Station Bulletin 118 June, 1914

OREGON AGRICULTURAL COLLEGE
EXPERIMENT STATION

Department of Bacteriology

Ammonification and Nitrification Studies of Certain Types of Oregon Soils

BY

T. D. BECKWITH, Bacteriologist
A. F. VASS, Research Assistant in Bacteriology,
and R. H. ROBINSON, Research Assistant in Chemistry

Corvallis, Oregon

Board of Regents of the Oregon Agricultural College and Experiment Station.

HON. J. K. WEATHERFORD, President ..... Albany
HON. E. E. WILSON, Secretary ..... Corvallis
HON. B. F. IRVINE, Treasurer ..... Portland
HON. OSWALD WEST, Governor of the State ..... Salem
HON. J. A. CHURCHILL, State Superintendent of Public Instruction ..... Salem
HON. BEN W. OLCOTT, Secretary of State, ... Salem
HON. CHARLES E. SPENCE, Master of State Grange ..... Canby
MRS. CLARA H. WALDO, ... Portland
HON. WALTER M. PIERCE, ... LaGrande
HON. J. T. APPERSON, ... Oregon City
HON. C. L. HAWLEY, ... McCoy
HON. H. VON DER HELLEN, ... Wellen
HON. G. M. CORNWALL, ... Portland

The Station Staff.

W. J. KERR, D. Sc., ... President
†A. B. CORDLEY, M. S., ... Director

Department of Agronomy.

H. D. SCUDDER, B. S., ... Agronomist
G. R. HYSLOP, B. S., ... Assistant Crops
W. L. POWERS, M. S., ... Assistant Irrigation and Drainage
M. M. McCOOL, Ph. D., ... Assistant Soils
J. E. COOTER, ... Assistant Soils Laboratory

Department of Animal Husbandry.

E. L. POTTER, B. S. ..... Animal Husbandman
G. R. SAMSON, A. B., B. S. ..... Assistant Animal Husbandman
O. M. NELSON, B. S. A. ..... Assistant Animal Husbandman
C. E. ROBINSON, ... Foreman College Stock Farm

Department of Bacteriology.

T. D. BECKWITH, M. S., ... Bacteriologist
A. F. VASS, M. S., ... Assistant Soil Bacteriology

Department of Botany and Plant Pathology.

H. S. JACKSON, A. B., ... Botanist and Plant Pathologist
H. P. BARSS, M. S., ... Assistant Research
F. D. BAILEY, A. B., ... Assistant Crop Pest Investigation
J. R. WINSTON, M. S. ..... Plant Pathologist, Hood River, Ore.
G. H. GODFREY ..... Laboratory Assistant

Department of Dairy Husbandry.

ROY R. GRAVES, M. S. ..... Dairy Husbandman
OTTO G. SIMPSON, B. S., ... Assistant Dairy Manufacturing
E. R. STOCKWELL, B. S., ... Assistant

Department of Chemistry.

H. V. TARTAR. B. S., ... Chemist
BERT PILKINGTON, B. S. ..... Assistant Research
R. F. BEARD, B. S. ..... Assistant Chemist
R. H. ROBINSON, M. S. ..... Assistant Chemist

Department of Entomology.

H. F. WILSON, M. S. ..... Entomologist
V. I. SAFRO, B. S. A., ... Assistant Research
A. L. LOVETT, B. S., ... Assistant Crop Pest Investigation

Department of Horticulture.

C. I. LEWIS. M. S. A., ... Horticulturist
V. R. GARDNER, M. S., ... Associate Pomology
E. J. KRAUS, B. S., ... Associate Research
A. G. B. BOUQUET. B. S., ... Assistant Olericulturist
F. C. BRADFORD, M. S., ... Assistant Research
F. R. BROWN. B. S., ... Assistant Crop Pest Investigation
G. S. RALSTON, B. S. ..... Assistant Research
C. C. STARRING, B. S., ... Assistant Hood River Horticulturist
A. M. WOODMAN B. S., ... Orchard Foreman

Department of Poultry Husbandry.

JAMES DRYDEN, ... Poultry Husbandman
C. C. LAMB, ... Foreman Poultry Plant

HELEN L. HOLGATE, B. S., ... Station Clerk
ROBERT WITHYCOMBE, B. S., ..Superintendent Eastern Oregon Substation, Union
D. E. STEPHENS, B. S., .Superintendent Eastern Oregon Dry-Farm Substation. Moro
L. R. BREITHAUPT, B. S. ..... Superintendent Harney Substation, Burns
F. C. REIMER, M. S., ... Superintendent Southern Oregon Substation, Talent
R. W. ALLEN. M. S. ..... Superintendent Umatilla Substation. Hermiston

---

†Elected Director of the Experiment Station and Dean of the School of Agriculture, April 8, 1914.

THE PURPOSE OF THE EXPERIMENTS.

1. The purpose of the experiments reported was to learn something of the number and kinds of bacteria, in a few widely different types of Oregon soils, and the effect of moisture and lime upon these numbers.

2. Also to determine the ammonifying powers of the soils with varying moisture content in order to ascertain the optimum condition.

3. To study the effect of soil acidity and of lime upon ammonification and nitrification.

4. To make a comparative study of the ammonifying and nitrifying powers of the six types of Oregon soils mentioned, and to correlate the number of bacteria present and the amounts of ammonia and nitrate nitrogen produced.

DESCRIPTION OF THE SOIL TYPES STUDIED.

Type I. Hermiston. This is a fine sandy soil, high in lime content, but very low in organic matter. The sample used was taken from the Umatilla Experiment Station at Hermiston, Oregon.

Type II. Redmond. This is a sandy loam soil, low in organic matter and high in lime. The sample was taken from near Redmond, Oregon, and was representative of the uncultivated soil.

Type III. Moro. A silt loam, upland soil, well supplied with lime and potash. The sample was sent in by Supt. D. E. Stephens from the sub-station at Moro, Oregon.

Type IV. O. A. C. This sample was taken from the College Farm at Corvallis. It is a silt loam deficient in lime and nitrogen.

Type V. Beaverdam. This is classified as muck soil, very low in lime and especially high in organic matter. The sample was sent in by Mr. Clyde Leedy from his farm at Sherwood, Oregon.

Type VI. Clatskanie. This is a peat soil, high in organic matter and low in lime content. It is representative of the fresh water tide land of the lower Columbia valley. The sample studied was taken from the gardens of the Columbia Agricultural Company of Clatskanie, Oregon.

TABLE A

THE CHEMICAL COMPOSITION OF THE SOILS.

	Total Potash K_2O	Total Lime CaO	Total Magnesia MgO	Total Phosphorus Pentoxide P_2O_5	Total Nitrogen N	Acidity Calculated as $CaCO_3$	Alkalinity in the form of $CaCO_3$
	%	%	%	%	%	%	
Hermiston	1.13	5.56	....	0.27	0.002	None	0.115
Redmond	1.59	5.76	3.11	0.22	0.05	None	0.125
Moro	2.04	2.15	0.78	0.27	0.12	None	0.042
O. A. C.	2.77	1.10	1.80	0.44	0.23	0.018	None
Beaverdam	1.032	3.38	0.72	0.29	1.46	0.015	None
Clatskanie	1.55	1.08	0.73	0.26	1.05	0.025	None

THE METHODS EMPLOYED.

The soils used in this work were shipped to the laboratory in clean sacks of double thickness. They were well mixed, uniform samples representative of the field from which they were taken. Upon reaching the laboratory they were placed in large containers and kept in a favorable condition until ready for use. The soil in each container was then thoroughly mixed, air dried, and possed through a twenty-mesh sieve.

In studying the effects of lime upon the number of bacteria, small amounts of the soils were treated at the rate of two tons per acre-foot of soil. Counts were made at the beginning of the experiment and at the end of four weeks, using checks. Modified synthetic agar[1] was used for pouring the plate. Dilutions of one to twenty thousand, and one to one hundred thousand were used, and all plates were incubated for three days at 28°C. Determinations were made of the amount of moisture in the samples and also the total number of bacteria per gram of air-dried soil.

In the ammonification experiments the soils were treated by the addition of lime, blood meal, and peptone. The "flask method,,[2], as we called it, was employed in these experiments, two hundred grams of the soil being used in each flask. All samples were air dried and had been passed through a twenty-mesh sieve. Known amounts of soil infusion were used, and the soils moistened to the desired moisture content. Lime, in the form of carbonate, was added to some of the samples, lime and blood meal to some, and lime and peptone to others. Lime was applied at the rate of two tons per acre-foot of soil. The samples were allowed to run for ten days when analyses were made to determine the total amounts of ammonia formed.

The rate of certain phases of the decomposition of protein substances in the soil may be measured approximately by estimating the amounts of ammonia formed. There are, however, two factors that enter into the computations making this determination difficult; the first is, the escaping of the ammonia into the air; and the second, the changing of the ammonia formed into nitrites and nitrates, or into protein substances by bacteria.

It was to overcome this first loss that we used what we called the "flask method"[2]. Two hundred grams of soil were placed in a 600 cc. flask moistened with soil infusion and water. The flask was plugged with a two-hole rubber stopper. In the stopper were fitted two pieces of glass tubing, one tube bent over and down, and open at the end to allow the air to enter the flask, and the second so connected with a wash bottle containing an acid solution of known

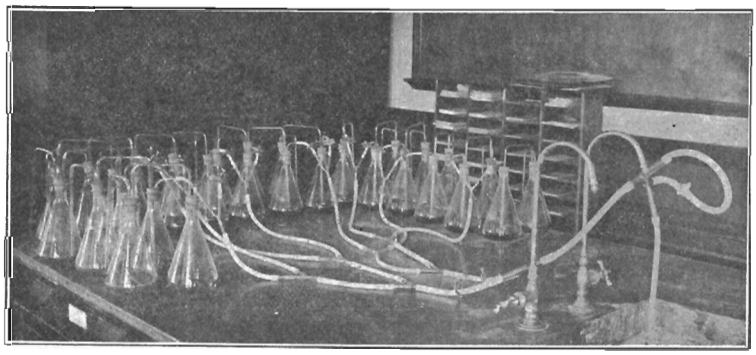

Figure A. The "flask method" as used in the ammonification experiments.

strength, that the end of the tube was below the surface of the liquid. Tenth-normal hydrochloric acid was used, with cochineal as an indicator. By means of a suction pump, a steady current of air was kept flowing through the flask. At the end of the experiment the acid solution was titrated, and the amount of ammonia determined. By this method, no ammonia was lost by escaping into the air.

The second factor was overcome to some extent by adding blood meal and peptone. This was done to encourage the intensity of the multiplication of the ammonifying bacteria, and for the time being, to check the activities of the nitrite and nitrate forming organisms, whose action changed the ammonia formed into nitrites and nitrates.

In the nitrification experiments the soils were prepared in the same manner as in the ammonification work. A method similar to the beaker method was used, two hundred grams of soil being introduced into pint Economy jars. The experiment was allowed to run four weeks. Determinations of total amounts of nitrate nitrogen were made at the beginning, in two weeks, and at the end of four weeks. Many nitrogenous materials may be used for this work, as has been shown by Wagoner and Dorsch[3], but, considering everything, it was deemed advisable to use ammonium sulphate and blood meal at the rate of one per cent. Distilled water was used in all the work, and varying moisture contents were maintained by weighings made every day. The samples were kept at room temperature. This might lead to some slight variation in results, due to changes in temperature, which has been found to have a marked effect upon the nitrification process. Davy[7], first found this to be true, and later Brown[5] and others.

The chemical methods employed were as follows: In the ammonification experiments the ammonia was determined by the A. O. A. C.[6] method. A fifty-gram sample of soil was introduced into an 800 c.c. Kjedahl flask with 150 c.c. of distilled water. An excess of magnesium oxide was added, the ammonia distilled off and collected in tenth-normal acid and titrated with alkali of the same strength.

