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THE EFFECT OF MOISTURE AND TEMPERATURE ON TRANSFORMATION OF
APPLIED AMMONIUM SULFATE IN SEVERAL WESTERN SOILS

by

Lloyd Richard Hossner

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Soil Chemistry

UTAH STATE UNIVERSITY
Logan, Utah

1961

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Lloyd Richard Hossner

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INTRODUCTION

The conversion of ammonia nitrogen to nitrite and nitrate forms of nitrogen has aroused much attention in the past and continues to receive much of the energy of the present day researcher.

It has been known for many years that the phenomenon of nitrification is almost in its entirety a biological process. In 1862, Louis Pasteur demonstrated the microbiological nature of the oxidation of alcohol to acetic acid. He suggested that the oxidation of ammonia might be of similar nature. In 1877 this suggestion was verified by Schloesing and Mintz, and for the next ten years the process received much attention, culminating in 1891 in the isolation of the responsible organisms by Winogradsky.

The conversion of ammonia to nitrate is a two-step process which is performed by two entirely different groups of nitrifying bacteria. The oxidation of ammonia to nitrite is performed primarily by the bacteria of the genus Nitrosomonas of which there are several species differing in their physiological makeup. The second step of the process is the oxidation of nitrite nitrogen to nitrate nitrogen which is carried on almost exclusively by a group of bacteria belonging to the genus Nitrobacter.

The nitrifying bacteria are autotrophic; that is to say, they are entirely dependent for energy on the oxidation reaction they carry on. They differ from the ordinary heterotrophic bacteria in that they cannot use the breakdown of organic materials as a source of energy. They are aerobic, requiring oxygen from the air to carry on the oxidation process

as opposed to the anaerobic and facultative bacterial forms, which obtain their oxygen either from combined substances or from oxygen gas or combined sources respectively. Another important aspect of the nitrifying bacteria is their inability to obtain their necessary supply of carbon for the building of their cell substances from any source other than carbon dioxide. They seem to be able to grow faster in darkness, can tolerate a fairly wide range of acidity and require certain inorganic nutrients such as calcium, copper, phosphorus and iron to carry on the oxidation process. Certain organic substances, when present in appreciable amounts, seem to be toxic to these bacterial types.

The increased use of ammoniacal forms of nitrogen fertilizers in the past several decades, and a better knowledge of the chemistry of nitrogen in the soil and how it is used by plants has focused greater attention on the nitrifying bacteria and the oxidation process which they are uniquely able to carry on.

Most calcareous or moderately acid soils, under optimum conditions of moisture and temperature, seem to have the capacity to support a population of nitrifying organisms to convert any applied ammonium fertilizer to the nitrate form in a comparatively short period of time. Yet some soils, even with adequate moisture and favorable temperature, do not have this ability. This is probably due to some adverse physical or chemical condition of the particular soil which must be overcome before the buildup of a sufficiently large nitrifying population can be realized.

In many areas it is the practice to apply ammonia or ammonia yielding fertilizers in the fall. This is done with the supposition that the

soil temperature will be low and, as a consequence, the activity of the nitrifying organisms will be impaired and the oxidation to nitrate with the possible attendant losses by leaching and by denitrification will be negligible before the subsequent cropping season in the spring. The success of this practice in sub-humid climates is based in part upon the knowledge of the rate of nitrification in various soils and the influence of environmental factors, chiefly temperature, in modifying the rate of nitrification. More information must be compiled on various soil types, as to their relative ability to support ammonium oxidation under a variety of environmental conditions. This information should lead to a more intelligent and economical use of ammonium fertilizers.

The experiments conducted were designed to determine the relative ability of several selected soils to oxidize applied ammonium sulfate fertilizer under carefully controlled conditions of temperature and moisture. The study was divided into two general phases. In the first study each soil was incubated for varying lengths of time at temperatures that might be found in the field during summer (25 and 35° C) and at field capacity (0.3 bar tension) and at the permanent wilting percentage (15 bars tension). In the second phase of the study the soils were incubated at 2° C and 0.3 bar tension. These conditions approximate winter conditions where ammonium fertilizers might be applied.

REVIEW OF LITERATURE

It is well known that soils differ greatly in their ability to support the nitrification process. Reasons for or against this ability have generally been attributed to one or a combination of the following factors: (1) moisture content of the soil, (2) pH of the soil, (3) soil temperature, (4) degree of aeration, and (5) initial population of nitrifying bacteria present.

