AN ABSTRACT OF THE THESIS OF

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The object of this study has been to learn the value of supplemental irrigation during the dry summer period in Western Oregon in its relation to promotion of nitrification and nitrogen fixation.

Two soil series, found in the Willamette Valley, were used in this study. One was Chehalis loam which is the most extensive and representative of the recent alluvial group of soils and constitutes much of the second bottoms. The other was Sifton gravelly fine sandy loam, and acid soil, which is found in the higher parts of the old valley flats or terraces. It has a high content of finely divided dark-colored organic matter, about 15 per cent, which is of a loose and fluffy consistency.

This investigation was conducted in two parts. One part was carried out in the greenhouse and the other in the laboratory.

The chief purpose of the greenhouse experiment was to determine whether supplemental irrigation results in any appreciable difference in the amount of nitrogen transformed or fixed and whether fixation of atmospheric nitrogen in amounts capable of measurement occurs in Chehalis loam and Sifton gravelly fine sandy loam. Treatments consisted of cropped and fallow soils, additions ofor organic matter to Chehalis loam and addition of lime to Sifton gravelly fine sandy loam.

Fixation in Chehalis loam with irrigation and without addition of organic matter amounted to 134 pounds per acre, which was three times that in the unirrigated soil. Fixation in soil receiving organic matter was relatively great both with and without irrigation. The greatest quantity of nitrogen in the crop resulted from treatment with organic matter and irrigation. Nitrification in this soil was insignificant.

Results show that nitrogen fixation in Sifton gravelly fine sandy loam is not influenced by irrigation or liming. Nitrification was active in Sifton soil. All pots receiving water showed greater quantities of nitrates with the greatest amount as a result of irrigation and liming.

The laboratory study was performed to study the effect of varying the moisture content upon nitrate formation, nitrogen fixation and carbon dioxide evolution under controlled conditions.

Soils were maintained at the wilting point, 25, 50, 75 saturation, and at the saturation point, One sample of Chehalis loam at each point received organic matter while lime was applied to Sifton soil in the same way.

Nitrification and nitrogen fixation are greatest in Chehalis loam when it contains moisture equivalent to 50 per cent of its water-holding capacity. Greatest carbon dioxide evolution occurred at 75 per cent saturation.

Greatest nitrification and carbon dioxide production in Sifton soil occurred at 75 per cent saturation. Ca O retarded nitrate production in Sifton soil until 75 per cent saturation was reached. In no instance, irrespective of treatment with moisture and lime, did any of the soils show and evidence of fixation with evidence of denitrification at the higher moisture contents.

SOIL NITROGEN TRANSFORMATIONS AS AFFECTED BY MOISTURE

by

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INTRODUCTION

Of all the fertilizer materials applied by the farmer those containing nitrogen are the most costly and remain for a shorter time in the soil, since they are most easily lost by percolation.

Nitrogen in the soil exists chiefly in an organic form. The supply of inorganic or available nitrogen in the soil is comparatively small due to the slow decomposition of the native soil organic matter to form ammonia and to the rapid removal of the nitrates subsequently formed. Changes from ammonia to nitrate are fairly rapid, but the removal of nitrates by plants and percolation keeps their concentration low at any one time. Due to this rapid removal it is necessary to facilitate means whereby the nitrate renewal is made a continuous process, especially during the period of rapid absorption by plants. This requires a system of nitrogenous organic matter maintenance and provision of favorable conditions for nitrogen fixation.

Moisture, temperature, and reaction play important roles in nitrification and nitrogen fixation. Climatic conditions in Western Oregon are such that 38 inches of a total 40-inch annual rainfall come outside the summer season, leaving a long, warm, dry, growing season. Therefore, the object of this study has been to learn the value of supplemental irrigation during the dry summer period in Western Oregon in its relation to promotion of nitrification and nitrogen fixation.

REVIEW OF LITERATURE

Practical value of nitrogen transformations were recognized long before their microbial nature was discovered. The process of nitrification made possible the manufacture of saltpeter to supply the large quantities of gunpowder consumed in almost incessant wars of Europe. In the eighteenth century the artificial production of saltpeter in beds of decaying organic matter reached a high degree of perfection.

Even though the process had reached such a high state of development, the underlying principles were entirely unknown until the latter part of the nineteenth century. At this time attempts were made to explain the oxidation of ammonia to nitric acid, on the strength of certain chemical reactions which could be brought about in the laboratory. All the work prior to that of Pasteur was purely chemical and it was not until his time that the biological explanation for accumulation of nitrates received consideration.

Bousingault, a French chemist, between 1860 and 1878 became interested in the naturally occurring nitre beds of Peru and Ecuador. His work established the fact that accumulation of nitrates resulted from organic matter, but it was explained as a chemical phenomenon in which the soil acted as a catalyzer.

To the chemists Schloesing and Muntz belong the credit of establishing by experiment the fact that nitrification is a biological process, when they observed the formation of nitrates in the purification of sewage by soil. The decade following the discovery of the important biological function resulted in a race between European and American scientists to determine to whom the honor should go of being the first to isolate and study in pure culture the organism responsible for this transformation.

Schloesing and Muntz were unable to isolate any specific organism. Warington of England proved that no matter what compounds of nitrogen are supplied to a plant as manure, they are rapidly transformed within the soil into nitrates. Furthermore, he found that ammonia is first changed to nitrite and then into nitrate. Berthelot first established the fact that nitrogen may be added to field soils through the activities of microorganisms. He also failed to isolate them.

At this point Winogradsky joined the search and after several years of untiring effort succeeded in isolating the first nitrogen fixing organism, a spore-forming anaerobe. In 1901 Beijerinck discovered an aerobic, non spore-forming organism which lived free in the soil and was capable of utilizing atmospheric nitrogen and building it up into complex compounds within its body.

Nitrogen, especially in the form of nitrate, is vitally essential in plant nutrition. The remarkable relationships existing between the microorganisms of the soil and growth of plants have given rise to numerous researches in this field. Early investigations were confined largely to methods of isolation, descriptions of organisms found, and to the study of their behavior. Current work is conducted along the line of alteration or modification of the environment and its effect upon the biological processes in the soil.

Is it possible that there exists a relationship or correlation between bacterial activities and actual crop producing power? In answer to this question, the work of several investigators who have in recent years associated the fertility of soils with their abilities to biologically produce ammonia, nitrates, and carbon dioxide will be reviewed.

Pendleton and Smith (1930), working with two Iowa soils, found that the soil considered least productive naturally also produced least nitrates. In field experiments by Reynolds (1931) the nitrifying power of the soil was correlated positively and significantly with the yields of cotton and corn. The nitrifying capacity was a better index of soil productivity than any other factor studied. Neller (1920) found a definite correlation between crop yield and nitrate accumulation. Data on work by Given (1913) at Pennsylvania show close correlation between high nitrifying power and high productivity. Burgess (1918), using the soil culture method, reported that nitrification is by far the most accurate biological soil test yet perfected for predicting fertility. Kellerman and Allen (1911), while working on the Truckee-Carson irrigation project in 1911, demonstrated that there was a close correlation between crop productivity and nitrifying power of good and poor soils. Lipman and Burgess (1915) used nitrification methods to determine relative availability of different forms of nitrogen in fertilizers and found that they are reliable indices to relationships between these forms of nitrogen in field soils. Brown

(1916), working with soils in Iowa, found that crop yields, ammonification, nitrification, and azofication varied within the plots in exactly the same relationship. Waksman (1923) states that a study of number of microorganisms, and of nitrification, yields results which can be used as indices of soil fertility. Gainey (1917) was a little more cautious in drawing his conclusions by stating that there is usually a correlation between nitrifying power and productivity, but this does not infer that the processes of nitrification are responsible for yield nor that yields on non-fertile soils are limited by the process of nitrification.

These investigations as a whole indicate that definite correlation exists between nitrifying power of a soil and its crop productivity.

Soil treatment is a major factor in modifying the activities of the soil microorganisms, and is the chief weapon used by man in his attack upon these problems. While the importance of climate cannot be subordinated, man is relatively helpless in changing it. Therefore it receives less consideration than those factors over which man has power. Jenny (1930), studying the influence of climate upon nitrogen and organic matter, ascribes to it a dominating effect on the amount of total nitrogen in soils; i.e., soils decrease in nitrogen content from north to south.