In the determinations of the total nitrate nitrogen, the colorimetric method was used. A weighed quantity of the soil, usually 50 grams, was titurated thoroughly in a mortar with a convenient amount of distilled water and then transferred to a beaker, using enough water to make the total amount 250 c.c. After stirring occasionally during half an hour, the heavy particles were allowed to settle and the supernatant liquid decanted through a filter. In cases where this soil extract did not come through clear, as was usually the case, pure carbon black was added to about 50 c.c., agitated a moment, and the liquid then filtered through a Gooch cruciblue, using suction. Twenty-five c.c. of the clear soil extract were then evaporated to dryness, and the amount of nitrogen in the form of nitrates was determined by the phenolsulfonic acid method. The results were calculated in terms of the nitrate radicle, NO_3.

THE INFLUENCE OF LIME ON THE NUMBER OF BACTERIA IN CERTAIN OREGON SOILS.

Lime has long been used as a soil amendment. The use of marl and ashes was common among the Greeks and Romans at an early date. In England lime was commonly used during the latter part of the nineteenth century; and pits were frequently sunk in a field from which chalk was taken to apply to the land. Lime has been used to some extent as a means of soil improvement in the North Atlantic States. The use of commercial fertilizers during the last few decades has to some extent checked the use of lime. Not that lime is a fertilizer, or that the fertilizer will serve the same purpose as the lime, but because the farmer will seldom add both. Not all soils require the application of lime, its benefits being confined almost entirely to those soils showing an acid condition. The effect of lime on the soil may be divided into the following divisions:

First. Physical effects. Lime flocculates the finest particles in a clay soil and promotes a granular structure. It improves the tilth, increases aeration, and improves the water circulation. Lime is supposed to produce porosity in a light sandy soil and increase the water-holding power.

Second. Chemical effects. Soils contain a large amount of plant food that is unavailable in its present condition. Especially is this true of potash and phosphorus. Lime acts as a liberating agent, changing these insoluble, unavailable substances into soluble, available plant food. It neutralizes acids and keeps the soil in an alkaline condition. Leaching may also be prevented, as the acid of humus substances is somewhat soluble, while their lime compounds are comparatively insoluble.

Third. Biological effects. Lime is an essential element for all of the higher plants and probably all of the lower forms. It also corrects those acid conditions in the soil, which cause a diminished food supply and have a direct physiological effect on the plants themselves, promoting that neutral or silghtly alkaline condition which is most favorable to the majority of cultivated plants. Lime aids the growth of many types of bacteria, especially those which convert organic nitrogen to nitrates, and those which assimilate atmospheric nitrogen.

Figure 1 shows the relative number of bacteria in Oregon soils and the effect of lime, blood meal, and peptone upon

these numbers. Beaverdam soil contains the greatest number of bacteria per gram in the normal and limited samples, but when blood meal is added, the increase in number is not so

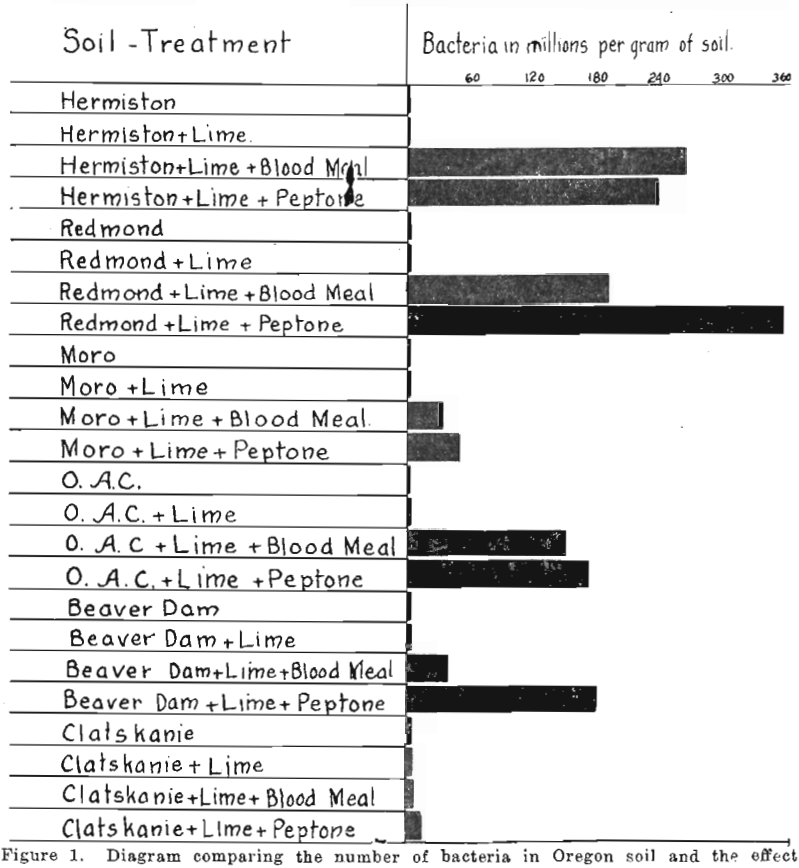

Figure 1. Diagram comparing the number of bacteria in Oregon soil and the effect of lime, blood meal, and peptone upon these numbers.

great in proportion as in the soils low in humus. The marked effect of lime upon the number of bacteria in Clatskanie, Beaverdam, and O. A. C. soils is shown in Figure 2. These soils being deficient in lime, the neutralization of the acidity brings about a favorable condition for the growth of the bacteria, which would result in an increase in the amount of available plant food. Blood meal and peptone had very little effect upon Clatskanie soil. This was perhaps due to the large amount of organic material already present. The plates poured from this sample showed a high count of moulds and a low

count of bacteria; but when lime was applied, the order was reversed, the bacteria making a marked gain and the moulds showing a decided decrease.

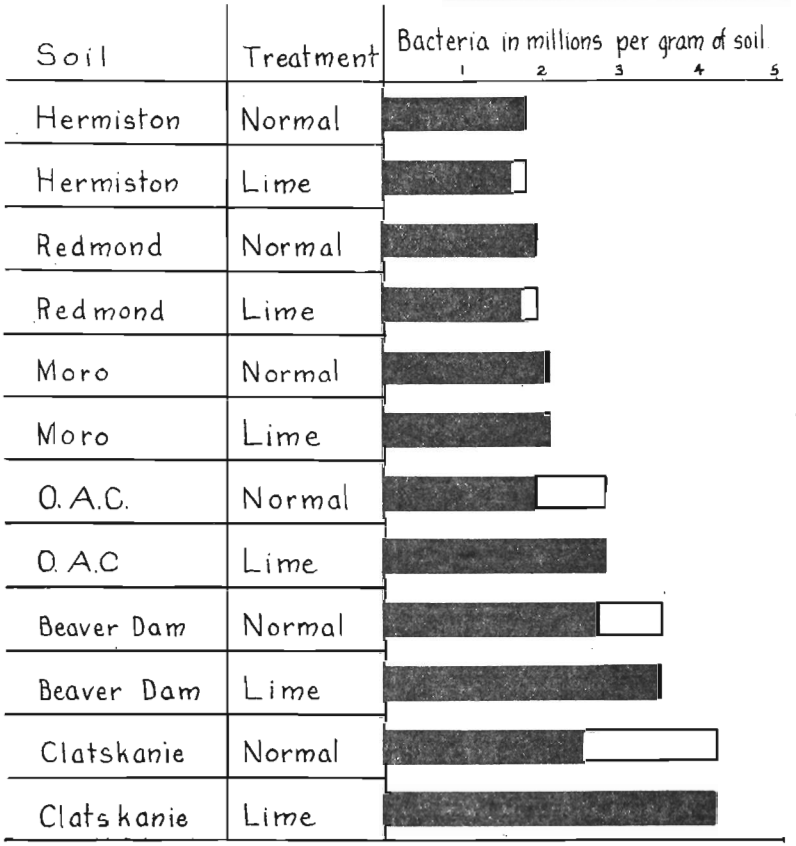

Figure 2. Diagram showing the number of bacteria in Oregon soils under normal conditions and when lime was added. The unshaded portions show the increase or decrease as influenced by lime.

Fewer bacteria were found in the Hermiston soil when peptone had been added than in the same soil when blood meal had been added. This was probably due to the fact that the Hermiston soil is sandy and well aerated, thus allowing a rapid decomposition of the peptone, so that at the end of the experiment, when the bacteriological count was made, the numbers and activities of the organisms were on the decline.

There has been considerable discussion as to the correlation between the number of bacteria and their physiological

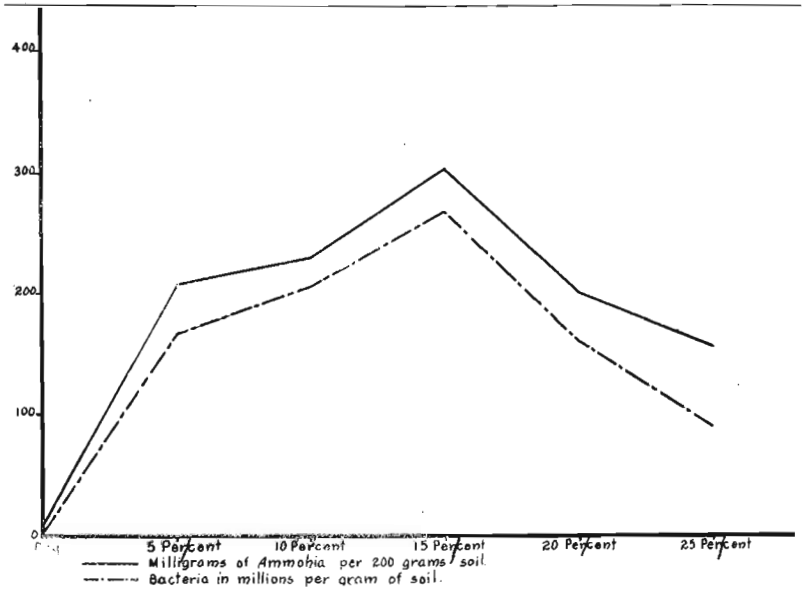

Figure 3. The effect of moisture content on number of bacteria and amounts of ammonia formed in Hermiston soil when blood meal was added. The correlation between the number of bacteria and the ammonia produced is shown.

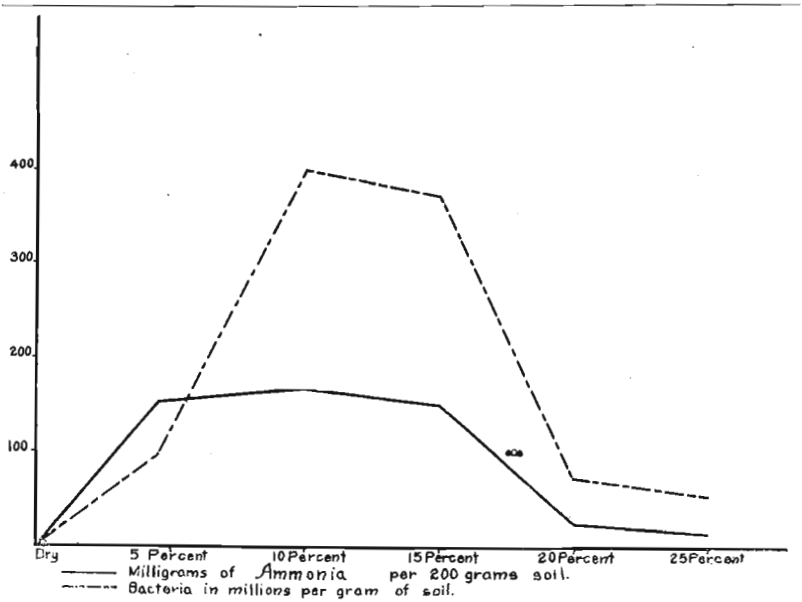

Figure 4. The effect of moisture content on number of bacteria and ammonia formed in Hermiston soil when peptone was added. The correlation between number of bacteria and ammonia produced is shown.

activities in the soil. Figure 3 shows the correlation of the milligrams of ammonia given off together with the number of bacteria, in Hermiston soil to which peptone had been added at the rate of one per cent, with varying amounts of moisture. Figure 4 shows the same thing in the same soil when blood meal was used as a fertilizer. Contrary to the findings of some investigators, there seems to be a close relationship between the increase in number and the amount of ammonia produced.