Soil Moisture

Russel *et al.* (1925), in their study of some Nebraska soils, found no nitrate production at the hygroscopic coefficient but observed that nitrate production increased with an increase in moisture up to 1.25 times the moisture equivalent. Robinson (1957) found similar results in the topsoil of the Kikuyu red loam coffee soil and reported that active nitrification of the natural soil nitrogen stopped at a soil moisture level just below the permanent wilting percentage. He found, however, that ammonification of natural nitrogen in this soil did not cease at this moisture level and ammonia nitrogen tended to accumulate but decreased substantially as the moisture value decreased to five-sixths and three-sixths of the permanent wilting percentage. Calder (1957) reported that between the roughly defined limits of air dryness and waterlogging in Uganda soil, there was a broad range of soil-moisture conditions under which large quantities of nitrate appeared in the soil. He concluded that accumulation of nitrate in unenriched tropical soils is not especially favored by any stable moisture content between the

limits of 15 percent and waterlogging. Change in moisture status during drying was accompanied by the appearance of much more nitrate than under steady moisture conditions. Bhaumik (1947) measured the amount of carbon dioxide evolved during 15 days for different soils and observed that for all soils the peak rate of carbon dioxide production was at or near to the moisture tension at the aeration porosity limit of that soil. Care should be taken in interpreting these results on the basis of nitrification since heterotrophic as well as autotrophic bacteria liberate carbon dioxide and their numbers in the soil generally greatly outnumber the autotrophic forms. Fitts (1955) reported that nitrate production took place over a fairly wide range of soil moisture. Adjustment of moisture by tension methods prove better than by addition of constant quantities per sample. The optimum moisture tension for nitrate production was about 100 cm of water which corresponds to a moisture level slightly above field capacity.

Soil Reaction

The pH of a given soil has been found to be an extremely limiting factor in the rate of nitrification of added ammonium fertilizers. The initial pH of the soil or how the pH may be changed upon oxidation of ammonium fertilizers has reportedly influenced nitrification. Various ideal ranges of pH values have been proposed by numerous workers, yet none seem to be fully acceptable over a variety of soil conditions. Meiklejohn (1953) reported that the optimum pH for Nitrosomonas activity was 8.5 to 8.8 and for Nitrobacter a maximum from 8.3 to 9.3. She also reported that Winogradsky and Winogradsky described 6 strains of Nitrosomonas with different pH optima ranging from 6.0 to 9.0 and 7 strains of Nitrobacter, with a pH optima ranging from 6.3 to 9.4. Engel (1958)

reported from his work with Nitrosomonas europea that there was no sharp pH optimum for NH_4^+ oxidation, and the reaction proceeded optimally from about pH 6.8 to 9.0 for this particular species. Frederick (1956) observed that a marked decrease in the rate of nitrification occurred as the pH dropped below neutrality. He found in Clermont silt loam (pH 5.0) that nitrification was influenced by changing the pH by addition of lime. Increase of pH from 5.0 to 6.2 and 7.4 with CaCO_3 showed marked increase in nitrate production and decrease of lag period in the Clermont soil. Anderson and Purvis (1955) found with Nixon sandy loam (pH 4.9) that samples treated with NH_4OH began to nitrify from 1 to 3 weeks sooner than those samples treated with $(\text{NH}_4)_2\text{SO}_4$. They found that by adjusting the soil pH to 6.8 with lime, nitrification began about 2 weeks earlier and there was no difference between the rate of nitrification of NH_4OH and $(\text{NH}_4)_2\text{SO}_4$ at 37, 42 and 47° F (2.8, 5.6 and 8.3° C). An interesting study by Martin (1942) on alkaline desert soils indicated that under the conditions of his experiment, a threshold pH value of 7.7 ± 0.1 existed above which the complete oxidation of ammonia to nitrate would not occur and to which the pH of such soils must first be reduced before nitrates accumulated in appreciable amounts. He also concluded that the activity of the bacteria which catalyze the oxidation of ammonia nitrogen to nitrite is reduced by high alkalinity but they are able to function at a higher pH value than those oxidizing nitrite to nitrate.

