered and many of the organisms die. Should heavy rains form a thick crust, they will be insufficiently supplied with oxygen. Should the crop be planted immediately after breaking, time enough will not ensue for the preparation and accumulation of sufficient plant food to start the crop.

Conditions Necessary for Nitrification.—The following conditions for the best production of nitrates are requisite: 1. The presence of nitrifying organisms in necessary quantities; 2. The proper temperature and moisture; 3. The aeration to supply the necessary oxygen for oxidation; 4. A base to neutralize the acids as they are produced.

Sufficient Number of Nitrifying Organisms.—That a sufficient number of nitrifying organisms is necessary for nitrification is seen in the sickly nature of the crop grown over a spot where an old building has stood. The first year very poor or no crop can be grown. Such a spot is said to be "dead soil," very few soil organisms are found; when the proper organisms are supplied large crops are raised.

Temperature and Aeration.—The effect of a low temperature in checking the formation of nitrates is readily noted in young cotton turning yellow and it may shed part of its foliage through "nitrogen starvation" whenever a cold wet time chills the ground in the spring. The bright green color returns when the warm soil, aerated by plowing, restores the activity of the nitrifying organisms in the surface layer. King found in the top foot of soil when oats were turning yellow only 0.026 parts of nitrogen per million of dry soil, whereas in soil where the oats were green on the same date there was 0.255 parts of nitric nitrogen per million.

The effect of aeration upon nitrification is seen in all processes of cultivating the soil. Nitrification is an oxidation process, both the change from ammonia to nitrite and from nitrite to nitrate are brought about by the addition of oxygen. The oxygen supply is obtained from the soil atmosphere. Thus a soil with a hard crust from a beating rain; a wet, soggy soil, or a hard, cloddy soil preventing the free supply of soil atmosphere interferes with nitrification. Cultivation by increasing the supply of oxygen aids nitrification.

It is known that the production of nitrates takes place almost exclusively in the cultivated surface soil. The researches at Rothamsted, England, show that drain gages situated at a depth of 40 inches and 60 inches respectively, yield no more nitrates in the drainage water than one at a depth of 20 inches; showing that nitrification goes on almost entirely at the surface.

Soil Acidity and Calcium Salts.—In the oxidation of ammonia to nitrite and of nitrite to nitrate, nitrous and nitric acids are formed. Beside these acids, carbon dioxide is generated in vast amounts in the life processes of most soil organisms and other organic acids are formed in the decomposition of carbohydrates, fats and proteins. Bacterial action goes on so rapidly in our southern soils with so much moisture and humidity, acids are formed very rapidly, and thus it often occurs that such soils are "sour." Soil bacteria become inactive and inefficient as the acidity increases. Thus if it were not for the presence of lime, magnesia, and other basic substances; an accumulation of acids would occur and hinder bacterial action. The addition of lime to correct this acidity thus becomes necessary.

The calcium salts formed by the action of these acids upon the lime are more or less soluble. In this manner enormous amounts of lime are annually carried to the ocean as bicarbonates, nitrates and sulphates. Thus soil organisms furnish shell fish and other marine life the necessary material for building their skeletons. Coral reefs are formed, also chalk cliffs, marl, and great limestone beds accumulate, islands are built, a new continent is made. In this manner calcium circulates in nature.

Uses of Lime.—Beside the correction of soil acidity and the beneficial effect to nodule and other bacteria, lime improves the tilth of a soil. It has the power of granulating the particles of a clay soil making it more porous; giving it more power to hold water; making it easier to cultivate; furnishing much more air space and thus provides better conditions for bacterial activity. Lime makes a sandy soil more compact by cementing the grains together and prevents the soil being blown about by the winds.

Lime makes available such mineral plant foods as potash and phosphoric acid. It also serves as a plant food; the chemical analysis of plants always show the presence of lime. Thus the chief uses of lime are: to correct soil acidity, to improve the tilth, to promote the growth of the soil bacteria, to make available mineral plant foods and to serve as a plant food.

Necessity for a Cover Crop.—The loss of soluble nitrates by leaching through heavy rains, especially when the land is not occupied by a crop may be very considerable. In the warm climate of our state, nitrification goes on at all times in the year. While it is slower during the winter months yet a considerable amount of nitrates are continually being formed by the bacteria. The use of a cover crop to use and thus fix this nitrogen as rapidly as the nitrate is formed is therefore necessary. The kind of cover crops and their use will be discussed under the general subject of green manures.

GREEN MANURES.

Elements over which the Farmer has no Control.—The atmosphere is the original source of 98½ per cent of the materials found in all green crops; the carbon, hydrogen, and oxygen are supplied in the form of water and carbonic acid gas. These substances in the humid climate in which we live, are furnished free of cost; the supply being largely beyond our control. To be sure it is necessary to conserve and control the water supply; but when this is done, we have an abundance of moisture for large crops.