The determination of nitrifying powers of soils as affected by moisture and by lime has been carried out by a number of investigators. In order to illustrate in a general way the nature of the results obtained by this line of research, a brief review of some typical investigations is reported.

Warington (1892) in experiments at Rothamsted in 1880 found that saturating ordinary soil with water caused it to rapidly lose the nitrate it contained. This work indicates that the importance of moisture is not of a recent origin. Work at New Jersey in 1908 by Lipman and Brown (1908) shows that a diminishing moisture content is followed by a gradual slowing down of the biological activities. The rate of decay of organic matter becomes less intense, and less nitrate, phosphorus, potassium and calcium become available for plant growth. Bizzel (1910), working with Dunkirk clay loam, found that moisture influenced nitrification. Moisture and aeration appear to be factors influencing nitrification under South African climatical conditions more than temperature as reported by Hall (1924). In Philippine soils Aquino

(1932) found that the number of bacteria in the soil increased with the moisture content, and that, for all the soils used, a definite correlation existed between production of nitrate nitrogen and bacterial counts. Whiting and Schoonover (1920) observed that as moisture conditions approached optimum in the spring, nitrate accumulation was most active. Prescott and Piper (1930) state that the rate of nitrification is governed primarily by the soil moisture conditions, for in South Australian soils during summer drouth there is little evidence of change, as the quantities accumulated the preceding period remain unchanged.

It is quite evident that moisture is essential if not limiting to biological processes of the soil, but it is also known that all the organisms participating in these processes are not affected similarly by the same quantity of moisture. Nor is it sound reasoning to assume that the same moisture content in all soils will produce comparable results. Waksman (1932) states that every soil has an optimum for nitrification and higher or lower amounts of moisture reduce this optimum. He found the optimum amount of moisture for the activities of most bacteria, fungi and protozoa to be between 50 per cent and 67 per cent of its water-holding capacity. Fraps (1908) found nitrification at its highest when the soil contained moisture equivalent to 55 per cent of its water-holding capacity. He also observed that excess water decreased nitrification down to practically zero in a water-logged soil. Greaves and Carter (1916) noted that the application of irrigation water up to 30 inches greatly increased the nitrifying power of the soil. In order to ascertain what effect keeping soils at different moisture contents without crop would have on nitrates present in the soil and nitrification tests, Noyes and Conner (1919) placed samples from five different acid soils in pots and incubated them for 10 months at varying moisture contents. They found that the amount of water present in the soil is concerned with its nitrification.

As a rule more nitrate was found in soils one-half saturated with water than in soils kept one-fourth saturated. Coleman (1908) noted the water requirement varied considerably with the soil. Maximum nitrification in a loam occurred with 16 per cent water; by reducing the water content to 10 per cent or increasing it to 26 per cent, nitrification was greatly retarded.

The effect of moisture in a nitrification study on sandy soil, loam and clay soil was demonstrated by Munter (1920). The moisture contents of the sandy soil was 6, 12 and 18 per cent; for loam, 8, 12 and 16 per cent; and 8, 18 and 28 per cent for the clay soil. In each case as the moisture content increased, nitrification increased. The per cent of material nitrified after three weeks' incubation in each case was three or more times greater at the upper moisture levels.

It is the general belief that excessive quantities of water are more injurious than insufficient quantities due to the anaerobic conditions arising and the loss of nitrogen by denitrification. Anaerobic fixation has been observed in recent years, and is believed to be of great importance, but evidence as yet has not been very conclusive. Pangiban (1925) in a study of temperature and nitrogen changes found higher fixation under anaerobic conditions. Walker (1930) states it is very probable that too little credit has been given to the anaerobic nitrogen-fixing bacteria of the Clostridium group in adding nitrogen to cultivated soils, and probably too much credit has been given to the aerobic organisms of the Azotobacter type.

Observations by Turk (1935) revealed the fact that 100 per cent of the soils studied showed significant anaerobic fixation in contrast to less than 60 per cent for aerobic fixation; and that anaerobic fixation is probably more important in helping maintain the stock of combined nitrogen in Michigan soils.

Whether they are aerobes or anaerobes, ammonifiers, nitrifiers or nitrogen-fixing organisms, it is quite evident that they are influenced by moisture. The problem of moisture under varying soil and climatic conditions is far from solved, thus making this still a fertile field for research.

The activities of microorganisms as affected by liming has been given consideration by investigators for many years. The effect of liming on nitrification has been the subject for study by many workers, but results obtained are not uniform in all cases. Lime is beneficial as a neutralizing agent, thereby making reaction more favorable for activity of the nitrifying organisms. However, their function is not limited by reaction because nitrification has been observed in acid soils.

Stephenson (1920) found that nitrification takes place in acid soils. According to results presented, lime in the form of carbonates does not stimulate the activities of the nitrifying bacteria in the soil, since no increase in nitrification of the soil's own nitrogen took place as a result of the addition of lime. He also states that the presence of acid salts, relatively insoluble acids, and amphoteric substances may cause the amount of base taken up by the soil to be high, when there is really only a small concentration of active acids, and that there is undoubtedly a difference in the effect of active acidity and potential acidity on nitrification. Brown and Houghland (1929), working on the effect of reaction on nitrification, state that measurements of nitrification or the nitrifying power of treated soils should be accompanied by data on reaction or hydrogen ion concentration if the results are to be correctly interpreted. If the reaction is varied, the treatment will probably show no effect. It is not impossible that treatments may entirely overcome acidity effects. Noyes and Conner (1919), working with five acid soils, found that with each soil the amount of nitrates after incubation were very much greater with lime than without lime. Hall (1924), studying nitrification of some South African soils, found nitrification in two of three acid soils quite good, and not much improved by liming, applications varying from 1000 to 15,000 pounds of limestone. Beckwith and associates (1914) found soils of Western Oregon, which are moderately acid, favorably influenced by the application of lime. In one instance the nitrifying power was increased 400 per cent. Reynolds (1931) found that liming greatly increased nitrate production in Texas soils; the soil that received lime produced about six times as much nitrate as the soil receiving no lime. The results of Walker and Thompson (1929) show the pronounced effect of lime on the nitrifying power of Grundy silt loam, nitrification being affected much more by lime than by manure and superphosphate. In fact, the nitrifying power was as high in the soils that received lime alone as it was where lime, manure, and superphosphate had been added. Waksman (1932) observed that the addition of lime increased the decomposition of organic matter added to the soil, whether this is determined by evolution of carbon dioxide or formation of nitrate. In a similar manner numerous other examples of work in this phase could be cited. Sufficient evidence is available in the literature to substantiate the fact that liming stimulates nitrification and the accumulation of nitrates in the soil. The work of Allen and Bonazzi (1915), and MacIntire and Shaw (1930) is worthy of mention in this respect.

The fact that the addition of basic material has a favorable effect on microorganisms, either by correcting soil reaction or by neutralizing the decomposition products, is well established. Since there is a great difference in the relative neutralizing value and reactivity of the various common basic materials used, as well as in amounts used, it is quite evident that these factors must be considered in conducting work involving the biological processes. Hutchinson and MacLennan (1914) state that calcium carbonate is preferred to calcium oxide since the latter tends to change rapidly the reaction of the soil and, therefore, bring about various uncontrolled chemical, physical, and biological changes. They also found that calcium carbonate did not. Walker and Brown (1935) state that the increase in nitrifying power of soil is a function of the amount of limestone added and the degree of fineness. Their results also show that where limestone was added in amounts less than the lime requirement or only slightly in excess of the lime requirement, the increased nitrifying power per ton was very great, but when the application greatly exceeded the lime requirement, additional limestone did not produce very great increases in nitrification. Studies conducted by Winters (1924) at Cornell University on soil conditions which promote nitrogen fixation show that in Volusia soil, with a high lime requirement, and in Dunkirk soil, with a low lime requirement, one ton of limestone per acre increased nitrogen fixation about as much as any heavier application. They found practically no difference between the effects on nitrogen fixing facteria of equivalent amounts of calcium as limestone or as burnt lime. Pepin (1925), studying the same soils, reported liming markedly increased the accumulation of nitrates in both soils. Lipman and co-workers (1931) made determinations over a period of 20 years on a variety of field crops grown on four five-year rotations, with varying amounts of calcium and magnesium limestone. In most cases the yield of nitrogen was almost as high with 2000 pounds of limestone as with 4000 pounds. In nearly all cases unlimed plots returned less nitrogen than the limed ones.