AMMONIFICATION EXPERIMENTS.

The transformation of the nitrogenous substances into ammonia is one of the most important phases of the nitrogen cycle in soils. It represents a certain stage in the process of decomposition and bears a close relation to soil fertility problems. The chemical reactions, of which amomnia is one of the end products, are quite complicated and depend upon many factors. For example, the moisture and temperature of the soil, as well as its physical and chemical composition, play an important part in determining the amount of ammonia produced. It is safe to say, nevertheless, that the rate of certain phases of the decomposition of protein substances in the soil may be measured approximately by estimating the amount of ammonia formed.

Ammonia determinations were made at the beginning and at the end of the experiment. The soil in each instance received the following treatment:

1st. Normal soil.
2nd. Normal soil plus lime.
3rd. Normal soil plus lime plus peptone.
4th. Normal soil plus lime plus blood meal.

The blood meal and peptone were added at the rate of one per cent; and the lime was applied at the rate of two tons per acre-foot of soil. The experiment was allowed to run ten days, after which ammonia determinations were made. The soils were kept at room temperature during the experiment. Varying amounts of moisture were used, which consequently controlled to a great extent the aeration of the soil. This in itself would have a marked effect upon the rate of ammonification, as has been noted by Pasteur[7] who was among the first to point out the effect of aeration on the rate of decomposition. Marchal[8] also found that temperature,

aeration, reaction, and concentration had a marked effect upon ammonification.

TABLE I.

Milligrams of ammonia produced in Hermiston soil with varying moisture content when rteated with Lime, Blood Meal, and Peptone.

TREATMENT	Air Dry	5%	10%	15%	20%	25%	TOTAL
Normal	.6	1.8	2.0	1.4	2.0	1.0	8.8
Limed	2.2	1.1	1.6	1.4	2.4	1.0	9.7
Lime and Blood Meal	4.4	156.6	166.8	153.1	21.9	17.5	520.3
Lime and Peptone ...	8.7	207.4	228.8	304.0	170.5	161.7	1080.6

The results of ammonification tests with Hermiston soil are given in Table I. There was very little difference between the unlimed and limed samples, the treatment having no noticable effect. The rate of ammonification in this soil was relatively high. As summarized by Löhnis and Greene[9], ammonification as a whole proceeds much more rapidly under aerobic than anaerobic conditions. It is believed that aerobic conditions favor more especially those latter stages in the breakdown which results in formation of ammonia itself. Rahn[10] finds that the aeration increases with the square of grain size. Hermiston soil being composed chiefly of sand, would

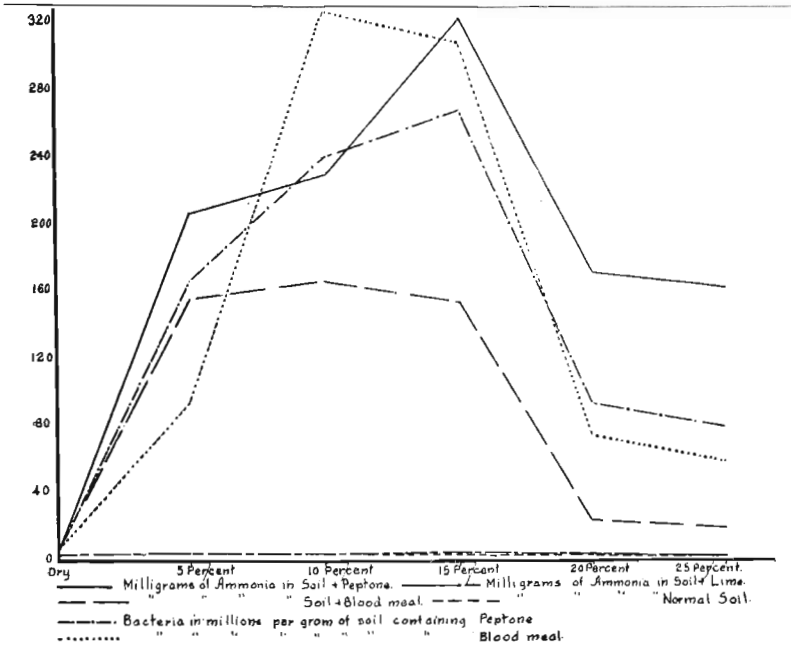

Figure 5. The effect of moisture content on ammonification and number of bacteria in Hermiston soils.

therefore be favorable to aerobic bacteria, as our findings in this soil have shown. This is in keeping with the results of the investigators mentioned above. Very small amounts of ammonia were formed in the normal soil, due to the fact that the samples were relatively low in organic matter. By the addition of blood meal and peptone, the ammonifying power is shown, indicating a very high efficiency for this soil.

By varying the moisture content, we find the optimum per cent of moisture is between ten and fifteen. Higher moisture content retards the ammonification, due, probably, to the creating of anaerobic conditions. In Figure 5 we find a comparison of the relative rate of ammonification in normal, limed, blood meal, and peptone soils. We find also the proportion of the number of bacteria to the amount of ammonia produced.

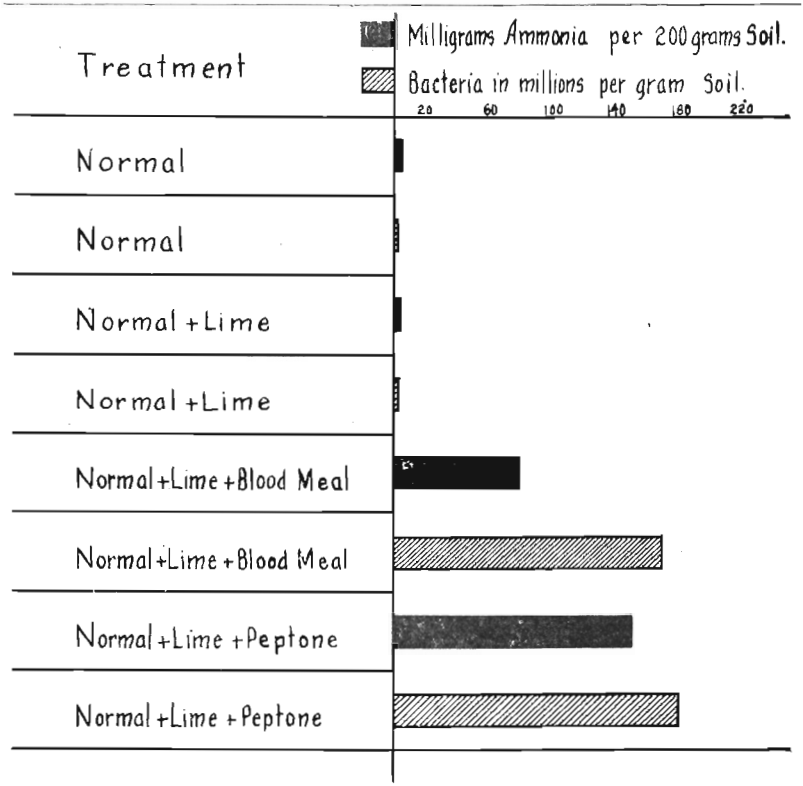

Figure 6. The effect of lime, blood meal, and peptone on the rate of ammonification and number of bacteria in Hermiston soils.

The optimum moisture content for the production of ammonia is also the content at which the number of bacteria is greatest.

Figure 17 shows the relative amount of ammonia produced in the Hermiston soil compared with the other types of Oregon soil. We find here that the ammonifying power of this soil is high when fertilizers are used, but that under normal conditions it is very low. In this experiment, however, the soils were kept at room temperature, which might lead to a slight variation in the results.

When the organisms in the Hermiston soil were considered according to number, their ammonifying power was low. Counts made at the end of the experiment showed that for every milligram of ammonia that had been produced there were then present in the soil 400,000,000 bacteria. When compared with the Clatskanie samples, we find that although the ammonifying power of the Hermiston soil is greater, the ammonifying power of the individual organisms is less.

TABLE II.

Milligrams of ammonia produced in Redmond soil with varying moisture content when treated with Lime, Blood Meal, and Peptone.

TREATMENT	Air Dry	10%	15%	20%	25%	30%	TOTAL
Normal	2.6	2.3	1.0	1.6	5.3	4.0	16.8
Normal and Lime....	2.8	2.9	2.4	2.5	2.0	5.3	17.9
Lime and Blood Meal	5.6	144.8	177.0	173.5	149.7	41.4	692.0
Lime and Peptone ...	5.6	280.7	281.2	280.7	288.3	272.9	1409.4

Table II shows the rate of ammonification in the Redmond soil. In this soil, as in the Hermiston sample, we find that lime has no effect upon the rate of ammonification, and that in some cases the production of ammonia is retarded when lime is applied. This is perhaps due to the fact that this soil normally contains a large amount of lime.

A comparison is made in Figure 7 of the quantity of ammonia in a normal and a limed soil, treated and untreated, and the proportion of the number of bacteria to the amounts of ammonia produced. As in the Hermiston soil, there is a very close relation between the number of bacteria present and the amount of ammonia formed.

Figure 8 shows the increase in the ammonification and the number of bacteria when blood meal and peptone were added. The Redmond soil leads all others in the rate of ammonification when fertilizers are present, as is shown in Figure 17. Evidently the two causes, good aeration and lime supply,

have been operative in bringing about this result. The available plant food already in the soil might be expected to bring about greater ammonifying efficiency than would be found in the Hermiston sample, and such appears to be the case.

The optimum moisture content for Redmond soil is between 15 and 20%, since at these two figures we find the greatest amount of ammonia produced. In case of the soil treated with blood meal, there was a gain of 32.2 mgs. of ammonia when the moisture was increased from 10 to 15%, and a drop

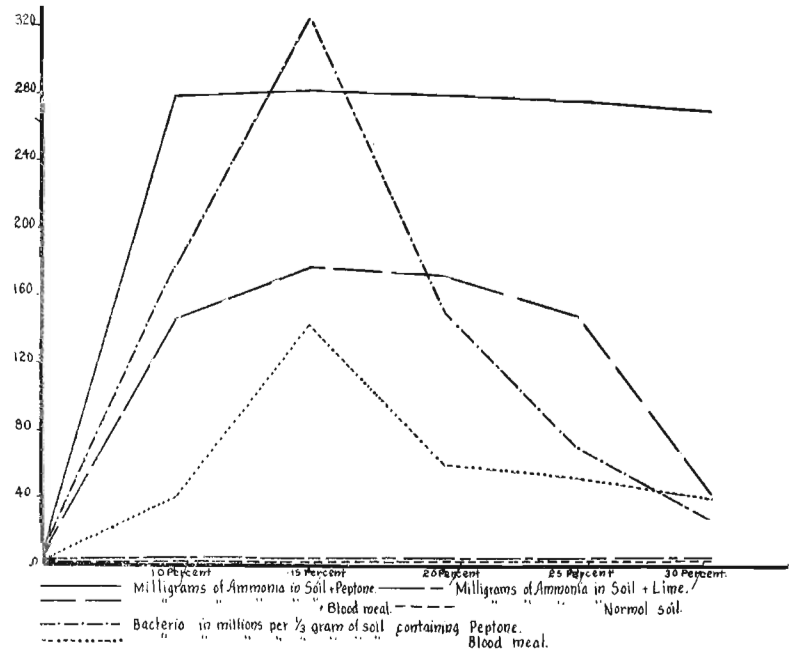

Figure 7. The effect of moisture content on ammonification and number of bacteria in Redmond soils.

of 23 mgs. ammonia when the moisture content was raised to 20%. There was very slight variation in the amounts of ammonia produced in the soil containing peptone when the moisture content was changed.