Table 1 gives the annual precipitation for Mississippi and for three of the large corn producing states in the Union. In each of these states, three sections were selected, which it was thought would give practically an average rainfall for that state.

The average rainfall for Mississippi is thus seen to be almost double that of Kansas and 15 and 17 inches more per annum than that of the states of Illinois and Iowa. Thus Mississippi has the advantage of our three great corn producing states in the amount of moisture, temperature and long growing season; three very important factors in the growing of crops.

Elements that the Farmer Needs to Furnish.—The farmer needs to concern himself about a very small per cent of the plant's food—less than 1½ per cent; since the other 98½ per cent, as has been said, is beyond his control. Of the seven elements included in this 1½ per cent,

TABLE 1.—Average annual precipitation in Mississippi compared with three of the best corn growing States in the United States.

	MISSISSIPPI			ILLINOIS			IOWA			KANSAS		
DATE	Agricultural College.	Meridian.	Vicksburg.	Urbana.	Springfield.	Cairo.	Sibley.	Ames.	Burlington.	Agricultural College.	Wichita.	Colby.
1902	53.4	50.1	47.3	[3]32.8	36.7	33.1	29.1	45.8	51.6	44.3	38.7	22.1
1903	40.9	44.6	38.0	32.5	28.3	33.0	35.4	26.4	35.0	37.0	31.1	21.1
1904	36.0	40.9	41.6	29.8	30.6	32.0	25.2	25.6	35.8	34.1	31.1	23.8
1905	[2]63.2	59.4	60.5	29.6	29.5	39.5	37.5	29.0	38.9	33.7	34.5	16.9
1906	[2]49.3	64.9	51.8	34.3	35.5	46.9	37.2	27.2	37.5	35.6	31.0	19.6
1907	45.9	54.2	51.6	40.2	41.1	45.6	25.3	27.6	37.6	28.5	31.1	13.0
1908	46.7	49.9	49.3	33.3	29.7	38.5	40.7	[1]35.9	38.1	44.1	37.7	14.7
1909	59.9	57.9	52.5	37.8	42.5	43.1	32.1	37.2	40.8	43.5	30.6	17.9
1910	37.5	43.4	50.4	28.0	27.5	45.9	16.1	18.7	28.0	33.8	17.8	6.0
1911	57.5	52.5	56.1	32.3	40.8	30.1	27.4	35.5	46.8	28.5	36.9	10.6
Av'ge	49.0	51.8	49.9	34.0	34.2	38.7	30.6	30.9	39.0	36.3	32.0	16.6
Av'ge for State		50.2			35.7			33.5			28.3	

1. Ames, Iowa, report incomplete; Des Moines report substituted.
2. Agricultural College, Miss., report incomplete; Aberdeen report substituted.
3. Urbana, Ill., report incomplete; Tuscola report substituted.

only four,—nitrogen, phosphorus, potassium and calcium—need claim his attention, since the other three, iron, sulphur and magnesium are present in the soil in sufficient abundance for the largest crops.

Calcium, phosphorus and potassium can be added only through purchase, however, often there is present in our soils a sufficient amount of the last two elements if they are only made available. The use of stable and green manures help greatly in making these available. Some soils are deficient in phosphorus and potassium then they should be added through purchase.

Nitrogen which is by far the most expensive to purchase and is the element most lacking in the soils of our state, need not be purchased, since it can be had from the inexhaustible supply in the air we breathe. There is, in the atmosphere, over every acre of land about 35,000 tons of nitrogen. The soil atmosphere like the air around us is about four-fifths nitrogen. Nitrogen in this form can not be used by crops; but the nitrogen fixing bacteria have the power to use atmospheric nitrogen in their life processes, and together with the legumes can supply it to our soils.

Nitrogen is furnished to our crops indirectly through the organic nitrogen of the soil. The organic nitrogen is furnished to the soil through the return of any organic material. The best means available to the farmer for the increasing of nitrogen is the plowing under of leguminous green manures. Where it is convenient it will be more profitable to feed the legumes and return the fresh stable manure to the soil. In many parts of the state there is not sufficient farm stock; and in these cases turning legumes to secure nitrogen and humus is not only a necessity in securing the humus, but much more profitable than the purchase of nitrogen in any form.

Since early times it has been known that legumes add fertility to the soil and when grown in crop rotations, the grain crops are benefited. That these crops are benefited when grown together with the legumes is indicated by the rank growth and dark green color. This may be noticed when oats are grown with vetch, timothy with red clover, or corn with soybeans or cowpeas.