Conditions existing in the Willamette Valley of Western Oregon prompted this particular investigation. In this area of nearly 5,000,000 acres, agricultural producers are confronted with problems involving moisture and lime, which form the basis of this work.

With a total annual rainfall of 40 inches, only 2 inches come during the summer. The moisture storage of the soil is thus taxed to the limit in the early growing season, and often reduced to the point which results in decreased crop yields. Likewise, as the moisture content falls from the optimum, microbial activity is reduced, resulting in decreased nitrification and nitrogen fixation.

Numerous streams traverse the valley floor and each year more and more of the waters are being diverted into irrigation ditches. This phase of agriculture is still in its infancy. Of the 500,000 irrigable acres, 10,000 receive water. This portion of the agricultural land with potentially controlled moisture justifies this investigation.

Some of the older Western Oregon soils have become acid from removal of bases by excessive percolation, due to winter rains. Frequently the pH drops below that favorable for activity of certain groups of organisms, especially the Azotobacter. For this reason studies on the effect of lime were included.

EXPERIMENTAL PLAN

Soils Studied

The soils used for the experimental work here presented are classified as Chehalis loam and Sifton gravelly fine sandy loam. Chehalis was selected because of its productiveness, extensiveness, and its suitability for irrigation. Sifton series is characterized by a relatively high percent of inert organic matter and produces well only with the aid of supplemental irrigation and barnyard manure to activate the organic matter.

Chehalis soil series occurs as smooth or slightly undulating, brown colored land of the river bottom of the Willamette Valley, and includes 218,715 acres. Chehalis is the most extensive and representative series of the recent alluvial group of soils and constitutes much of the second bottoms. These soils are derived from basaltic and other alluvial material deposited so recently that it has undergone little modification in profile since deposition. Soils of Chehalis series, being of recent origin, have been only slightly leached and are still comparatively high in bases such as calcium, potassium, and magnesium. In reaction, Chehalis soils are nearly neutral and show only a slight lime requirement, usually one-half to one ton an acre. Samples for this experiment were collected on the East College Farm.

Sifton soil series occupies the higher parts of the old valley flats or terraces. It is commonly known as "loose land." Typical areas of Sifton are very dark brown or black when moist. When the dry soil is cultivated it raises a cloud of dust which settles as a sooty deposit. The soil has a high content of finely divided dark-colored organic matter, about 15 per cent, which is of a loose and fluffy consistency. This dark color and high organic content may extend to a depth of 3 feet, but as a rule does not extend below 15 or 20 inches. Below this the size and content of cobbles increase. Gravel of various size, ranging from one-half inch to 2 inches in diameter, is scattered throughout the surface soil. Twenty-five per cent of the sample collected for this work would not pass through a one-quarter inch screen. Reaction varies between pH 5.2

and 6.0. The lime requirement is 4 tons calcium carbonate per acre as determined by the Troug method. Samples used in this work were collected on the West Stayton irrigation district in the northwest quarter of Section 7, Range 2, West; Township 9 South, Willamette Meridian, Oregon.

Greenhouse Study

Soils for this work were collected in September, before the winter rains, and stored in large earthenware pots. All gravel and debris larger than one-quarter inch was removed by screening. Moisture was determined by drying 100 gram samples for 24 hours at 105°C. Chehalis and Sifton soils contained 19 per cent and 31 per cent moisture respectively when sampled for use.

Twelve earthenware pots of one-gallon size used in this experiment were filled with 4 pounds of soil, moist basis, for each of the two soils. Six of the pots were cropped with oats. The other six remained fallow. The cropped pots were seeded heavily enough to permit thinning so as to leave six strong, uniformly distributed plants in each pot. Three pots of each, cropped and fallowed, were irrigated frequently. The remaining jars of the cropped series were irrigated only when condition of plants indicated need for water. Corresponding pots of remaining fallow series received even less water than the lightly irrigated cropped pots.

For the Chehalis soil, one pot of heavily and one of lightly irrigated cropped soil received an application of organic matter equivalent to a 3-ton green manure crop, dry basis. Two pots of fallow soils were treated likewise. In a similar manner the acid Sifton soil received an application of calcium oxide at the rate of $1\frac{1}{2}$ tons per acre. Organic matter added to the Chehalis soil was Ladino clover. Soils were weighed and placed in pots immediately after being collected, but organic matter was not added, and cropped pots were not seeded until the first week in November.

Samples for total nitrogen determination were taken from fallow pots after 8 weeks. Top growth of crop was harvested March 15. The low yield of dry matter is attributed to the short, dark days prevail-

SOIL RESPIRATION APPARATUS

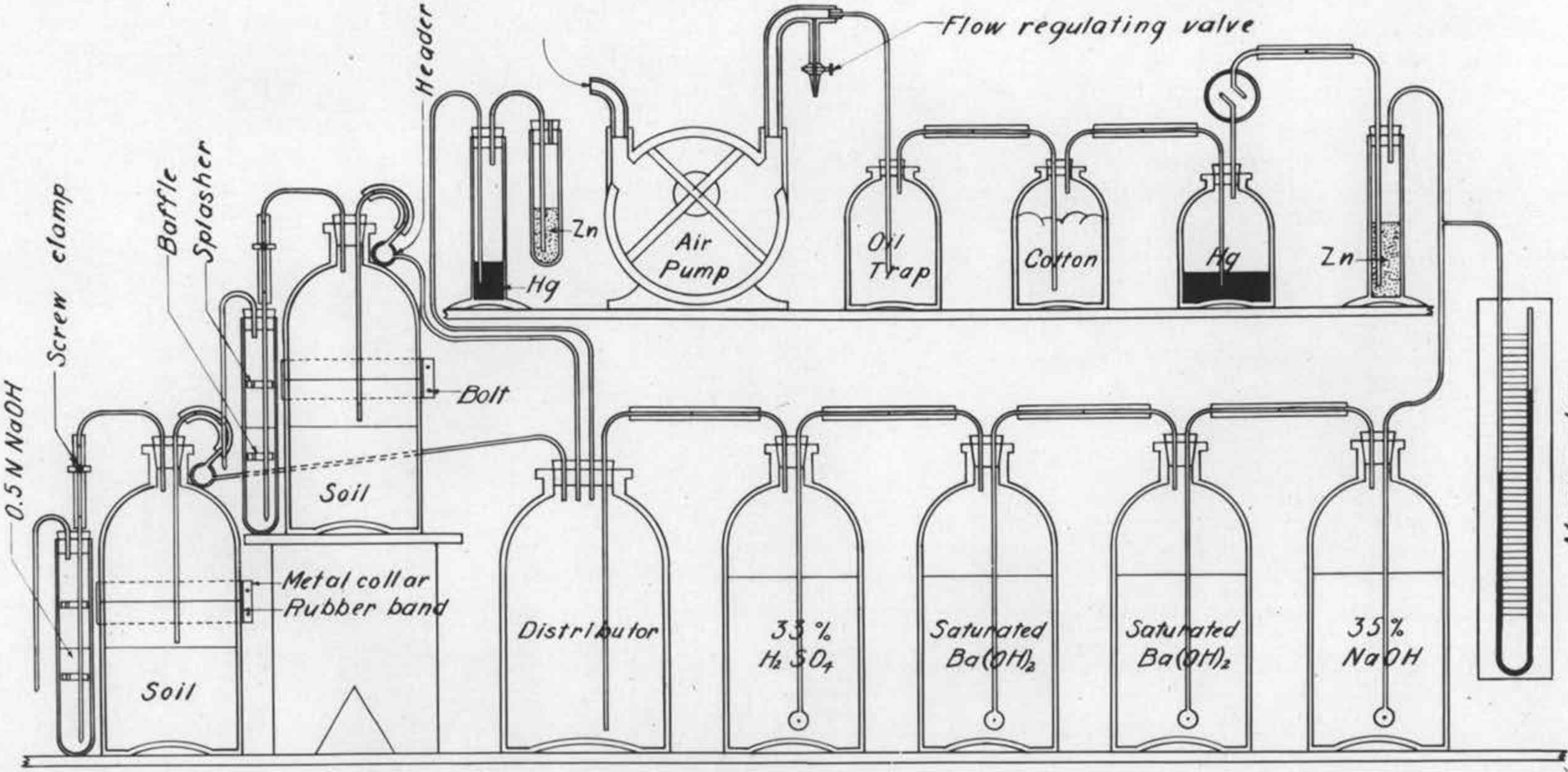


PLATE I.

ing in this section during the winter season.