The ammonifying power of the soil was very high, as was also the number of bacteria. When the organisms were considered according to number, their ammonifying power was rather low, being in the class with the Hermiston soil.

TABLE III.

Milligrams of ammonia produced in Moro soil with varying moisture content when treated with Lime, Blood Meal, and Peptone.

TREATMENT	Air Dry	10%	15%	20%	25%	30%	TOTAL
Normal	3.4	0.8	2.4	6.7	1.9	3.7	18.9
Limed	3.4	1.1	3.0	7.5	2.7	4.9	22.6
Lime and Blood Meal	6.1	157.8	184.6	168.1	117.6	31.6	665.3
Lime and Peptone .	6.1	248.0	263.8	267.6	254.0	192.9	1232.4

The results of ammonification experiments with the Moro soil are tabulated in Table III. This sample responded slightly to the application of lime, and ranks high in the ammonifying efficiency as shown by the text. Normally, the soil contains plenty of lime, hence no marked results would be expected by such treatment.

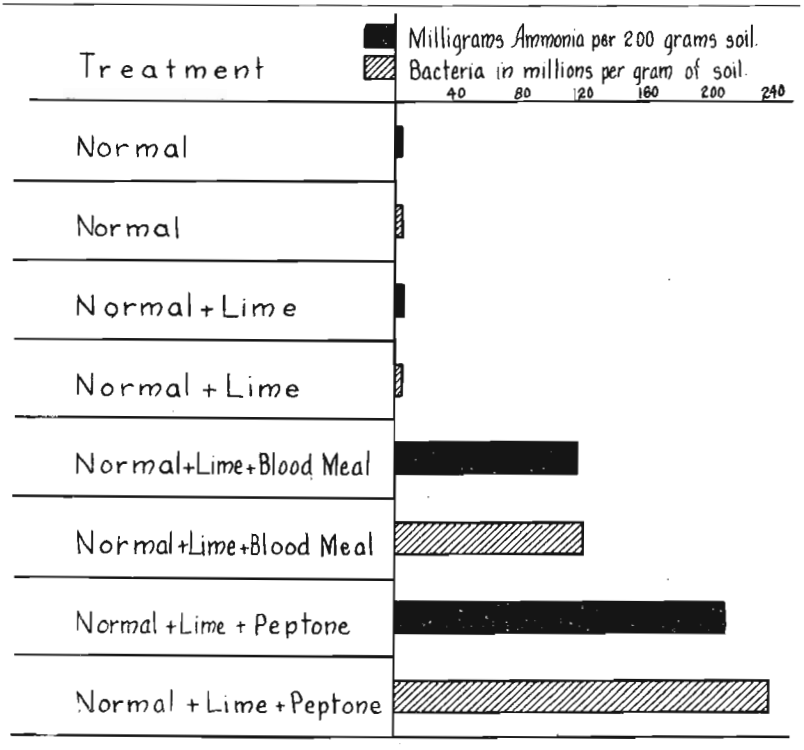

Figure 8. The effect of lime, blood meal, and peptone on ammonification, and number of bacteria in Redmond soils.

The correlation between the amount of ammonia produced and the number of bacteria, is shown in Figure 10. There is a direct relation between these two, and the results compare favorably with the results from the other types studied.

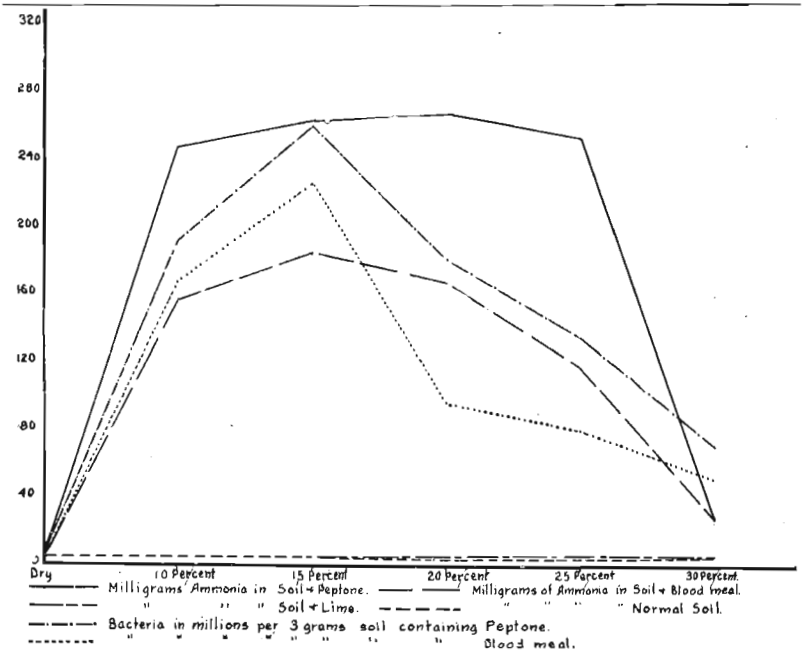

Figure 9. The effect of moisture content on ammonification and number of bacteria in Moro soils.

The ammonifying power of the organisms considered according to number is very much greater than the organisms in the Redmond and Hermiston soils. There is a marked drop in the number of bacteria per gram of soil when the moisture content goes above 15%. The rise and fall in the curves, showing the number of bacteria, is always more abrupt than the curve illustrating the amount of ammonia formed.

The rate of ammonification of the peptone did not vary to any marked extent from 10 to 25%. There was an abrupt drop when the moisture was increased to 30%. The maximum amount of ammonia was produced in the peptone and normal soils when 20% moisture was present.

The blood meal sample showed its greatest gain at 15% moisture. At 20% there was a drop of 9% in ammonia pro-

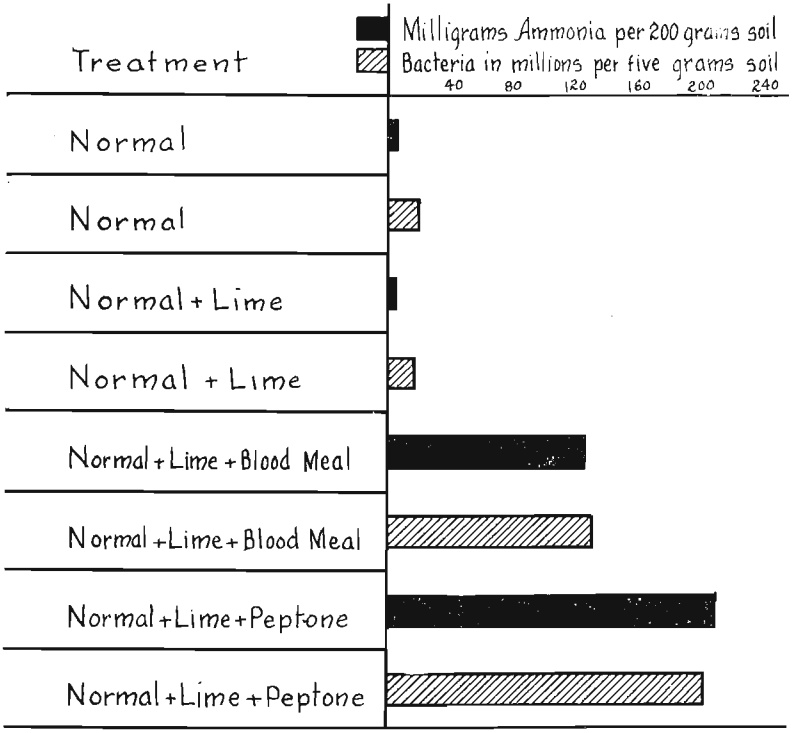

Figure 10. The effect of lime, blood meal, and peptone on ammonification and number of bacteria in Moro soils.

duction, and above that point the drop was greater.

We find in Figure 17 that the ammonifying power of Moro soil ranks next to Redmond, placing it second in the series of the types of Oregon soils studied. In the normal and limed soils only small amounts of ammonia were produced, but when blood meal and peptone were added, large amounts of ammonia were given off, thus showing the strong ammonifying powers of the soil.

The O. A. C. sample used in these experiments was slightly acid. Table IV contains the results of the ammonification

TABLE IV.

Milligrams of ammonia produced in O. A. C. soil with varying moisture content when treated with Lime, Blood Meal, and Peptone.

TREATMENT	Air Dry	10%	15%	20%	25%	30%	TOTAL
Normal	6.0	6.9	10.4	8.6	8.3	9.4	49.6
Limed	6.0	6.9	10.4	9.4	9.0	9.0	50.7
Lime and Blood Meal	6.4	139.4	142.5	122.3	62.0	18.3	490.9
Lime and Peptone ...	10.3	258.6	266.9	229.7	223.8	147.5	1136.8

experiments with this soil. Up to 15% moisture there was no difference in the amount of ammonia produced in the normal and the limed sample. Above that point there was a noticeable increase in the amount of ammonia formed when lime was added.

Figure 11 shows the rate of ammonification with different moisture content in normal and treated soils; it shows also the effect of these factors upon the number of bacteria. The ammonifying efficiency of O. A. C. soil is normal, being lower

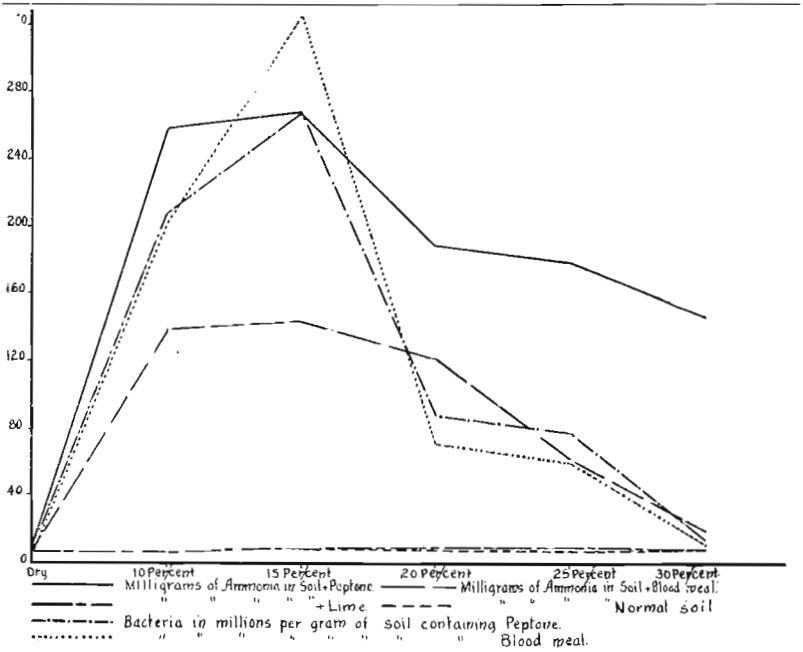

Figure 11. The effect of moisture content on ammonification and number of bacteria in O. A. C. soils.

than the coarse grained soils of Eastern Oregon, and higher than the muck soils in the Willamette and Columbia River Valleys.