Amount of Nitrogen Taken from the Air.—From experimental data obtained by the University of Illinois Experiment Station, it was found that on an average plants take about one-third of their nitrogen from the soil and two-thirds from the air through the aid of the root tubercle bacteria. If one-third of the nitrogen remains in the roots and stubble and two-thirds is removed with the crop, which is approximately true; it is evident that the soil would neither lose nor gain in nitrogen.

Amount of Nitrogen in Tops and Roots.—From the results of the Delaware Agricultural Experiment Station it was found that about 6 per cent of the nitrogen is in the roots of cowpeas and soybeans; 11 per cent in vetch; 28 per cent in crimson clover; 32 per cent in red clover; and 42 per cent in alfalfa. The University of Illinois Agricultural Experiment Station finds the percentage of nitrogen in the roots of sweet clover to be 19 per cent of the total. It is seen, therefore, that leguminous crops with extensive root systems like alfalfa, red clover, crimson clover, and sweet clover may be removed and yet leave behind in the roots and stubble nitrogen equivalent to the amount taken from the soil by these plants. Now if stable manure is added or an occasional crop of green manure is turned to compensate for the loss of nitrogen by leaching, the nitrogen in the soil will be maintained. When leguminous crops like cowpeas or soybeans are cut and removed there does not remain sufficient nitrogen in the roots to compensate for that taken from the soil by the crop so the land becomes poorer in nitrogen.

Soil Inoculation.—When the nodule bacteria are not present in a soil, they may be added by inoculating the seed with pure cultures of these organisms or by inoculating the seed or soil by use of well infected soil taken from a plot of ground where the legumes have been growing well and are known to contain an abundance of tubercles on their roots.

Pure cultures of these organisms can readily be obtained, by one trained in bacteriological work, by aseptically opening a tubercle from the root of the legume and smearing some of the nodular substance on a prepared agar jelly medium. The organisms in a day or two grow on this medium until they are plainly visible to the eye in a raised, watery, jelly mass.

The Soil Bacteriological Division of the Bureau of Plant Industry of the United States Department of Agriculture grow these cultures and distribute them free to the farmers. There are also a number of commercial firms that sell them at a very nominal price.

The advantage claimed for the use of pure cultures are: 1. The organisms are more virulent. 2. There is no danger in distributing noxious weed seed as may be done in soil inoculation.

In practice many failures have followed the use of pure cultures. The cultures die readily and are easily contaminated and then killed by the overgrowth of other bacteria and moulds. Their application must be made with much more care than with infected soil. However, use of pure cultures is at present proving to be much more successful than formerly; this is because of the better prepared cultures, with more careful directions, and the farmers have followed directions in more detail.

The inoculation with soil may be done by making an emulsion with well infected soil, and when the soil settles, sprinkle the seed or the dry soil with this emulsion and thoroughly mix; sow broadcast or with seed drill. Where the infected soil can be had in abundance near the field to be inoculated, the more usual way is to spread this soil over the plowed field at a rate of from 200 pounds to a ton per acre. The better time to apply, whatever method used, is on a cloudy day when the soil is moist and harrow in well. Sunshine readily kills the bacteria; and they are also killed by drying. For this reason it is better to apply in the afternoon.

The question is often asked whether the organisms that produce tubercles on one legume will produce tubercles on another. It is known that the organisms producing nodules on sweet clover will produce nodules on alfalfa or bur clover; and that the organisms producing nodules on red clover will do so on alsike, white and crimson clover; in the same way common vetch and hairy vetch are associated and cowpea with partridge pea. The organisms are not interchangeable for alfalfa, red clover, cowpea, soybean or vetch. Only on the near related species of legumes do the organisms produce nodules interchangeably.

The organisms for the common clovers, cowpea and vetch are widely distributed throughout the South and rarely is inoculation necessary. For alfalfa and soybean it is often necessary to inoculate. Soil from ground where sweet clover, or bur clover as well as alfalfa grows with an abundance of nodules is satisfactory for the inoculation of alfalfa.

When no infected soil is available on the farm or in the neighborhood, it is advisable to plant only a small plot, say one-tenth acre, with great care, using pure culture or soil shipped in for inoculation.

How long the nodule bacteria live in the soil without the presence of a leguminous crop is not definitely known; probably in five or six years they will be so reduced in numbers that inoculation will be necessary, or at least helpful.

COVER CROP.

A cover crop or catch crop is one occupying the ground before or after the money-making crop or principal crop of the season; such as one following early potatoes, oats and vetch, corn or cotton. The uses of a cover crop are to protect the soil from the burning sun, and prevent the injury to the soil bacteria and the small rootlets of the crop in the surface soil; to utilize the soluble nitrates that would be lost by leaching and to add organic matter to the soil.