After crop removal, soils were permitted to dry. When thoroughly dry the soil was removed from the pot as one lump, and worked gently between the hands to remove soil from crown and the greater portion of the root system. Roots were cleaned as thoroughly as possible in this manner, then washed and dried and weighed with the tops. This procedure was followed so as to remove as much organic matter as possible from the soil and facilitate obtaining a representative sample for total analysis. Representative samples were collected and taken to the laboratory for final nitrate and total nitrogen determinations on both soils and plant growth.

Laboratory Study

In this phase of the work the object was to maintain a definite moisture content throughout the duration of the incubation period. This was accomplished to a very marked degree of success, through the use of a respirator of the type employed by Dr. W. B. Bollen (1936) Oregon State College, delivering purified, moisture-controlled air, Plate I. The wilting point and maximum water-holding capacity for both soils were determined prior to work on this study.

The wilting point was determined by raising sunflower seedlings in baking-powder cans. After they were well established, water was withheld and plants were permitted to wilt until they failed to recover after 24 hours in a moist chamber. Tops were removed, weighed, dried, and reweighed. Dry roots equal to 10 per cent of fresh weight of sunflower tops resulted. Cans were placed in an oven at 105°C. for two days, after which weighings were made and soil moistures were calculated in the usual manner.

For maximum water-holding capacity two weighed porcelain Gooch crucibles were filled to within one-eighth from the top with soil and placed in a vessel to which water was added until level with the surface of the soil. Gooch crucibles remained in the water for two hours, after which they were removed and permitted to drain in a moist chamber. When free water failed to appear on the bottom of the Gooch crucible after being wiped dry they were placed in an oven at 105°C., and dried for twenty-four hours. Calculations were made as usual. This procedure was checked with the Hilgard and Bouyoucus methods. Chehalis loam was found to have a wilting point of 8 per cent and a maximum water-holding capacity of 40 per cent. The Sifton gravelly fine sandy loam was found to have a wilting point of 14 per cent and maximum moisture-holding capacity against gravity of 98 per cent.

All soil used was screened through a 2 m.m. screen. Thirty per cent of the Sifton would not pass 2 m.m. Twenty-five per cent was gravel larger than one-quarter inch. Six hundred grams of water-free soil, were placed in $2\frac{1}{2}$ liter bottles used on the respirator. Ten bottles were used for each of the two soils. The moisture content was maintained at five different points, viz., wilting point, 25 per cent saturation, 50 per cent, 75 per cent, and at the saturation point. One bottle at each moisture point received no other treatment. Duplicate bottles were treated similarly to the greenhouse study, i.e., the Chehalis received organic matter at the rate of 3 tons per acre, and the Sifton received $1\frac{1}{2}$ tons of lime an acre. Soils were incubated for 60 days.

Carbon dioxide evolved was determined daily for 4 days, and every second day until the tenth day, after which determinations were made weekly. After 30 days' incubation the bottles were opened and 200 grams of soil, water-free basis, were removed and analyzed for water soluble nitrates, phosphates, sulphates, and nitrites. Reaction was determined and plates were poured for bacteria, actinomyces and mold counts. After 60 days the same routine as for 30 days' incubation was followed. In addition to these, total nitrogen determinations were made.

Methods of Analysis

Carbon dioxide evolution was used as one criteria of biological activity. Apparatus, shown by diagram in Plate I, and procedure used in work is considered worthy of special mention (Bollen, 1936). Long has the production of carbon dioxide been recognized as a measure of biological activity. From the very beginning of this discovery there was controversy over methods and apparatus used in its determination. The chief difficulties were the lack of a source of slow, though continuous, aspiration; too high a vacuum; and incomplete removal of the carbon dioxide from the air entering. The apparatus devised by Neller (1918) overcame many of the previous obstacles, but still there was the feature of vacuum which had not been modified. This objectionable feature was removed by apparatus used in this work, for instead of vacuum, pressure was used to move air over the surface of the soil. Air was purified similarly to methods used by others by passing through two wash bottles of sodium hydroxide and two of barium hydroxide to remove all carbon dioxide present. Finally, before passing over soil the purified air was passed through a 33 per cent sulphuric acid solution to regulate vapor pressure. Carbon dioxide was collected in 1/N sodium hydroxide and was determined by double titration using phenolphthalein and brom phenol blue as indicators.

Nitrate in the soil was determined by the phenoldisulfonic acid method, as described by Harper (1924). A slight modification in classifying procedure was used in results presented, Bollen 1936. Copper sulphate used in the Harper method was replaced by copper acetate, since it was desired to determine sulphates. This proved to be an effective clarifying agent to remove interfering tints caused by the presence of organic coloring matter, especially prevalent in the Sifton soil. A 1:5 water extract was employed, using 100 grams of soil on the dry basis, and shaking intermittently for 30 minutes. Before adding flocculating agents it was found that a clearer resultant solution could be obtained if water extract were permitted to settle and supernatant solution decanted. Flocculating agents were then added to this solution. One gram of copper acetate was added, followed by 0.4 gram calcium hydroxide to precipitate the copper as copper hydroxide, which is an effective clarifying agent. One gram of magnesium carbonate was added to remove the excess calcium hydroxide. For all comparisons 100 c.c. aliquots were evaporated to dryness, flooded with 3 c.c. phenoldisulphonic acid, made mildly alkaline with ammonium hydroxide, and compared with a standard of 1 p.p.m. nitrogen as nitrate.

Total nitrogen was determined by the Kjeldahl-Gunning-Hibbard procedure, according to the official and tentative methods of analysis, A.O.A.C., 1930. Ten grams of soil were digested with 40 c.c. of sulphuric acid and approximately 10 grams of salt mixture composed of: 10 parts of potassium sulphate, 1 part ferrous sulphate and $\frac{1}{2}$ part copper sulphate. About 200 c.c. of the distillate was collected in saturated boric acid solution.

Nitrites were determined by the alpha-naphthylamine sulphanilic acid method, according to A.O.A.C., 1930.

Sulphates were determined turbidimetrically by acidifying a quantity of the water extract with HCl and precipitating with powdered barium chloride, according to the method of Schreiner and Failyer, 1906.

The procedure of Truog and Myer (1929) was used to determine water doluble phosphorus in the 1:5 water extract. The pH values were obtained by means of the quinhydrone electrode, using suspensions of one part of soil to five parts of water. The acre weight of soil used in making comparative treatments and calculations was 2,000,000 pounds.

EXPERIMENTAL RESULTS

Greenhouse Study

Realizing fully the limited interpretative value of data that cannot be submitted to statistical treatment, and also the large experimental error resulting from a limited number of nitrogen determinations upon soil, it is believed, nevertheless, that the data submitted in Tables 1 and 2 indicate certain tendencies in the nitrogen content of cropped and fallow soils. Before any effort is made to compare the effect of various treatments upon the nitrogen content of the soil in this phase of the work, it is wished to call attention to the fact that the procedure followed in this portion of the experiment is subject to many corrections, but time did not permit alterations to be made in this instance. It is only with these preliminary remarks in mind that this work is presented.

The chief purpose of this experiment was to determine whether supplemental irrigation results in any appreciable difference in the amount of nitrogen transformed or fixed, and whether fixation of atmospheric nitrogen in amounts capable of measurement by analytical methods occurs in

TABLE 1. EFFECT OF MOISTURE ON NITRIFICATION AND NITROGEN FIXATION

IN CROPPED AND UNCROPPED CHEHALIS LOAM

Soil No.	Treatment	N as NO_3 at end	Gain or loss in NO_3 over start(1)	Kjeldahl N in crop	Kjeldahl N in soil	Kjeldahl N in soil and crop	Gain or loss over Start(2)	Total N including NO_3±(3)
		p.p.m.	p.p.m.	mgm.	mgm.	mgm.	mgm.	p.p.m.
				CROPPED				
C-1	Irrigated	19	8	54	1511	1565	83	65
C-2	"	21	10	54	1540	1594	112	86
C-3	" + organic matter	23	12	85	1599	1684	187	139
C-4	Dry + " "	20	9	48	1638	1686	189	138
C-5	"	17	6	25	1482	1507	25	23
C-6	"	17	6	26	1496	1522	40	33
				FALLOW				
C-1	Irrigated	3	-8		1511		29	20
C-2	"	3	-8		1511		29	20
C-3	" + organic matter	7	-4		1599		102	69
C-4	Dry + " "	5	-6		1687		190	136
C-5	"	4	-7		1482		0	0
C-6	"	4	-7		1452		-30	-20

(1) N as NO_3 at start 11.