In this soil, as in the Redmond sample, the amount of moisture present did not seem to have a very marked effect on the rate of ammonia formed in soils containing peptone. The soils containing blood meal, however, responded more readily. The normal and limed soils showed a noticeable increase at 15% moisture, but above that point the amount

remained about the same. The blood meal and peptone samples also reached a maximum efficiency at this moisture content.

The comparative amount of ammonification of O. A. C. soils with other Oregon types is brought out in Figure 17. Both when treated and untreated, the Clatskanie and Beaverdam soils ranked above it in the unfertilized tests; and Redmond and Moro soils ranked above it when blood meal and

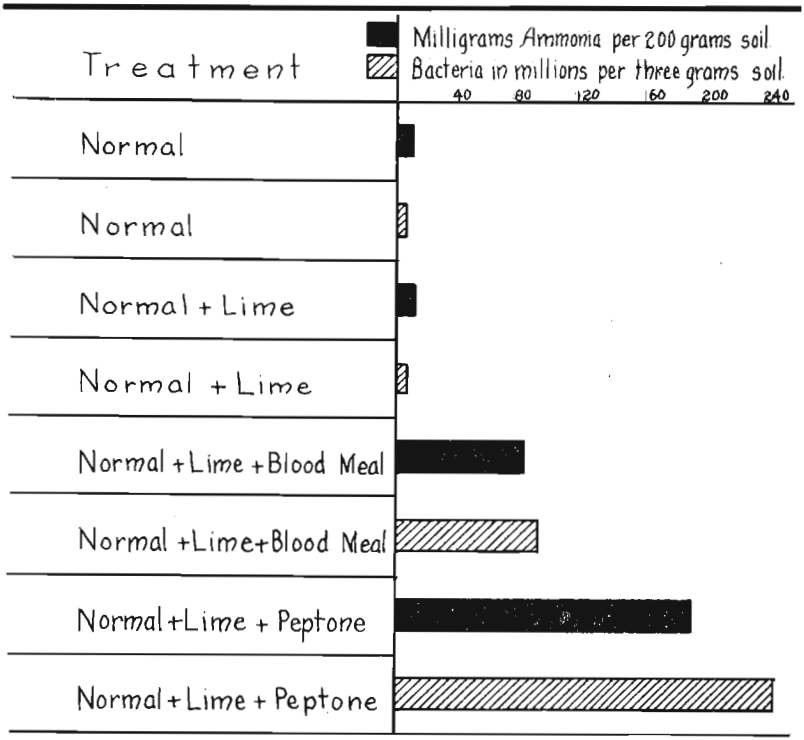

Figure 12. The effect of lime, blood meal, and peptone on ammonification and number of bacteria in O. A. C. soil.

peptone were added. The peptone was acted upon more readily than was the blood meal. There were two hundred million bacteria at the end of the experiment for every milligram of ammonia that had been formed. The ammonifying power of the bacteria considered according to number, was relatively lower than the organisms in the Moro soil, where there were only forty million bacteria per milligram of ammonia produced.

TABLE V.

Milligrams of ammonia produced in Beavedam soils with varying moisture content when treated with Lime, Blood Meal, and Peptone.

TREATMENT	Air Dry	10%	20%	30%	40%	50%	TOTAL
Normal	40.2	34.2	39.4	30.0	47.2	56.4	247.4
Normal and Lime ...	34.6	33.6	40.2	34.4	45.8	66.0	254.6
Lime and Blood Meal	43.6	33.2	65.2	135.2	140.8	77.8	495.8
Lime and Peptone ...	46.6	97.8	205.4	259.6	278.0	234.4	1116.8

In Table V are reported the results of the ammonification experiments with Beaverdam soil. In this soil, which contains a large amount of organic matter, we find the rate of ammonification normally high. This is probably due to the large amount of organic matter present in the soil. The relative rate of ammonification when lime, blood meal, and peptone were added, is shown in Figure 13. The difference due to the application of lime stands out very prominently. The sample containing 30% moisture gave 30 mgs. of ammonia, and 34.4 mgs. of ammonia for the normal and limed soil respectively, showing the marked gain of over 13% due to the application of lime.

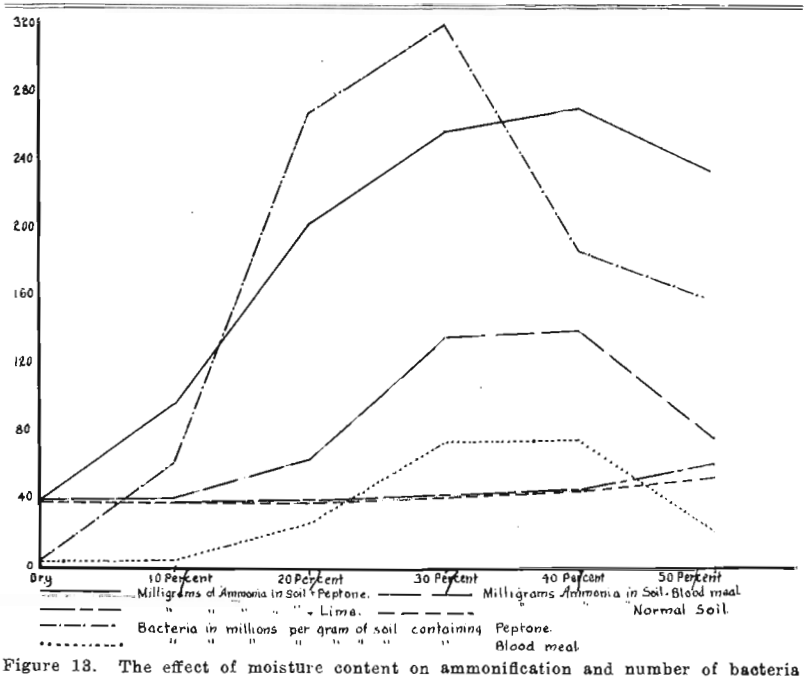

Figure 13. The effect of moisture content on ammonification and number of bacteria in Beaverdam soil.

The peptone was acted upon far more readily than the blood meal. Figure 14 shows this relation when an average of the soils at varying moisture contents was taken. The total amount formed in the six samples was 495.8 mgs. and 1116.8 mgs. ammonia in the blood meal and peptone soils respectively. The large amount of ammonia formed in the normal soil was probably due to the high percentage of organic matter already present in the sample.

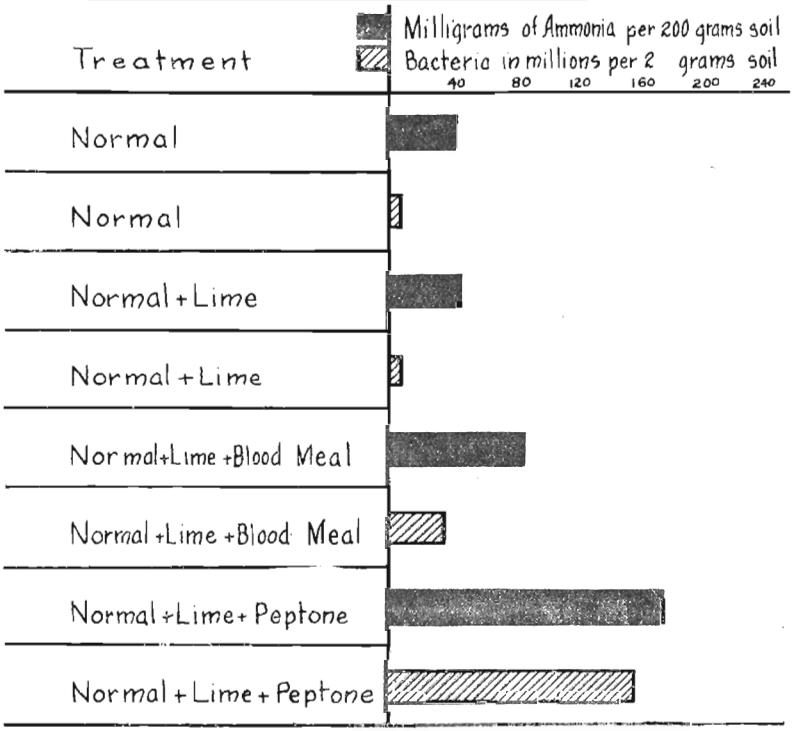

Figure 14. The effect of lime, blood meal, and peptone on ammonification and number of bacteria in Beaverdam soils.

The relation of the number of bacteria to the amount of ammonia produced, is given in Figure 14. This correlation is again very noticeable. The ammonifying power of the organisms in Beaverdam soil is relatively high, there being 1 mg. of ammonia produced for every one hundred sixty million bacteria in the soil at the end of the experiment.

The optimum moisture content for production of ammonia was between 30 and 40% in the soils containing blood meal,

the ammonifying bacteria also reaching the maximum number at these same figures. Thirty per cent moisture seems to be more favorable to the bacteria in the peptone samples. This may have been due to the fact that at a higher moisture content most of the peptone had been broken down before the end of the experiment, and as a result, the number of bacteria per gram of soil had decreased because of the lack of food.

In all of the experiments there was a tendency for the bacteria to reach the maximum number before the ammonifica-

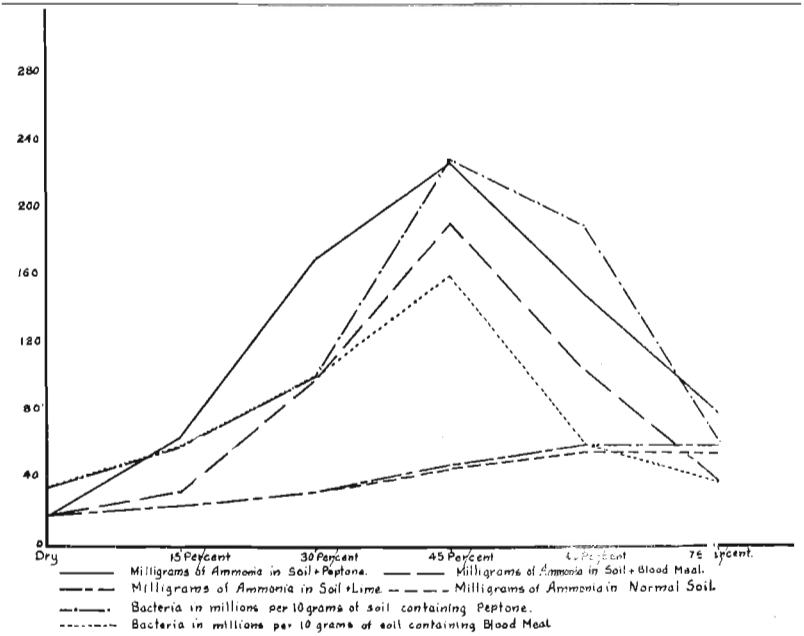

Figure 15. The effect of moisture content on ammonification and number of bacteria in Clatskanie soil.

tion had reached the highest point. Figure 17 gives a comparison of the ammonifying efficiency of Beaverdam soil compared with the other Oregon types. The unfertilized samples gave very high returns; but when blood meal and peptone were added the amounts of ammonia formed were relatively low, indicating the poor ammonifying power of the soil as compared with the Eastern Oregon types.

In Table VI are given the results of ammonification experiments with Clatskanie soil. This is a peat soil which is very high in organic matter. The normal soil contains a com-

TABLE VI.

Milligrams of ammonia produced in Clatskanie soil with varying moisture content when treated with Lime, Blood Meal, and Peptone.

TREATMENT	Air Dry	15%	30%	45%	60%	75%	TOTAL
Normal	18.4	24.6	35.4	46.0	56.4	55.2	236.0
Limed	19.8	25.8	35.4	48.0	60.6	60.2	249.8
Lime and Blood Meal	22.4	31.8	98.6	192.6	105.4	38.4	489.2
Lime and Peptone ...	17.0	65.0	168.8	226.2	149.4	79.6	706.0

paratively large amount of ammonia, but when blood meal and peptone were added there was not the marked increase in ammonia production that we had in the other soils.