It is very necessary to increase the organic matter or humus in most of our Mississippi soils. Usually one feels that he can not afford to give up a season's growth for the improvement of the soil alone; so when this can be done with a catch crop occupying the ground during the part of the season after the money crop is harvested a saving of time is made and our soil is improved also.

Kind of Cover Crop.—The kind of a crop to use for a cover crop will depend upon circumstances; and only general advice can be given. There are two general kinds to consider the legumes and the non-legumes. Other things being equal the legume cover crop will be the better of the two; since the legume has the power, not possessed by the non-legume to use and thus to add to the soil nitrogen from the atmosphere. Circumstances may occur when non-legumes, like rye, rape, oats, or buckwheat may be better suited than a legume for a cover crop. The seed of these may be available, when that of the legumes are not. The soil, time of planting and the purpose for which the crop is to be used are all to be considered. It is far better to use a non-legume than to allow the soil to be unoccupied. The use of a non-legume holds the nitrogen that is present, adds organic matter, prevents the washing of the soil by the mass of roots present, and a mulch is formed protecting the soil and holding moisture.

The legumes well suited for catch crops are, cowpeas, soybeans, vetch, crimson clover, red clover, or bur clover. When these are to be used as green manures which is their most important use, the cowpea or soybean do well after corn: planting these at the last plowing of the corn. By late fall they have made an immense growth and will be ready by or before Christmas to plow under together with the stalks for the next cotton crop.

In turning them, the use of a stalk cutter to mash down the legume and to cut the corn stalks in short lengths will be very necessary. It would be better to turn them while the legume is still green and full of sap. The bacteria will then act much more effectively on the green material and reduce the organic material more efficiently.

FIG. 1.—Cowpeas in Corn Seeded at Last Planting.

For cover crops after cotton the clovers or rye are better suited. These stand the tramping, necessary in the picking of cotton, and continue their growth through the winter and early spring. They may be turned in the spring and a silage crop of corn planted. The silage crop can be planted later than the cotton or a corn crop planted for grain. This gives time for the clover crop to get a good growth and makes a large amount of organic matter which can be turned sometime in April or May.

Drying Effect of Cover Crop.—The Mississippi farmer has little to fear from the danger of the drying effect in the use of green manures. In sections of the country where the average annual rainfall is less than thirty inches there is much danger that the green manure will use the moisture and leave the soil too dry to start the succeeding crop. Or there may be insufficient moisture in the soil when the green manure is turned to bring about the decomposition of the organic matter. This would result in a serious injury to the physical condition of the soil. With an average annual rainfall of more than 50 inches there is no danger from a lack of moisture for the cowpeas or soybeans which are turned in the winter and but little danger that any crop would suffer for this reason.

Kind of Legumes to Grow.—The kind of legumes to plant will depend upon the climate, the soil, and the use for which the crop is desired. A very large variety of legumes can be successfully grown in

Mississippi. Alfalfa grown in the limestone soils has no superior as a hay crop and soil builder. As many as five cuttings per season may be obtained giving ten tons of cured hay. Its extensive root system makes it one of the best soil builders among legumes. For catch crops and late summer crops cowpeas or soybeans are unexcelled. They may be planted after oats and vetch, a potato crop, or in corn at the last plowing. These may be hogged off or turned for green manure. For a winter crop hairy vetch with oats, crimson or bur clover are excellent. The oats and vetch make an abundance of excellent hay; the crimson clover or bur clover are splendid early pastures or to turn as green manure. In the southern part of the state the velvet bean makes an immense amount of forage and is highly prized. Lespedeza for its hardiness, and as a pasture is very highly recommended.

EXPERIMENTAL.

Purpose of the Work.—The general purpose of the soil bacteriological studies that are being carried out at this Experiment Station are: 1. To add something to the knowledge of Soil Bacteriology; and, 2. To arouse an interest in the farmers of Mississippi to largely extend the use of green manures as a means of increasing the fertility of their soils. The special object of this experiment was to determine the effect of micro-organisms in the fermentative destruction of a green manure and the advantage of adding a light dressing of stable manure, a bacterial culture, or both in the utilization of this green manure as a plant food.

Plan of the Work.—Two series of jars were arranged; one for the bacteriological tests, and the other cropped to oats in order to correlate the results of the bacteriological tests with the vegetative tests. Each series is done in duplicate.

Jars.—The three-gallon stone jars, were selected with care, so they all have practically the same height and diameter. The ones used for the series cropped to oats have a drain, those used for the bacteriological tests are not drained.