(2) Total N " " 1482.

(3) N in organic matter, 14.7 mgm. per pot, deducted.

Chehalis loam and in Sifton gravelly fine sandy loam. The effect of treatments with lime and organic matter was also studied for the same purpose. The data obtained when soils were irrigated and unirrigated and further handled as outlined in the methods of procedure for this experiment are recorded in Tables 1 and 2.

The results show a striking difference in the quantities of nitrogen fixed in the two soils. The data in Table 1 indicate that fixation in Chehalis loam, with irrigation and without addition of organic matter, amounted to 134 pounds per acre, which was three times that in the unirrigated soil. Fixation in the cropped series receiving organic matter was extremely great, indicating stimulative effects of organic matter, but irrigation here evidently played no part in the process, since the quantity of nitrogen fixed in the unirrigated pot was equally as great as in the pot receiving water. This suggests that fresh carbonaceous organic residue as well as the organic matter of the soil has a direct or indirect influence upon the activity of microorganisms.

As may be seen in Table 1, the nitrogen contained by crops receiving water is two times that of the unirrigated crop. The greatest quantity of nitrogen in the crop resulted from treatment with organic matter and irrigation. This was probably due to increased nitrification and assimilation of nitrogen in the nitrate form. All of the cropped soils, irrespective of treatment, showed an increase in nitrogen content for the respective systems over those at the outset of the experiment.

The results with the fallow soils of Chehalis loam presented in Table 1 also are of interest from the viewpoint of irrigation. Irrigated pots C-1 and C-2 are identical and both show an increase of 29 mgm. nitrogen over the original soil. Pots C-5 and C-6, treated the same as C-1 and C-2 in all respects but irrigation, show no fixation in one instance and a loss in the other. This increase can be directly attributed to the water applied. Results of the organic matter treatments of the fallow soils are not so easily accounted for. Total nitrogen fixed, after deducting the quantity added in the organic matter, is greater in the unirrigated than in the irrigated soil. This may be due to excessive irrigation or to leaching or some unknown factor since the results are not comparable with those of the cropped soils.

TABLE 2. EFFECT OF MOISTURE ON NITRIFICATION AND NITROGEN FIXATION

IN CROPPED AND UNCROPPED SOILS

Sifton gravelly fine sandy loam

Soil No.	Treatment	N as NO_3 at end	Gain or loss in NO_3 over start(1)	Kjeldahl N in crop	Kjeldahl N in soil	Kjeldahl N in soil and crop	Gain or loss over start(2)	Total N including NO_3*
		p.p.m.	p.p.m.	mgm.	mgm.	mgm.	mgm.	p.p.m.
				CROPPED				
S-1	Irrigated	38	10	57	7595	7652	-17	-7
S-2	"	38	10	56	7607	7663	-6	5
S-3	" + lime	38	11	57	7719	7776	7	17
S-4	Dry + "	50	22	29	7731	7760	-9	15
S-5	"	44	16	45	7744	7789	20	32
S-6	"	47	19	28	7744	7772	3	21
				FALLOW				
S-1	Irrigated	68	40		7682		-87	-30
S-2	"	71	44		7756		-13	34
S-3	" + lime	75	47		7793		24	66
S-4	Dry + lime	63	35		7719		-50	-5
S-5	"	62	34		7694		-75	-26
S-6	"	60	32		7856		87	102

(1) N as NO_3 at start 29.

(2) Total N " " 7769

Nitrification in this study was insignificant as seen by final analysis in Table 1. The small quantity of nitrogen or nitrate present in the cropped soils could be accounted for, since the crop would readily assimilate this form, but the loss of nitrate nitrogen over the original content in the fallow soils rather specifically indicates that the process of nitrification was not active or that the nitrates were being removed by means other than the crop.

Table 2 bears evidence that nitrogen fixation in Sifton gravelly fine sandy loam is not influenced by irrigation, nor by liming below the lime requirement of the soil. Water applied to both cropped and uncropped pots resulted in loss of total nitrogen amounting to 20 p.p.m. The loss was two to five times greater in the fallow soils. The cropped pots remaining dry showed no loss in nitrogen. Liming to the extent of the application in this work had no effect on nitrogen fixation. An equivalent of a $1\frac{1}{2}$ ton per acre application was used in this work, whereas tests indicated a 4-ton lime requirement. This curtailment was made to comply with the financial status of the farmer in estimating his ability to purchase lime at the present time. The pH of Sifton gravelly fine sandy loam at the outset of this work was 5.3 and with a

TABLE 3. FORMATION OF NITRATES AND FIXATION OF NITROGEN

IN CHEHALIS LOAM CONTAINING VARYING QUANTITIES OF MOISTURE

No.	Treatment	30 days pH	30 days N as NO_3	30 days Gain or loss over start	67 days pH	67 days N as NO_3	67 days Gain or loss over start	Kjeldahl N at end of experiment	N balance including NO_3 *
			p.p.m.	p.p.m.		p.p.m.	p.p.m.	p.p.m.	p.p.m.
	Original soil	6.8	11		6.8	11		1010	
C-1	Wilting point	6.6	54	43	6.6	30	19	1070	79
C-2	" " + organic matter	6.8	14	3	6.8	17	6	1100	98*
C-3	25% saturation	6.7	35	24	6.7	31	20	1080	90
C-4	" " + organic matter	6.8	17	6	6.8	23	21	1130	143*
C-5	50% "	6.6	44	33	6.7	55	44	1070	104
C-6	" " + organic matter	6.5	45	34	6.6	75	64	1150	196*
C-7	75% "	6.7	47	36	6.6	53	42	1050	82
C-8	" " + organic matter	7.2	4	-7	7.1	21	10	1070	62*
C-9	Saturated	7.3	None	-11	7.3	None	-11	1030	9
C-10	" + organic matter	7.0	None	-11	7.3	None	-11	1130	101*

* N in organic matter, 8 p.p.m., deducted.