Lime had a marked effect upon the rate of ammonification and the increase in the number of bacteria. The total amounts of ammonia produced with varying moisture contents were 236.0 mgs. of ammonia in the normal soil, 249.8 mgs. of ammonia in limed soil, 489.2 mgs. ammonia in soil containing blood meal, and 706.0 mgs. ammonia in soil to which peptone

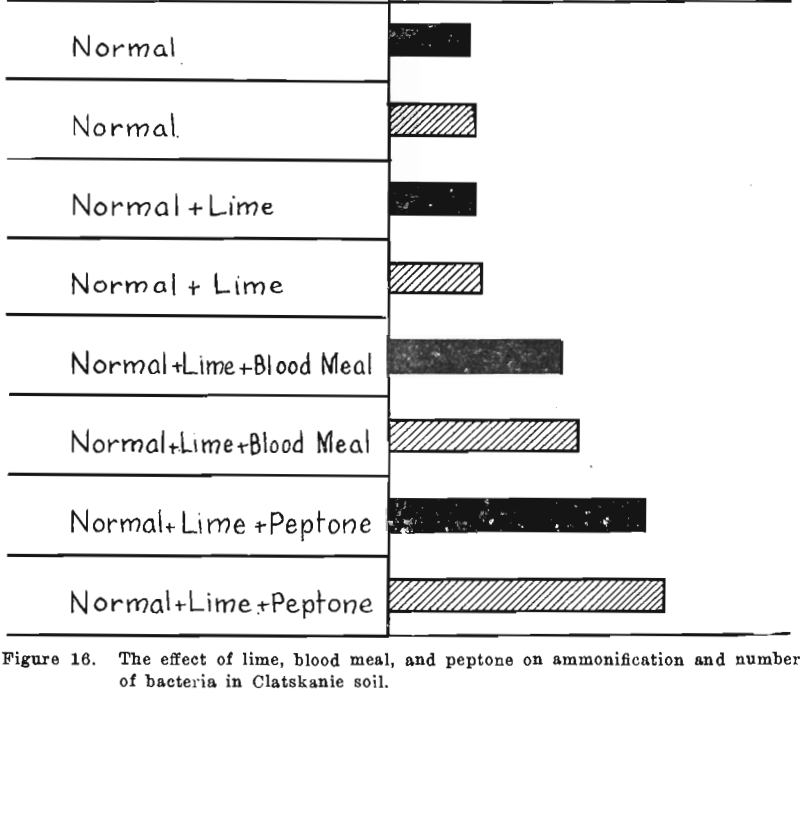

Figure 16. The effect of lime, blood meal, and peptone on ammonification and number of bacteria in Clatskanie soil.

had been added. These figures show that there was a large amount of ammonia in Clatskanie soil under normal conditions; but that the ammonifying efficiency of the soil is very low is indicated by the fact that there was not the marked increase in ammonia formation, as in the Eastern Oregon types, when peptone and blood meal were added.

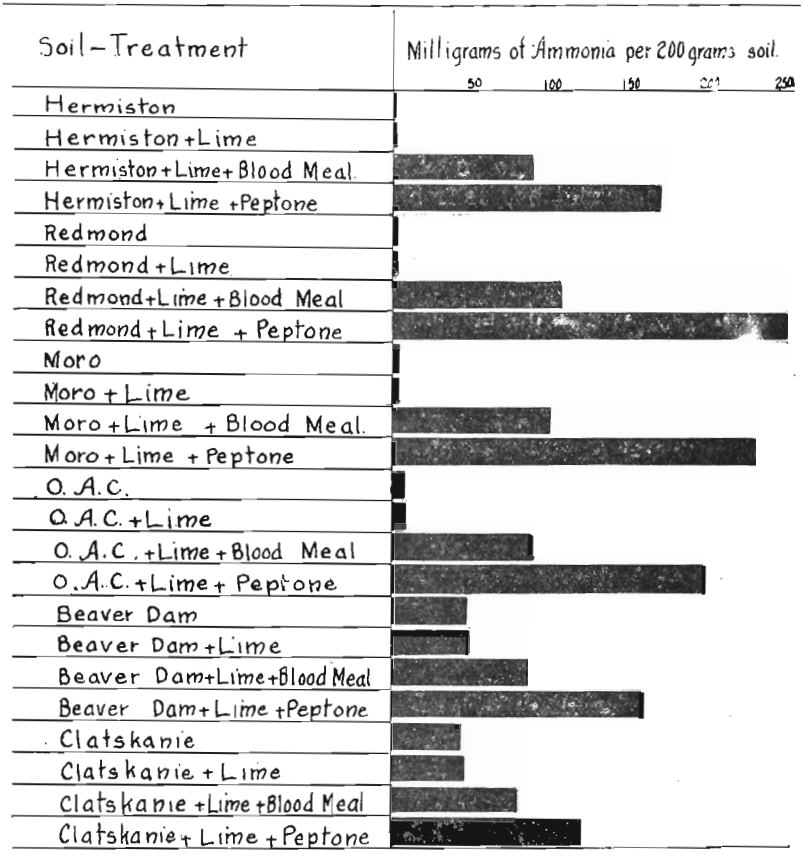

Figure 17. Diagram showing the relative amounts of ammonia produced in the different soils under normal conditions and when treated with lime, blood meal, and peptone.

A comparison of the rate of ammonification and bacterial increase, as affected by the moisture content, is shown in Figure 15. By studying these results it will be noted that the optimum moisture condition of this soil is 45%, 75% being required for complete saturation.

Again we have a very close relation between the number of bacteria and milligrams of ammonia produced. The ammonifying power of the soil was very low, but the ammonifying power of the bacteria considered according to number was very high, there being one milligram of ammonia produced for every twenty million bacteria.

THE NITRIFICATION EXPERIMENTS.

For the growing crop the question of nitrification is of vital importance, especially where fertilizers containing organic and ammonium compounds are used. The rate at which this change takes place determines to a certain degree the time of year for adding nitrogenous fertilizers other than nitrates. If the nitrifying efficiency of the soil is low, the fertilizers may remain for some time before undergoing the change into available plant food. If, on the other hand, the soil has a high nitrifying power, care must be exercised regarding the best time for applying fertilizers, as the nitrates, because of their solubility, are readily leached out of the soil.

While nitrogen in forms other than nitrates may be used by plants, it is generally conceded that nitrate nitrogen is the most valuable form for plant use. Considerable work has been done along this line by Hutchinson and Miller[11] of the Rothanstead Station, Stephens and Withers[12] of the North Carolina Station, and others.

In the work here reported, we used ammonium sulphate and blood meal at the rate of one per cent, basing the nitrifying power of the soil on the ability of the micro-organisms to change these nitrogenous substances over into nitrates.

Table VII contains the results of nitrification experiments with Hermiston soil. In Figure 18 is shown the rate of loss in nitrates when lime was added to the sample. It is well known

TABLE VII.

Milligrams of nitrate nitrogen produced in Hermiston soils when Lime, Ammonium Suphate, and Blood Meal are used.

	Normal		Ammonium Sulfate		Blood Meal	
TREATMENT	No Lime	Lime	No Lime	Lime	No Lime	Lime
Beginning	2.09	2.08	3.59	2.56	3.36	2.61
Two Weeks	.68	.34	2.74	1.82	.54	.22
Four Weeks	1.45	.69	1.10	1.86	1.38	.97
TOTAL	4.22	3.11	7.43	6.24	5.28	3.80
Increase due to lime	— 1.11		— 1.19		— 1.48	

that the nitrifying bacteria are influenced by the reaction of the soil, an acid condition being inhibitory to nitrification. On the other hand, too much lime does not seem best for their growth.

It is interesting to note that in all cases with the Hermiston soil the application of lime had a retarding effect upon the nitrifying power. This difference was not so marked with ammonium sulphate. Temple[13] has found that ammonium sulphate does not nitrify as readily in acid soil as does tankage, but when calcium carbonate is added the ammonium sulphate

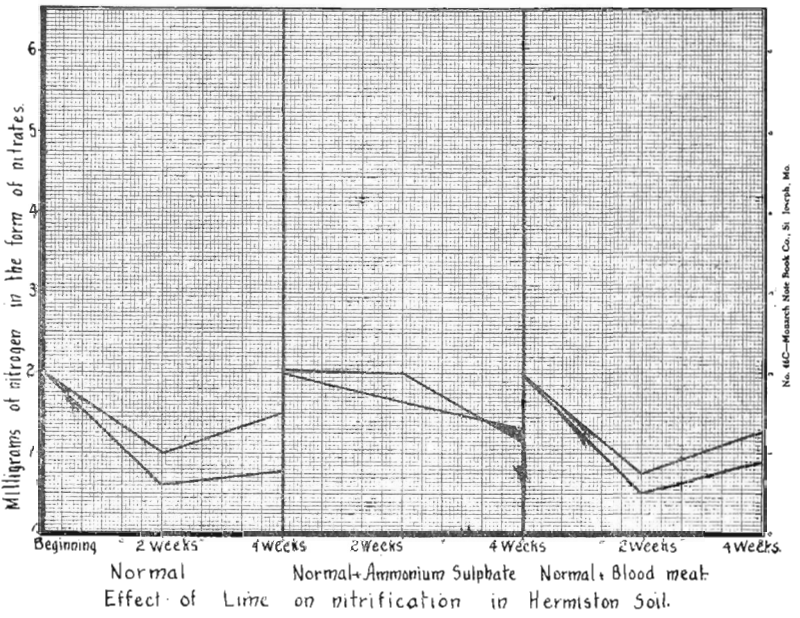

Figure 18. Diagram showing the rate of loss in nitrification due to the addition of lime. The unshaded portions show this loss.

nitrifies as readily as the other nitrogenous substances. He has attributed this to the fact that in the decomposition of organic substances the ammonia given off neutralizes the acidity of the soil to a certain extent.

Figure 19 shows the decrease in the nitrification of ammonium sulphate and blood meal when lime was added. The comparative results of nitrification in the six types of Oregon soils experimented with are given in Figure 30. It will be seen from this diagram that the Hermiston soil ranks very low in nitrifying power. Lohnis[9] found that in respect to

nitrification, aeration is of great importance. Schloesing[14] in his experiments also showed that the amount of nitrate nitrogen formed was in direct proportion to the amounts of oxygen supplied. This condition was found to be true in regard to ammonification, but such was not the case in the nitrification experiments using Hermiston soil, which is the best aerated of all the soils studied. This sample, when organic materials were added, gave a very high ammonifying efficiency;

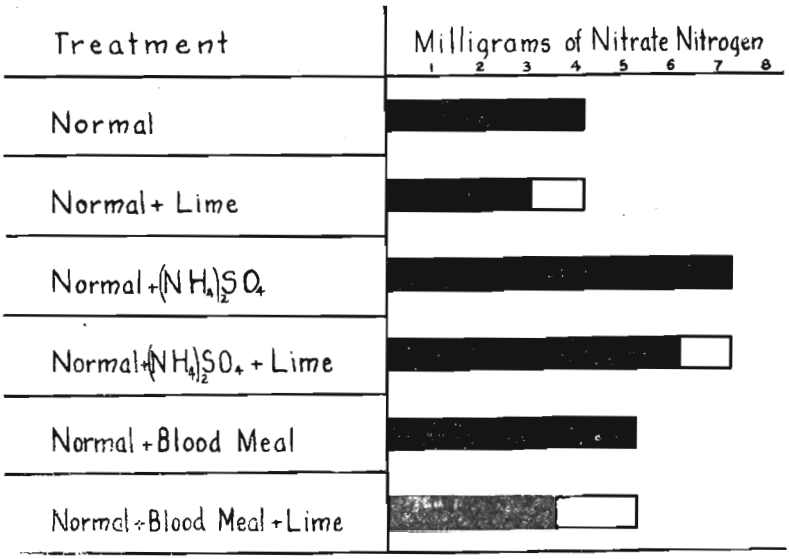

Figure 19. Diagram comparing the amounts of nitrate nitrogen formed in Hermiston soils, when lime and fertilizers were added. The unshaded portions show the decrease due to lime.

but not when ammonium sulphate and blood meal were added in nitrification work. There seemed to be no relation, therefore, between the ammonifying and the nitrifying powers of this soil.