Soil.—The soil used in the jars was obtained from a tilled field on the Experiment Station Farm. It is poor in plant food and is a mixture of sandy silt and a sandy loam soil. An analysis showed it to contain only 648 pounds of total nitrogen per acre for the plowed stratum, 6 2-3 inches. The soil was put through a one-fourth inch mesh seive and thoroughly mixed before putting it in the jars. The water holding capacity of this soil was 23.7 per cent. The soil in the jars used for the bacteriological test was kept at a moisture content of 12 per cent by adding distilled water to weight from time to time. The jars cropped to oats were watered to maintain a good tilth. The scheme of the treatment is given in Table 7. Jars in Series I were cropped to oats; in Series II, were used for bacteriological tests.

Fertilizers.—The ground limestone used was furnished us from the Casper Stolle Quarry and Contracting Co., East St. Louis, Ill. The finely ground raw rock phosphate came from the Mt. Pleasant Fertilizing Co., Mt. Pleasant, Tenn. Limestone was used in all jars save the first four in each series at a rate of three tons per acre. (See

TABLE 7.—Treatment and arrangement of the jars.

No. of Jar Series 1.	No. of Jar Series 2.	(Treatment Same for both Series).
1 & 2	21 & 22	Nothing Added.
3 & 4	23 & 24	No lime & 2 T. A1.
5 & 6	25 & 26	3 T. G. L. & 2 T. A1.
7 & 8	27 & 28	3 T. G. L. & 4 T. A1.
9 & 10	29 & 30	3 T. G. L. & 4 T. A1. & 2 T. H. M.
11 & 12	31 & 32	3 T. G. L. & 4 T. A1. & 2 T. C. M.
13 & 14	33 & 34	3 T. G. L. & 4 T. A1. & 1 T. R. P. & 2 T. H. M.
15 & 16	35 & 36	3 T. G. L. & 4 T. A1. & Bacterial Culture.
17 & 18	37 & 38	3 T. G. L. & 4 T. A1. & 1 T. R. P. & B. Culture.
19 & 20	39 & 40	3 T. G. L. & 4 T. A1. & 1 T. R. P. & 2 T. H. M., and Bacterial Culture.

T. G. L.............................Tons of ground limestone per acre.
T. A1.................................Tons of alfalfa per acre.
T. R. P..............................Tons of raw rock phosphate per acre.
T. H. M.............................Tons of horse manure per acre.
T. C. M.............................Tons of cow manure per acre.

Table 7.) The rock phosphate was used at the rate of one ton per acre. The limestone and the rock phosphate were intimately mixed with the soil on a sterile oil cloth and then returned to the jar. Horse manure and cow manure were used at the rate of two tons of the fresh manure per acre. Alfalfa was used as the green manure. It was collected from a patch that was beginning to bloom, cutting the stalks off close to the ground. No roots but stalks only were used. The moisture of the green alfalfa was determined, and the green alfalfa was added to all but the check jars at a rate equivalent to two and four tons of the dry weight per acre. The alfalfa and the stable manure were added at a depth of eight inches.

The bacterial culture was prepared by growning, on meat juice peptone agar, in four, six inch Petri dishes, from a seeding of an infusion, made from garden soil and horse manure. The Petri dishes were incubated for thirty-six hours at 30 deg. C. A very heavy growth was obtained. Sterile distilled water was added to the agar plates and the surface gently rubbed with a sterile bent glass rod in order to detach the bacteria. The emulsion from the four plates was placed in a flask and made up to 1,200 cc. and thoroughly shaken. One hundred cc. of this emulsion were poured over and mixed with the top soil in each of the twelve jars as indicated in Table 7.

Bacterial Tests.

Two kinds of bacteriological tests were made. A quantitative test made by determining the number of bacteria per cubic centimeter; and a physiological test made by determining the amount of ammonia produced with uniform samples of the different soils when seeded into equal amounts of a good ammonifying medium.

Quantitative Tests.—The quantitative tests are made by seeding on a specially prepared solid jelly medium uniform quantities of soil infusions. After trying out several of the different solid jelly media recommended for this purpose the following formulae recommended by Lipman and Brown was used:

1000 cc. distilled water.
0.5 g. K_2HPO_4
0.2 g. Mg SO_4
0. 05g. peptone.
10.0 g dextrose.
trace, $Fe_2(SO_4)_3$.
15 gs. Agar.

While this medium is not all that one could desire, it is as good as any we have tried. We have reason to suspect that a medium may be found that will be better to differentiate the fermentative power of soils on green manures. We believe that peptone gelatine would do this; but the difficulties of getting harmonious results with the use of gelatin in as warm a climate as we have here are almost insurmountable. A search for a better medium will be continued; but until one is found the one given will be used.