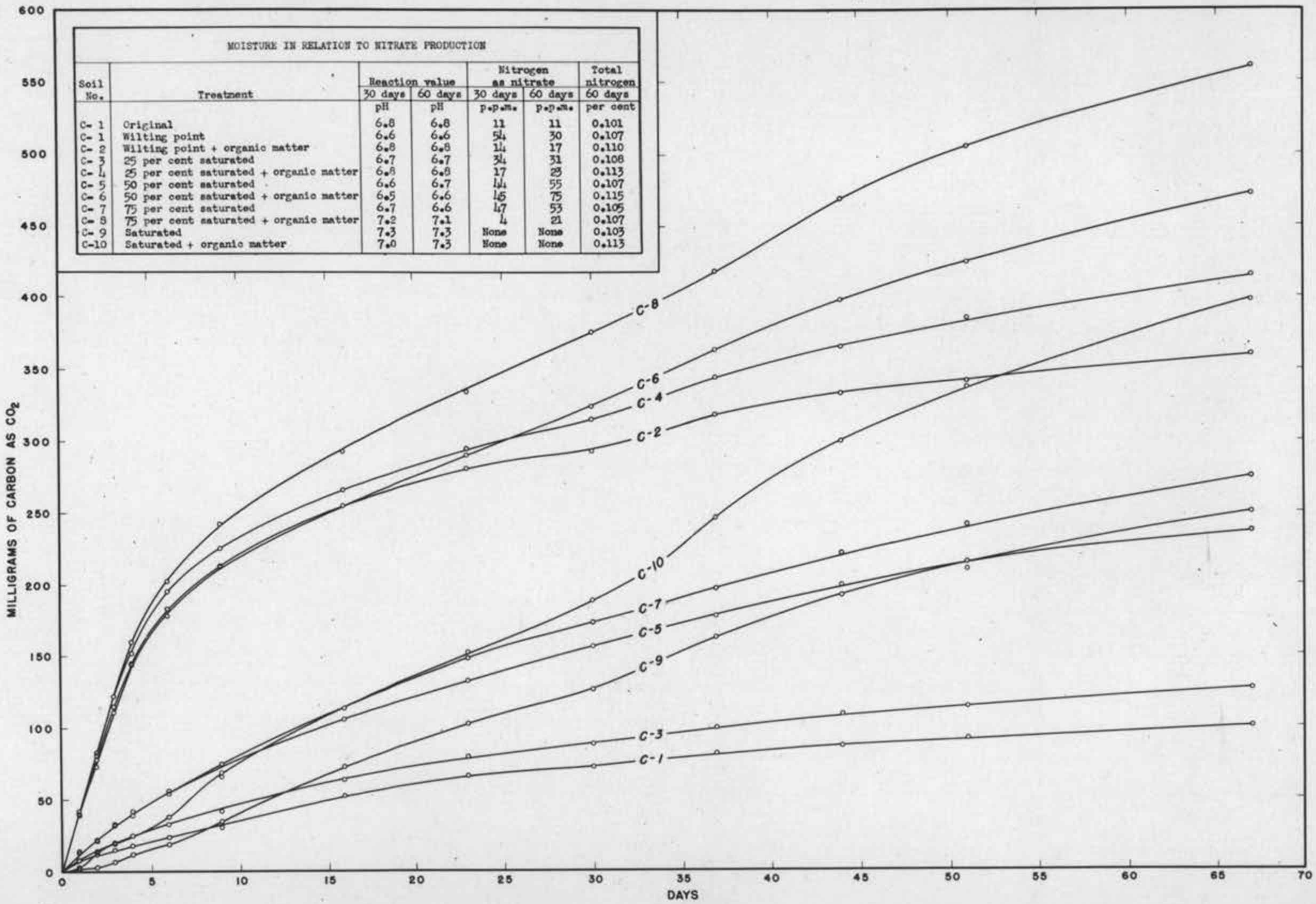
lime requirement of 4 tons it is probable that reaction played a major role in limiting nitrogen fixation.

Nitrification was active in Sifton gravelly fine sandy loam, as shown in Table 2. The relatively large quantity of nitrate accumulated in the fallow soils may be used as a criteria. All pots receiving water showed greater quantities of nitrates with the greatest amount as a result of irrigation and liming. Nitrates remaining in cropped soils after harvest were very small. This is to be expected, since nitrogen as nitrate is readily available to plants. Greater quantities of nitrates remained in the dry cropped soils. In the irrigated cropped soils only an increase of 10 p.p.m. was found over the quantity present at the start of the experiment.

Laboratory Study

This experiment was performed to study the effect of varying the moisture content upon nitrate formation, nitrogen fixation, and carbon dioxide evolution, under controlled conditions. Procedure and methods of analysis were conducted as previously described. The results for Chehalis loam are recorded in Table 3 and Figure 1.

FIG. I. INFLUENCE OF MOISTURE AND ORGANIC MATTER ON CO_2 PRODUCTION OF CHEHALIS LOAM



MOISTURE IN RELATION TO NITRATE PRODUCTION

Soil No.	Treatment	Reaction value 30 days	Reaction value 60 days	Nitrogen as nitrate 30 days	Nitrogen as nitrate 60 days	Total nitrogen 60 days
		pH	pH	p.p.m.	p.p.m.	per cent
C- 1	Original	6.8	6.8	11	11	0.101
C- 1	Wilting point	6.6	6.6	54	30	0.107
C- 2	Wilting point + organic matter	6.8	6.8	14	17	0.110
C- 3	25 per cent saturated	6.7	6.7	34	31	0.108
C- 4	25 per cent saturated + organic matter	6.8	6.8	17	23	0.113
C- 5	50 per cent saturated	6.6	6.7	44	55	0.107
C- 6	50 per cent saturated + organic matter	6.5	6.6	45	75	0.115
C- 7	75 per cent saturated	6.7	6.6	47	53	0.105
C- 8	75 per cent saturated + organic matter	7.2	7.1	4	21	0.107
C- 9	Saturated	7.3	7.3	None	None	0.103
C-10	Saturated + organic matter	7.0	7.3	None	None	0.113

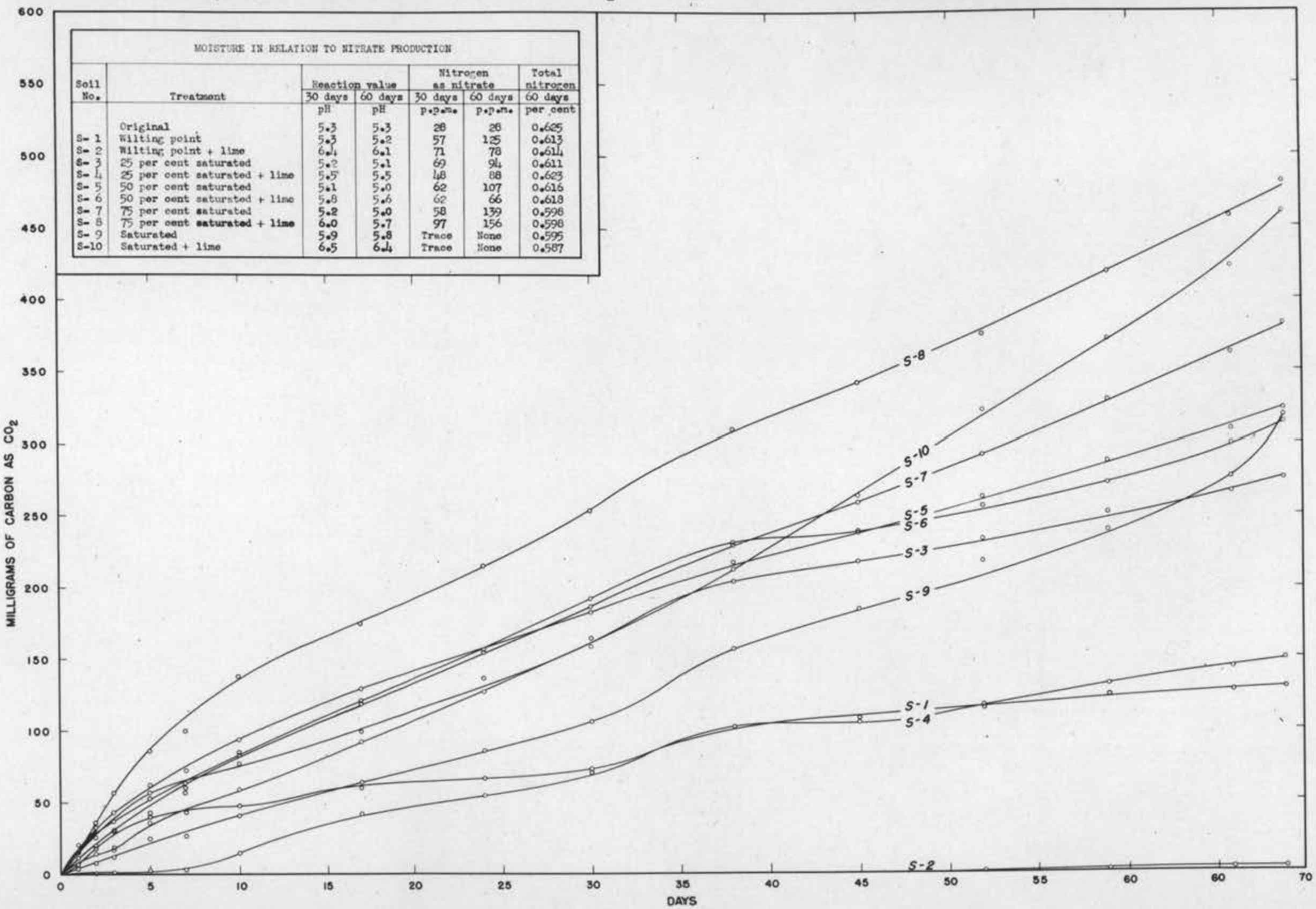
The production of carbon dioxide was uniform, giving typical curves. Varying the moisture between the wilting and saturation points caused very great difference in the amount of carbon dioxide formed. Beginning with the wilting point, each increase in moisture up to and including 75 per cent saturation caused a corresponding increase in carbon dioxide evolution. At the saturation point carbon dioxide formation was greatly reduced. The same trend occurs both in soils receiving no other treatment than varying moisture and in those which received organic matter in addition. Soils kept at 75 per cent saturation showed the greatest biological activity. A sharp decline occurred in the soil maintained at the saturation point. The maximum carbon dioxide production from soils receiving organic matter took place during the first 7 days. By the end of this time approximately 50 per cent of the carbon dioxide evolved during the 67 days of the experiment had been given off. This does not hold true for soils receiving no organic matter in which case there was an apparent constant rate of decomposition, as shown by the gradual but steady rise in the curves in Figure 1.