The loss due to the application of lime to the normal soils was 26%: 10% when ammonium sulphate was added, and 28% in the blood meal sample, there being a slight increase of the limed over the unlimed at the end of four weeks when ammonium sulphate was used as a fertilizer. It is interesting to note that ammonium sulphate seemed to counteract the influence of the excess of the lime in the soil and in that way bring about a more favorable condition for the nitrifying bacteria. Way[15] found that ammonium sulphate was more or

less completely decomposed in the soil with the formation of free acid in the soil solution.

In Table VIII are listed the results of the nitrification experiment with Redmond soil. This soil, like the Hermiston sample, has a very low nitrifying power. Figure 20 shows the

TABLE VIII.

Milligrams of nitrate nitrogen produced in Redmond soils when Lime, Ammonium Suphate, and Blood Meal are used.

	Normal		Ammonium Sulfate		Blood Meal	
TREATMENT	No Lime	Lime	No Lime	Lime	No Lime	Lime
Beginning	1.17	1.03	1.74	2.78	1.08	1.49
Two Weeks	3.22	2.45	5.54	17.17	4.85	44.76
Four Weeks	7.63	4.89	10.77	16.64	30.56	46.41
TOTAL	12.02	8.37	18.05	36.59	36.49	92.66
Increase due to lime	— 3.65		18.54		56.17	

effect of lime on the rate of nitrate formation in the soil. In the normal soils there was a decrease, and in the fertilized samples there was a marked increase in the amounts of nitrate nitrogen formed. Especially was this true in the case of the blood meal sample at the end of two weeks. This noticeable

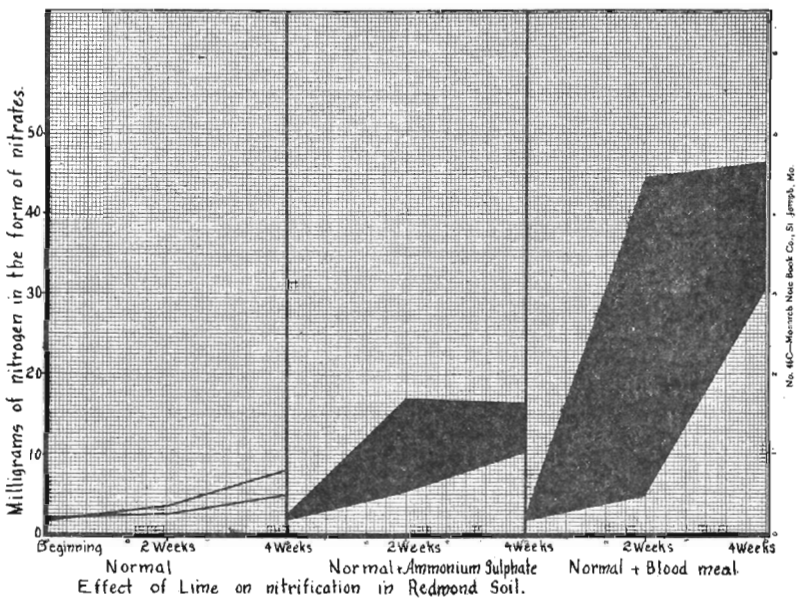

Figure 20.. Diagram showing the rate of loss in nitrification in normal soils, and the gain in fertilized soils due to the addition of lime. The unshaded portions show the loss and the shaded portions the gain, in nitrificaton.

effect of lime upon the nitrification of nitrogenous substances in soil that is already neutral may perhaps be attributed to the formation of acid in the decomposition of these substances, the lime neutralizing the acidity thus formed, and consequently aiding nitrification.

Figure 30 shows the relative rate of nitrification of Redmond soil to be very low compared with the soils of the Willamette and Columbia River Valleys. The relative amounts of nitrate nitrogen produced are shown by Figure 21. The slight increase in normal soils and a marked increase in the fertilized

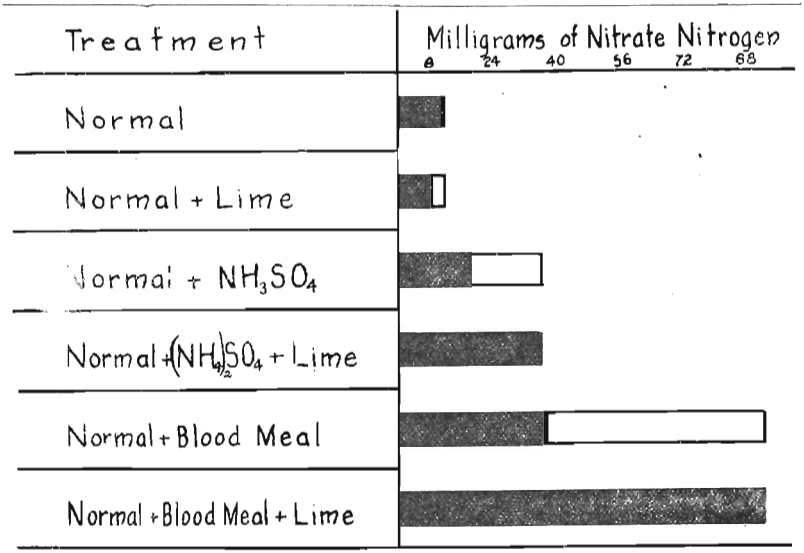

Figure 21. Diagram comparing the amount of nitrate nitrogen formed in Redmond soil when lime and fertilizers were used. The unshaded portions show the loss or gain due to lime.

samples is of interest. In the normal soil there was a decrease of 3.65 mgs. of nitrate nitrogen when lime was added, whereas in the soils to which ammonium sulphate and blood meal were applied there was an increase of 18.54 mgs. and 56.7 mgs. of nitrate nitrogen respectively.

Lime seemed to have its most marked effect during the first two weeks of the experiment, 74% of the total gain being made during that period. The blood meal sample made a very marked gain the last two weeks in the unlimed soil, which may have been due to the neutralization of the acidity by the ammonia produced.

TABLE IX.

Milligrams of nitrate nitrogen produced in Moro soils when Lime, Ammonium, Suphate, and Blood Meal are used.

	Normal		Ammonium Sulfate		Blood Meal	
TREATMENT	No Lime	Lime	No Lime	Lime	No Lime	Lime
Beginning	4.64	5.72	4.21	5.00	4.38	5.26
Two Weeks	4.15	13.43	3.59	4.39	.64	1.60
Four Weeks	6.79	16.36	2.19	4.83	1.30	10.40
TOTAL	15.58	35.51	9.99	14.22	6.32	17.26
Increase due to lime	19.93		4.23		10.94	

Moro soils showed a noticeable gain when lime was applied, as shown in Table IX. The increase due to lime was 19.93 mgs. of nitrate nitrogen for the untreated soil, 4.23 mgs. of nitrate nitrogen and 10.94 mgs. of nitrate nitrogen for the soils treated with ammonium sulphate and blood meal respectively. Figure 22 shows the rate of nitrification, the anlyses being made at the end of two and four weeks.

The relative amounts of nitrate nitrogen produced are shown in Figure 23. Ammonium sulphate seemed to have a retarding influence on the production of nitrates. The blood meal also seemed to have the same retarding effects until after the first two weeks, when there was a noticeable gain.

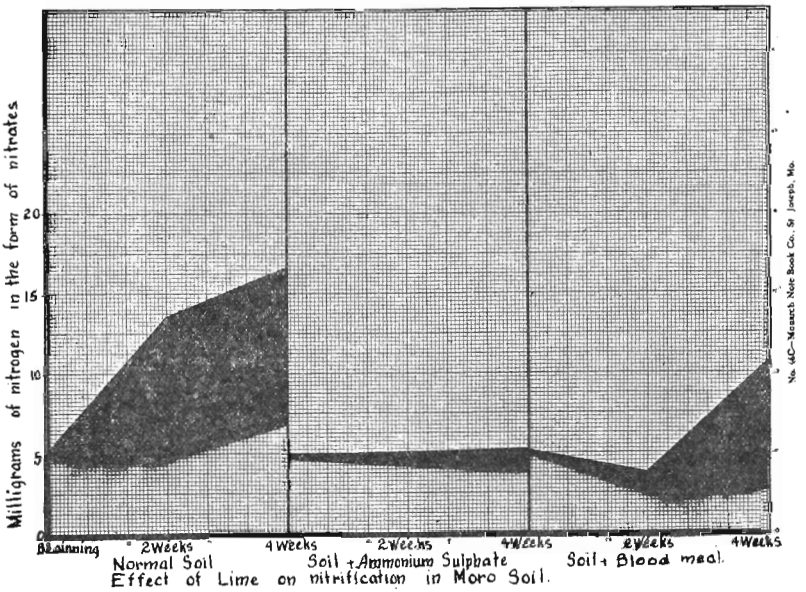

Figure 22. Showing the rate of increase in nitrification in normal and fertilized soils due to the addition of lime. The shaded portions show this increase.

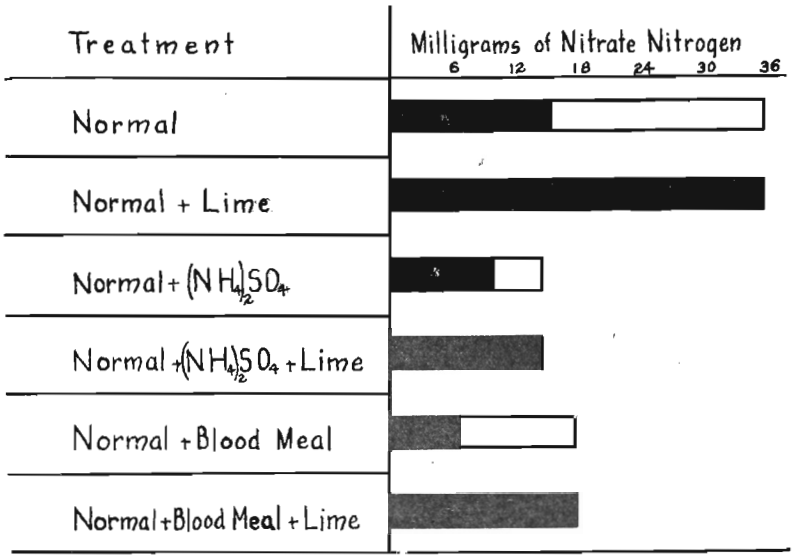

Figure 23. Diagram comparing the amount of nitrate nitrogen formed in Moro soils, when lime and fertilizers were applied. The unshaded portions show the increase due to the application of lime.

The nitrifying power of Moro soil was rather low compared with the valley soils of Western Oregon, the comparison being shown in Fig. 30. The increase due to lime was greatest in the normal soil and least in the soil treated with ammonium sulphate, there being an increase of 125% in the normal soil, 42% in the ammonium sulphate sample, and 170% in the soil to which the blood meal had been added.

The addition of fertilizers had very little effect upon the formation of nitrate nitrogen. In most cases, indeed, they seemed to retard the nitrifying activities.