Plating—The soil infusion is obtained by first removing two inches of the top soil and then mixing the next six inches and carefully weighing off one hundred grams of soil from each pot. This sample is placed in a 500 cc. shaking bottle, 200 cc. of sterile tap water is added, and shook for fifteen minutes in a shaking machine; this is dilution A. In a few moments after being removed from the shaking machine, the sand settles out and one cc. of the supernatant fluid is pipetted off with a sterile one cc. pipette and put into 99 cc. of sterile tap water; this is dilution B. After thorough shaking, place 5 cc. of dilution B into a small flask containing 45 cc. of sterile tap water; this is dilution C. After shaking C place 10 cc. of this dilution into a flask containing 90 cc. of sterile tap water; which gives dilution D. Again shaking and place 10 cc. of dilution D into a flask of 90 cc. of sterile tap water; which gives dilution E.

Duplicate one cc. samples are placed in sterile Petri dishes from dilutions D and E, 1 to 20,000 and 1 to 200,000 respectively, and 10 cc. of the melted synthetic agar, cooled to 45 deg. centigrade, are poured into the dishes and intimately mixed. After the agar has solidified, the dishes are placed in the incubator at 28 deg. centigrade and incubated for three days. The plates are counted using a small tripod lens.

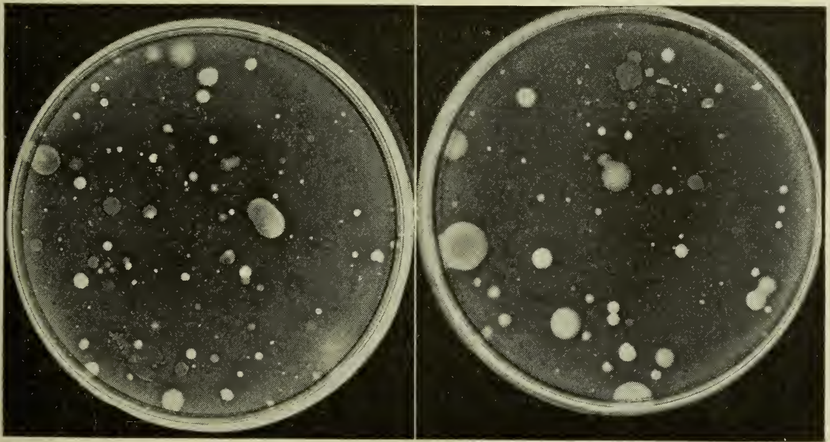

FIG. 2.—Colonies of Soil Bacteria, duplicate plates; dilution 20,000.

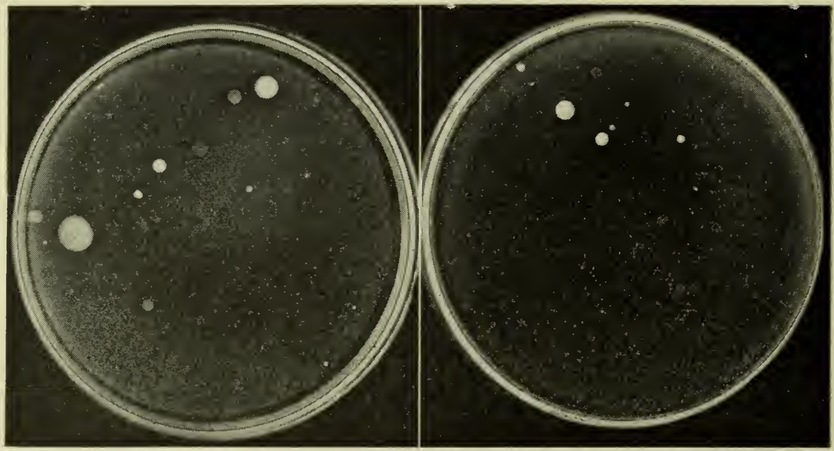

FIG. 3.—Colonies from same soil emulsion as in Fig. 2; dilution 200,000.

(See Figs. 2 and 3.) All colonies occurring on the agar were included in the count. Table 8 shows the average counts made from samples taken on Jan. 2, Jan. 28, and March 9, 1914.

On the whole the results correspond fairly well with the result of the vegetative tests. (See Table 9.)

It will be noted that on Jan. 2, No. 3 shows a depression in numbers where the ground limestone was added. The opposite result is noted Jan. 28 and March 9. The result from the last two dates correspond with the vegetative test and is what would be expected from the addition of the ground limestone. The reason for the depressed result on Jan. 2 is not evident. This lack of correspondence of the bacterial counts and the vegetative tests is only noted in a few instances. It may be due to the difficulty in making accurate counts. But since the correspondence far outweighs the discrepancies; and the fact that the general average has a tendency to harmonize the results, puts it beyond question, that there is an intimate relation between the fertility of the soil and the bacterial counts. (See Table 9.)

TABLE 8.—Average Bacterial Counts at Three Testings.