Stirring at the end of 30 days to draw a sample for analysis caused increased biological activity, resulting in greater quantities of carbon dioxide produced. This is indicated by the slight break in the regularity of the curves immediately following the 30-day determinations.

The influence of moisture upon nitrification in Chehalis loam is shown in Table 3. Optimum moisture for nitrification does, no doubt, depend upon the nature of the soil to a large extent. For this reason the moisture content is expressed in per cent of the maximum water-holding capacity of the soil. Nitrification is at its highest in Chehalis loam when it contains moisture equivalent to 50 per cent of its water-holding capacity. Excessive amounts of water appeared to be more injurious than too small quantities, as seen by the total absence of nitrates in those soils maintained at the saturation point, while small gains were recorded even at the wilting point. Soil alone at 75 per cent saturation showed large increases in nitrates, while those at the same moisture content treated with organic matter showed insignificant amounts. This condition is probably due to the organic matter supporting a denitrifying flora.

Table 3 indicates that nitrogen fixation appeared to be influenced more by the presence of energy material than by

FIG. 2. INLUENCE OF MOISTURE AND LIME ON CO_2 PRODUCTION OF SIFTON GRAVELLY FINE SANDY LOAM



MOISTURE IN RELATION TO NITRATE PRODUCTION

Soil No.	Treatment	Reaction value		Nitrogen as nitrate		Total nitrogen
		30 days	60 days	30 days	60 days	60 days
		pH	pH	p.p.m.	p.p.m.	per cent
	Original	5.3	5.3	28	28	0.625
S- 1	Wilting point	5.3	5.2	57	125	0.613
S- 2	Wilting point + lime	6.4	6.1	71	78	0.614
S- 3	25 per cent saturated	5.2	5.1	69	94	0.611
S- 4	25 per cent saturated + lime	5.5	5.5	48	88	0.623
S- 5	50 per cent saturated	5.1	5.0	62	107	0.616
S- 6	50 per cent saturated + lime	5.8	5.6	62	66	0.618
S- 7	75 per cent saturated	5.2	5.0	58	139	0.598
S- 8	75 per cent saturated + lime	6.0	5.7	97	156	0.598
S- 9	Saturated	5.9	5.8	Trace	None	0.595
S-10	Saturated + lime	6.5	6.4	Trace	None	0.587

fluctuations in moisture content. The utilization of energy results in an increase in the number of organisms able to utilize atmospheric nitrogen for synthesis of protoplasm. In each instance where energy material was added, the quantity of nitrogen fixed greatly exceeded that fixed by the soils receiving no organic matter. Greatest fixation occurred in soil maintained at 50 per cent saturation where an increase of 132 p.p.m. was found after 67 days' incubation. Progressive but small increases in nitrogen were found in soils receiving organic matter with increases in moisture up to 50 per cent saturation. At 75 per cent saturation the least amount of fixation was recorded. Soils receiving no treatment other than variation in moisture showed about equal quantities of nitrogen fixed up to 50 per cent saturation. Above this less fixation occurred with each increase in moisture. The smallest amount was found in saturated soil.

Curves showing carbon dioxide evolution from Sifton gravelly fine sandy loam are not nearly as regular as those produced by Chehalis loam, as shown in Figure 2. Lime as CaO apparently has a decided influence on carbon dioxide production, both in increasing it at the higher moisture levels and in decreasing it in the lower moisture contents.

Maximum carbon dioxide was given off in soil treated with lime and maintained at 75 per cent saturation. Where no lime was used the rate of production increased progressively with each increase in moisture up to 75 per cent saturation. Unlimed saturated soil produced carbon dioxide equal to that of soil maintained at 50 per cent saturation. Limed soil held at the wilting point showed no traces of carbon dioxide production until the 52d day, and gave only 3 milligrams of carbon as carbon dioxide for the entire 69 days. Unfortunately the liming material used in this work was calcium oxide. This, no doubt, accounts for the absence of carbon dioxide at the wilting point, and also for the comparatively low rate at 25 per cent saturation. The carbon dioxide was probably absorbed by calcium oxide used. At 50 per cent saturation this liming factor was overcome when carbon dioxide evolved nearly equal to that of soil alone at the same moisture content. Limed soils maintained at 75 per cent saturation and at total saturation produced carbon dioxide in excess of that of soil alone at similar moisture points. The sudden increase in carbon dioxide production from the saturated soils after 30 days is attributed to the stirring which occurred when bottles were opened and samples removed at the end of that time. Increases in some of the

TABLE 4. FORMATION OF NITRATES AND FIXATION OF NITROGEN

IN SOILS CONTAINING VARYING QUANTITIES OF MOISTURE

Sifton gravelly fine sandy loam

No.	Treatment	30 days pH	30 days N as NO_3	30 days Gain or loss over start	69 days pH	69 days N as NO_3	69 days Gain or loss over start	Kjeldahl N at end of experiment	N balance including NO_3±
			p.p.m.	p.p.m.		p.p.m.	p.p.m.	p.p.m.	p.p.m.
	Original soil	5.3	28		5.3	28		6250	
S-1	Wilting point	5.3	57	29	5.2	125	97	6130	-23
S-2	" " + lime	6.4	71	43	6.1	78	50	6140	-60
S-3	25% saturated	5.2	69	41	5.1	94	66	6110	-66
S-4	" " + lime	5.5	48	20	5.5	88	60	6230	-40
S-5	50% "	5.1	63	35	5.0	107	79	6160	-11
S-6	" " + lime	5.8	63	35	5.6	66	38	6180	-32
S-7	75% "	5.2	59	31	5.0	139	111	5980	-150
S-8	" " + lime	6.0	98	70	5.7	156	128	5980	-142
S-9	Saturated	5.9	Trace		5.8	None		5850	-300
S-10	" + lime	6.5	Trace		6.4	None		5870	-380

other curves are noticeable but not nearly as evident as those for the saturated soils.

Nitrification in Sifton gravelly fine sandy loam was generally good (Table 4), with liming or variations in moisture having little effect until 75 per cent saturation was reached. Unlimed soil at the wilting point presented an unreasonably high figure. Nitrate content of other unlimed soils increased with each additional increase in moisture. Limed soils did not show this same trend, since the nitrate content of the 50 per cent saturated soil was lower than that at 75 per cent saturated. Greatest nitrification occurred in both limed and unlimed soils maintained at 75 per cent saturated in which 111 p.p.m. and 128 p.p.m. over the original content were recorded respectively.

The unusual results obtained for total nitrogen at the end of the experiment for Sifton gravelly fine sandy loam are difficult to explain. In no instance, irrespective of treatment with moisture and lime, did any of the soils show any evidence of fixation. In a soil not rich in organic matter apparent loss in nitrogen may be accounted for by increases in nitrates which are not included in Kjeldahl nitrogen. This would not be applicable here, for the losses exceed the nitrates. Where much organic matter is present, loss of nitrogen may occur by evolution of nitrogen gas or its simple gaseous compounds as a result of denitrification; the abundance of organic matter stimulates the general microbial flora, a portion of which becomes denitrifying whenever aeration is limited, locally or generally. Since Sifton soil contains about 14 per cent organic matter, some of the losses are probably due to such causes.

Denitrification may occur to a marked degree in a saturated soil, and to a less marked degree in soil with moisture as low as 50 per cent saturated. The enormous losses recorded in Table 4 for 75 per cent saturated and for saturated soil are probably due to losses in this manner.

The equipment used in this work should, with a slight modification, provide good means for determining to some extent the amount and form of nitrogen lost under favorable conditions from highly organic soils.