Table X gives the result of the nitrification test with Beaverdam soil. This sample, which is high in organic matter, gave a very marked gain when lime was used, this addition being

TABLE X.

Milligrams of nitrate nitrogen produced in Beaverdam soil when Lime, Ammonium Suphate, and Blood Meal are used.

	Normal		Ammonium Sulfate		Blood Meal	
TREATMENT	No Lime	Lime	No Lime	Lime	No Lime	Lime
Beginning	13.32	22.20	13.40	28.10	12.52	22.40
Two Weeks	19.92	113.00	52.37	56.16	15.82	62.50
Four Weeks	47.72	112.84	91.56	154.00	143.34	118.46
TOTAL	80.96	248.04	157.33	233.26	171.68	203.36
Increase due to Lime	167.08		75.93		31.68	

especially noticeable in the unfertilized soil, for at the end of two weeks the amount of nitrate nitrogen in the soil had increased from 19.92 mgs. to 113.0 mgs. At the end of four weeks the unlimed soil had increased to 47.72 mgs. of nitrate nitrogen. When lime was added to the soil, there was an increase of over 200% during the first two weeks, but no increase during the last two weeks of the experiment.

When ammonium sulphate was added to the soil, the limed sample, at the end of two weeks, showed very little increase over the unlimed sample, but at the end of four weeks showed a marked gain. The unlimed and limed samples had 91.56 and 154.0 mgs. of nitrate nitrogen respectively, thus showing a gain of over 160%.

Figure 24 shows by diagram the rate of nitrification of Beaverdam soils treated with ammonium sulphate and blood meal. There was a noticeable increase in nitrification the first

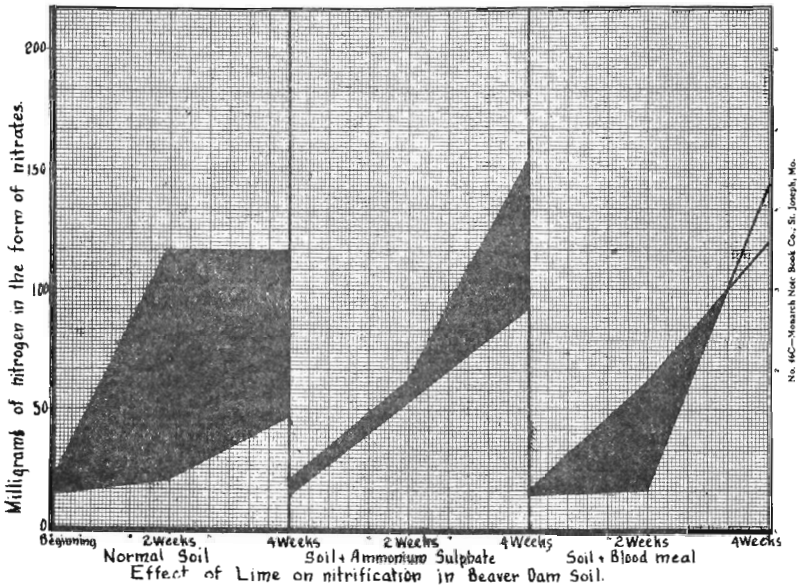

Figure 24. Showing the rate of increase in nitrification in normal and fertilized soils due to the application of lime. The shaded portions show this increase.

two weeks when lime was added to the soil, but at the end of four weeks the increase was very slight. The total increase was 167.08 mgs. nitrate nitrogen for the untreated soil, 75.93 mgs. in the soil treated with ammonium sulphate, and 31.68

mgs. in soil to which blood meal had been added. Figure 25 illustrates the amount of this nitrification.

It is of interest to note that lime, when added to the soil containing blood meal, had very little effect upon the amount of nitrification. The explanation of this may be due, perhaps,

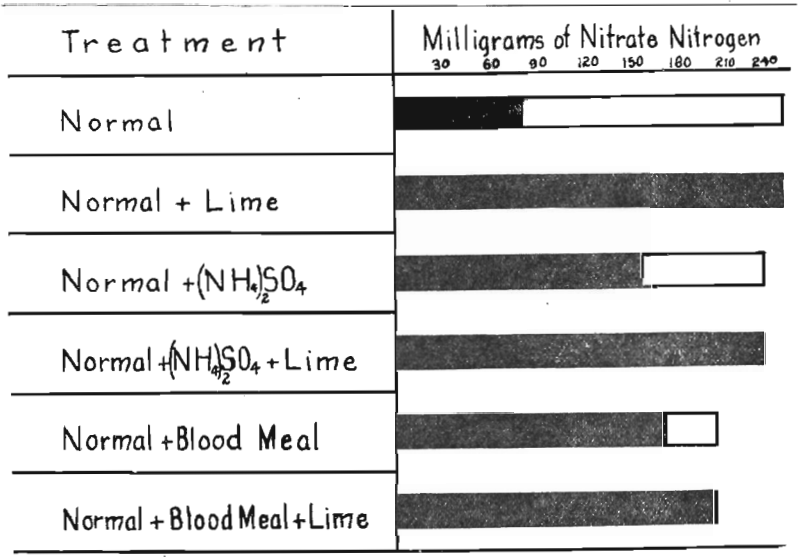

Figure 25. Diagram comparing the rate of nitrate nitrogen formed in Beaverdam soils when lime and fertilizers were added. The unshaded portions show the gain due to lime.

to the neutralization of the acidity in the soil by the ammonia formed in the process of decomposition. The effect of lime is quite evident the first two weeks, but after that time it is not so noticeable.

The opposite is true when ammonia sulphate is used, the addition of lime, until after two weeks, having very little effect upon the rate of nitrification. The acid condition resulting from the transformation of ammonium sulphate to nitrates might account for this lack of nitrification.

The results indicate the advisability of using lime on the soils of the Beaverdam type. In Figure 30 we find that the nitrifying power of this soil is very high. This is especially true the first two weeks of the test. There was a total increase of 143.5 mgs. nitrate nitrogen at the end of two weeks in all of the samples, and a total gain of 102.68 mgs. at the end of four weeks due to the application of lime.

TABLE XI.

Milligrams of nitrate nitrogen produced in Clatskanie soils when Lime, Ammonium Suphate, and Blood Meal are used.

	Normal		Ammonium Sulfate		Blood Meal	
TREATMENT	No Lime	Lime	No Lime	Lime	No Lime	Lime
Beginning	10.80	40.00	11.6	39.4	12.20	43.20
Two Weeks	11.44	57.54	14.2	42.02	27.28	58.82
Four Weeks	48.24	295.14	54.18	649.02	324.42	788.66
TOTAL	70.48	392.68	79.98	730.44	363.90	890.68
Increase due to lime	322.20		650.46		526.78	

Table XI gives the results of the nitrification experiments with Clatskanie soil. Soils from this locality are classified as peat soils, being composed chiefly of organic matter, and are very deficient in lime. We find that the increase due to the lime was 322.2 mgs. nitrate nitrogen in the normal soil, 650.5 mgs. in the soil containing ammonium sulphate, and 526.78 mgs. in the sample containing blood meal. The rate of nitrification was greatest when blood meal was used, but lime had the most noticeable effect when used with ammonium sulphate.

Figure 26 compares the rates of nitrification. When ammonium sulphate was used without lime there was no noticeable

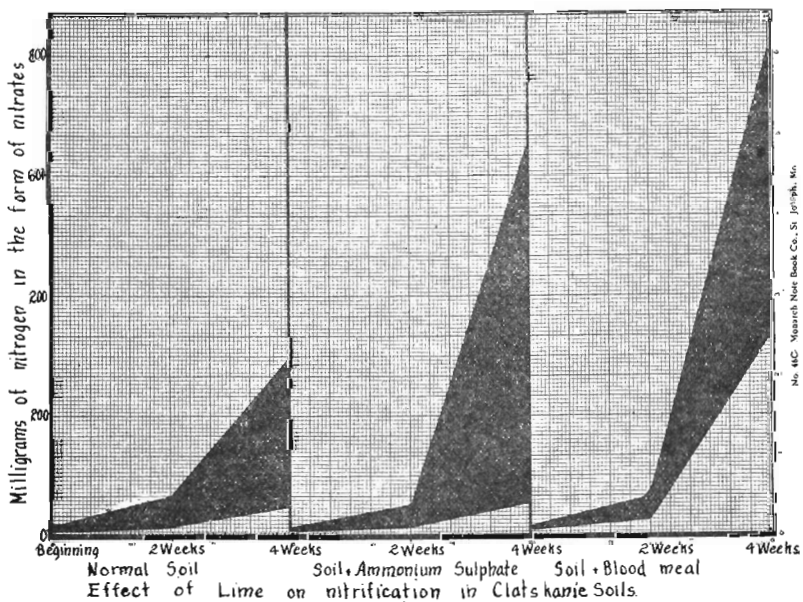

Figure 26. Showing the rate of increase in nitrification in normal and fertilized soils due to the application of lime. The shaded portions show this increase.

increase over the normal soil. When lime was added, however, there was a marked increase in the total amount of nitrate nitrogen formed, 79.98 mgs. nitrate nitrogen being present in the unlimed sample and 649.02 mgs. nitrate nitrogen in the limed sample, showing an increase of over 800%. The increase

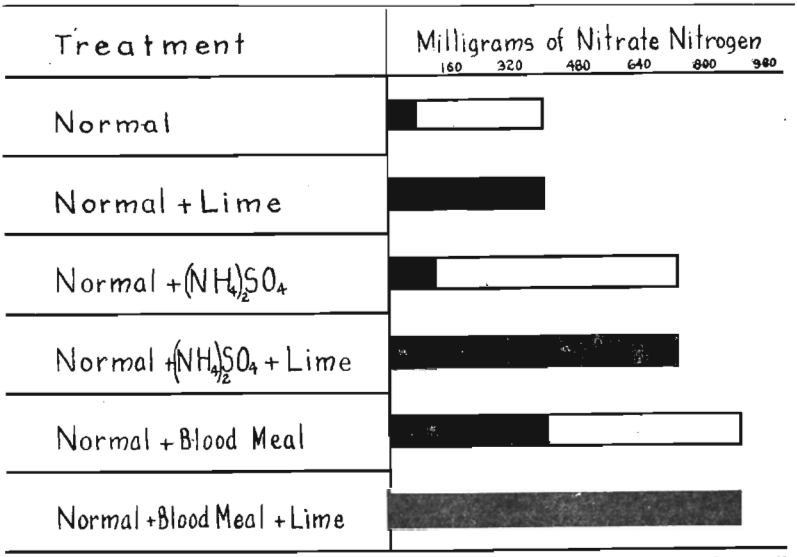

Figure 27. Diagram showing the rate of nitrate nitrogen formed in Beaverdam soils when lime and fertilizers were used. The unshaded portions show the gain due to lime.

in normal soils, due to the lime, was 580%. In the blood meal samples the increase was from 363.9 mgs. nitrate nitrogen in the unlimed sample to 890.68 mgs. nitrate nitrogen in the limed samples, an increase of 245%.

Figure 27 shows the relative effect of lime when ammonium sulphate and blood meal were added, the greatest gain taking place when ammonium sulphate was used. The rate of

TABLE XII.

Milligrams of nitrate nitrogen produced in O. A. C. soil when Lime, Ammonium Suphate, and Blood Meal are used.

	Normal		Ammonium Sulfate		Blood Meal	
TREATMENT	No Lime	Lime	No Lime	Lime	No Lime	Lime
Beginning	4.00	7.10	3.32	6.68	2.4	6.42
Two Weeks	12.92	13.86	19.92	12.10	5.2	5.90
Four Weeks	10.95	19.17	47.72	32.73	134.4	97.40
TOTAL	27.87	40.13	70.96	51.51	142.0	109.72
Increase due to lime	12.26		19.45		32.28	