TREATMENT	Date of Testings.		
	Jan. 2.	Jan. 28.	Mar. 9.
Nothing added	6,443,000	2,230,000	4,143,000
No lime & 2 T. Al.	7,560,000	3,329,000	5,145,000
3 T. G. L. & 2 T. Al.	7,155,000	4,438,000	5,610,000
3 T. G. L. & 4 T. Al.	6,044,000	4,220,000	6,890,000
3 T. G. L. & 4 T. Al. & 2 T. H. M.	7,476,000	5,780,000	8,220,000
3 T. G. L. & 4 T. Al. & 2 T. C. M.	6,478,000	5,560,000	8,445,000
3 T. G. L. & 4 T. Al. & 1 T. R. P & 2 TH.M.	8,637,000	5,109,000	7,780,000
3 T. G. L. & 4 T. Al. & Bact. Culture	7,778,000	5,560,000	7,560,000
3 T. G. L. & 4 T. Al. & 1 T. R. P. & Bac. Cul.	8,188,000	4,439,000	7,986,000
3 T. G. L. & 4 T. Al. & 1 T. R. P. & 2 T. H. M. & Bact. Culture	6,010,000	5,001,000	8,560,000

Abbreviations same as for Table 7.

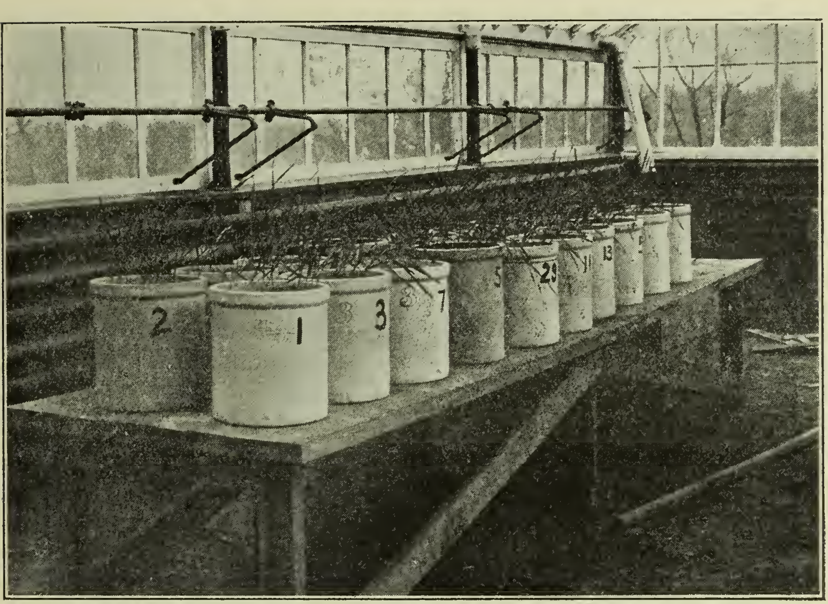

FIG 4.—Oats in Series I, January 9, note the uniform start.

Physiological Tests.—These tests were made each time with the quantitative bacterial determinations. The results were not satisfactory. This was due, in part, to faulty apparatus that has been remedied by purchasing new; and probably also to some fault in the technique which is being improved. The results for this reason are not included.

Vegetative Tests.

The vegetative tests were made by planting a second series of jars to oats. This series was treated just like the one used for the bacteriological tests already described. The oats were planted in duplicate jars Dec. 3, 1913. They came up well and were thinned to ten stalks per jar. They were watched each day and watered when necessary to keep them in good tilth. Rain water from a glass roof was used for the watering.

The oats were harvested, March 26, 1914, cutting the stalks just above the ground. The green and dry weights, and the number of milligrams of nitrogen per dry gram of substance were determined. The results are given in Table 9.

It will be seen that these results harmonize almost perfectly with the bacterial counts. It is very evident that the main thing lacking in this soil is organic matter. Where two tons of the alfalfa, only, were added the oats crop was practically five times that of the check jars with nothing added. (See Fig. 6.)

FIG. 5.—Oats in Series I, February 28, note the difference in growth due to treatment. (See Table 7.)

The addition of limestone in this particular case gave a very slight increase. It will be noted that the light dressing of horse and cow manures each gave a marked increase in the crop. It is further noted that where the bacterial culture was added in place of the light dressing of stable manure the increase in the oats crop was as great, in this case

TABLE 9.—The average bacterial counts, the weights and nitrogen content of the oats straw.