Tests for nitrites, sulphates and phosphates were made in accordance with the methods described previously. In no instance, in either of the soils used, were the results significant enough to warrant further comment. Nitrites never accumulated above 0.1 p.p.m. Water soluble phosphates in Chehalis loam reached 0.2 p.p.m. in some cases. At no time during the experiment did Sifton gravelly fine sandy loam indicate the presence of water soluble phosphorus. This may be attributed at least partially to the acid reaction of the soil. Sulphates were entirely absent from Sifton soil and present in traces in Chehalis loam, but these varied throughout the samples, making it difficult to say they were due to any specific treatment.

Supplementary Study

Interest in the ultimate effect of alternate wetting and drying on nitrification prompted a supplementary study under conditions indicated in Table 5 to be made in conjunction with the major problems. Extreme deficiency of available phosphorus added further interest in the effect of phosphate fertilization on Sifton gravelly fine sandy loam.

Alternate wetting and drying as studied in this work was not similar to previous work described in the literature nor does it in any way duplicate conditions which exist in the field. Samples were brought to optimum moisture, placed in an incubator at 28°C. and permitted to dry down as rapidly

TABLE 5. EFFECT OF WETTING AND DRYING ON NITRIFICATION

IN SOIL RECEIVING VARIOUS TREATMENTS

Sifton gravelly fine sandy loam

No.	Treatment	Optimum moisture				Alternate wetting and drying			
		3 weeks		12 weeks		3 weeks		12 weeks	
		pH	NO_3	pH	NO_3	pH	NO_3	pH	NO_3
			p.p.m.		p.p.m.		p.p.m.		p.p.m.
	Original soil	5.3	2	5.3	2	5.3	2	5.3	2
1	Soil alone	5.2	47	5.0	179	5.2	32	5.1	75
2	$CaCO_3$	5.5	57	5.4	134	5.8	28	5.6	85
3	$(NH_4)_2\ SO_4$	4.7	215	4.6	313	5.0	57	4.9	147
4	$CaCO_3 + (NH_4)_2\ SO_4$	5.0	267	4.8	300	5.4	99	5.1	197
5	$CaCO_3 + (NH_4)_2\ SO_4 + Ca(HPO_4)_2.H_2O$	4.8	267	4.8	313	5.3	67	5.0	163
6	Dried blood	4.9	179	4.7	326	5.2	83	5.5	58
7	Sulphur	4.6	0	4.0	0	5.0	0	3.8	0

as possible. Dishes filled with concentrated sulphuric acid were placed in the incubator to absorb moisture given off. Just as soon as approximate air dry weight was obtained the samples were again raised to optimum moisture.

Results in Table 5 show that neither alternate wetting and drying nor phosphate fertilization had any effect on nitrification. Addition of phosphorus shows no increase in nitrates over those soils receiving similar treatment with the exception of phosphorus. About 50 per cent less nitrate is found in all soils subjected to alternate wetting and drying than in those maintained at optimum moisture capacity. This does not coincide with the work of Waksman (1932) in which he states that a soil moistened after drying will show an increase in accumulation of nitrates over a soil allowed to remain moist. To obtain partial sterilization from drying probably prolonged periods of drying are necessary.

Prescott (1920) studied the extreme drying and cracking of soils in Egypt following flooding by the Nile river. He found that partial sterilization of soil is followed by increased bacteriological activity and after moistening, dried soils showed an increase in biological activity over untreated soil. Lebedjantzev (1924) also found drying caused partial sterilization followed by increased fertility as expressed by crop growth. Four successive treatments of wetting and drying resulted in additional crop yields. Results of the laboratory work here reported does not correlate with field results of Prescott and Lebedjantzev.

DISCUSSION

It is well known that any given substance of vegetable or animal origin may decay faster in one soil than in another, and that in the same soil decomposition of any given substance may be hastened or retarded as conditions of moisture, temperature, and aeration become more or less favorable. For these reasons the nitrogen placed at the disposal of the plant varies from soil to soil as well as within various soils.

The importance of these factors is stressed because the farmer has it within his power to modify moisture, temperature, and aeration conditions sufficiently to hasten decomposition processes and to increase materially the rate of availability of nitrogen from organic matter in, or added to, the soil.

Increases in quantities of nitrogen fixed by Chehalis loam as a result of additions of organic matter and moisture indicate that these factors are both essential for fixation in the soil. This fixation is probably due largely to the free-living nitrogen-fixing organisms belonging to the azotobacter group, since the pH is above 6.0, generally accepted as the lower limit for their activity.

The nitrogen balance for Chehalis loam receiving organic matter as indicated in Table 3, shows a steady increase in nitrogen up to and including 50 per cent saturation, followed by a decrease at 75 per cent saturation, with an appreciable increase again at total saturation. This suggests that there were two maxima, one for aerobic organisms, the other for anaerobic.

Fixation in Sifton soil is less readily attributed to Azotobacter, because the reaction under aerobic conditions never exceeded pH 6.0. Under greenhouse conditions fixation was indicated in five of the six cropped soils and three of the fallow soils, but did not correlate well with either liming or irrigation. In the laboratory, under more controlled conditions, losses were recorded for all Sifton soils irrespective of treatment. This indicates either inactivity of nitrogen fixers or, more probably, excessive activity of denitrifying flora.

Both Chehalis and Sifton soils show that moisture has a pronounced effect on nitrification. Greatest nitrification occurred at 50 per cent saturation in Chehalis loam and at 75 per cent saturation in Sifton gravelly fine sandy loam.

Liming had no stimulative effect on nitrification in Sifton soil until 75 per cent saturation capacity was reached. If the nitrifying power of the soil is a measure of fertility then moisture is certainly the most needed treatment for better crop growth in this soil. Unless sufficient water accompanies the use of lime it appears to be of little value; however, liming in this work was not sufficient to correct the acidity. If it were, the effects of its use might have been altered.

While the optimum moisture content for microbial activity in soil is usually considered to be 50 to 60 per cent of the saturation capacity, results obtained in the laboratory experiment indicate that the optimum is between 75 and 100 per cent saturation. A supplementary experiment is now in progress to test this point with Chehalis loam. While the work is not completed, preliminary results indicate that for this soil the optimum, as indicated by carbon dioxide evolution, is in the neighborhood of 80 per cent.

The nitrification studies of Chehalis loam show that the organic matter added was sufficient to influence the accumulation of soil nitrates. At the end of 30 days it showed a depressing effect at the lower and high moisture contents and no resulting effect at 50 per cent saturation. This decrease is due to the fact that microorganisms use up the available nitrogen compounds in the process of growth and multiplication.

Both insufficient moisture and excessive moisture retard carbon dioxide and nitrate formation, the latter much more markedly. Optimum moisture conditions for maximum carbon dioxide production are also optimum for production of nitrates.

In view of these results, attempts to control moisture under field conditions by supplemental irrigation would undoubtedly be justified. In order to secure greatest returns, frequent applications of water would be essential so as to maintain the moisture content at a comparatively high level.

SUMMARY

1. Nitrate formation and carbon dioxide evolution in Chehalis and Sifton soils were similarly influenced by moisture, the maximum in each case appearing at 75 per cent saturation.

2. Sifton gravelly fine sandy loam, which is high in so-called inert organic matter, produced the greatest amount of nitrate and carbon dioxide.

3. The addition of lime to Sifton soil stimulates the production of nitrate and carbon dioxide.

4. Insufficient moisture as well as excessive moisture retarded nitrate and carbon dioxide production.

5. Organic matter added to soil increased nitrate formation only at optimum moisture content.

6. Organic matter decomposition, as measured by carbon dioxide production, is speeded up by additions of moisture up to 75 per cent saturation.

7. No increase in total nitrogen in Sifton soil occurred in the laboratory experiment. This indicates an apparent absence or insufficient activity of nitrogen fixing flora.

8. In the greenhouse, fixation in cropped soils exceeded that in fallow soils.

9. Irrigation increased the quantity of nitrogen fixed in Chehalis loam.

10. Denitrification was very active in Sifton soils with high moisture contents.

11. Alternate wetting and drying in tumblers had no effect on nitrate accumulation in Sifton soil after 12 weeks.

12. Phosphorus and lime showed no stimulative effect on nitrification in Sifton soil.

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