	TREATMENT.	Average of three Bacterial Counts.	OATS STRAW		Pounds of nitrogen per ton of Straw.
			Green Weight.	Dry Weight.	
1	Nothing added	4,272,000	29.85	7.8	11.2
2	No lime & 2 T. Al.	5,345,000	127.65	32.7	16.8
3	3 T. G. L. & 2 T. Al.	5,734,000	135,55	32,75	16,4
4	3 T. G. L. & 4 T. Al.	5,718,000	161,00	36.50	25.0
5	3 T. G. L. & 4 T. Al. & 2 T. H. M.	7,155,000	186.15	38.15	26.8
6	3 T. G. L. & 4 T. Al. & 2 T. C. M.	6,827,000	174.85	37.30	24.4
7	3 T. G. L. & 4 T. Al. & 1 T. R. P. & 2 T. H. M.	7,176,000	194.50	42.00	23.2
8	3 T. G. L. & 4 T. Al. & Bac. Cul.	6,966,000	191.00	41.15	23.8
9	3 T. G. L. & 4 T. Al. & 1 T. R. P. & Bac. Culture	6,871,000	186.10	40.95	23.8
10	3 T. G. L. & 4 T. Al. & 1 T. R. P. & 2 T. H. M. & Bac. Culture	6,524,000	171.90	38.55	23.0

Abbreviations same as for Table 7.

slightly greater, than where the horse manure or cow manure was applied. This indicates that the benefit of a slight dressing of stable manure, when added with a green manure is due in large part at least to the increased bacterial activity induced by adding the bacteria with this manure (See Fig. 4 and 5).

FIG. 6.—Oats in Series I, January 26; Nos. 1 and 2 nothing added, 3 and 4 two tons of alfalfa per acre.

The beneficial effect of the bacterial culture was readily noted in the oats crop to which these cultures had been added by the darker green color and the ranker growth.

The pots receiving both the light dressing of stable manure and bacterial culture; did not give as large a growth as where these were applied separately. This effect is also noted in the smaller bacterial count. (See Table 9.) Just to what this effect was due is not evident. It may be that there was some antagonism between the organisms of the bacterial culture and those in the stable manure. It does not appear that it could have been due to an acid reaction of the soil since there was an abundance of limestone added.

It should be noted that the nitrogen content of the oats straw varied with the amount of fertilizer added. In the jars where nothing was added the analysis shows only 11.2 pounds of total nitrogen per ton of oats straw; where the two tons of alfalfa was turned the analysis showed 16.8 and 16.4 pounds of nitrogen respectively; and with 4 tons of green manure gave an analysis of 25 pounds of nitrogen per ton of straw. Not only does the better fertility give a better growth; but also a better quality of feed as shown by the greater amount of nitrogen present.

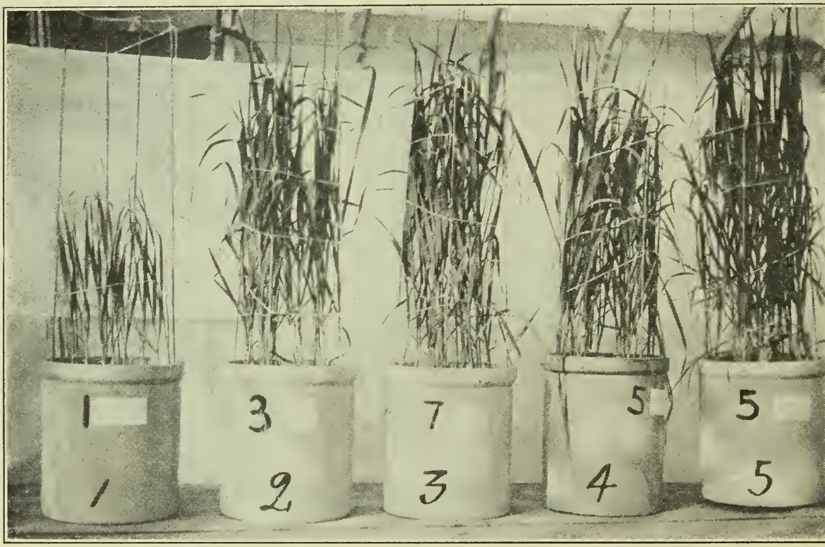

FIG. 7.—Oats in Series I, January 26 (Lower Numbers apply).

1. Nothing added.
2. Two tons of alfalfa per acre. (No lime.)
3. Two tons of alfalfa and three tons lime per acre.
4. Four tons alfalfa and three tons lime per acre.
5. Four tons alfalfa, three tons lime per acre and bacterial culture added

CONCLUSIONS.

1. There is a direct relation between the bacterial count and the amount of organic matter added.

2. The quantitative bacteriological test and the vegetative test agree very uniformly.

3. A light dressing of stable manure with a green manure gives a marked effect as shown both by the crop grown and the bacterial counts.

4. The addition of a bacterial culture along with the green manure has as great an effect as the addition of the light dressing of stable manure which indicates that the benefit of the addition of the stable manure is due largely to the addition of the bacteria contained in the manure.

5. The addition of organic matter gives not only a larger growth but a better quality of feed as shown by the analysis for total nitrogen in the straw.