Temperature

Active nitrate production has been reported at soil temperatures varying from as low as 2 to as high as 40° C. Meiklejohn (1953) reported that nitrification did not proceed at 40° C on tropical Uganda soil. Sabey et al. (1959) reported that no nitrification occurred at

0° C and that the maximum rates of nitrate production increased and the delay periods decreased as temperatures were increased from 0 to 25° C. Frederick (1956) found that the optimum temperature for nitrification of ammonium nitrogen lies between 27 and 35° C with no sharp break until the temperature goes below 20 or above 35° C. Similar results were obtained by Waksman and Madhok (1936) who reported that between 27 and 37° C is the best temperature for nitrate production. Russel et al. (1925) noted some nitrification at as low as 5° C with a maximum of nitrates produced at 35° C. Anderson and Purvis (1955) found some nitrification at 37° F (2.8° C) at 6 weeks in all soils tested. Tyler et al. (1959) found no minimum temperature for nitrification and observed that the process continued at a slow rate at 37° F (2.8° C). In an attempt to correlate data obtained in the laboratory under controlled conditions with that which exists in the field, Sabey et al. (1956) showed that field data agreed closely with laboratory data at temperatures above 8° C.

Initial Population of Nitrifying Bacteria

Meiklejohn (1953), in her recent review of the nitrifying bacteria, writes:

It has been claimed that the united activities of some organisms account for the nitrification observed in soil, but Nitrosomonas, or a related autotrophic organism, has been found in every soil where it has been seriously looked for, and in which nitrification takes place; and it produces much greater amounts of nitrite than any of the heterotrophs, so the hypothesis seems unlikely. It remains true to say that the only bacteria which have been adequately described, and certainly known to produce nitrite in quantity from ammonia, or to produce nitrate by oxidation are the autotrophic species described by Warington and the Frandlands, and isolated by Winogradsky.

The importance of initial population of nitrifying bacteria can readily be realized and the limitations of some soils to nitrify ammonium can

be explained from this standpoint. Sabey et al. (1959) reported that the influence of temperature and nitrifying population on delay periods and maximum rates varied greatly among soils. They found that increasing the initial population of nitrifiers in a soil which normally supported slow nitrification decreased the delay period but did not increase the maximum rate of nitrate production. Frederick (1957) reported similar conclusions in his studies at low temperatures. He found that a lag phase usually indicated a time during which an initial inhibition is overcome or the population is built up. The results of his experiment seem to indicate that differences in the population of nitrifiers in the original soils rather than that of an inactivation of the nitrification process at low temperatures are responsible for the paucity of nitrifying micro-organisms and a very slow rate of development. Anderson and Purvis (1955) report that differences in nitrification between soils as related to initial population of nitrifying bacteria cannot be correlated with the soil type, texture, organic matter content, total N, and the exchange capacity or base saturation other than through the influence of bases on soil reaction.

Degree of Aeration

Since the nitrifying bacteria are strictly aerobic, it is important that sufficient amounts of oxygen be available to them at all times. Amer and Bartholomew (1951) indicated that the optimum concentration of oxygen for nitrification in soil is about that contained in ordinary atmospheric air. Investigators have not found oxygen concentration below 1 percent except in lower horizons in soils.

EXPERIMENTAL PROCEDURE

Soils Used

Four soils varying in physical and chemical properties (table 1) were collected from various areas in the Western region; namely, Yolo loam from Northern California, Walla Walla silt loam from Eastern Washington, Willamette silt loam from Western Oregon and Elfrida sandy clay loam from Southern Arizona.

Incubation at 25 and 35° C

Preparation of soil

Upon receipt of the soils from the various areas, they were air dried and ground to pass a 2 mm sieve. Moisture percentage of the soils in the air dry state was calculated. Moisture percentages at 0.3 and 15 bars tension were determined by use of the pressure plate and pressure membrane apparatus.

Soil samples weighing 33.3 grams on an oven dry basis were weighed into 250 ml Erlenmeyer flasks. Ammonium sulfate solution was added to each sample to give a N level of 150 ppm. Distilled water was then added to bring the sample up to the predetermined moisture level. The soil was well mixed by hand to insure that the N fertilizer and soil moisture were consistent throughout the sample.

Temperature control

To obtain the desired temperatures of 25 and 35° C at which all soils were to be incubated, two large water baths as described previously by Justice and Smith (1960) were used. A 500 watt heating element was mounted in the center of each bath. A bimetallic thermoregulator was

Table 1. Some physical and chemical properties of soils used in the incubation study^a

Soil	pH	O.M.	O.C.	N	C/N	Moisture	Tension	EC x 10 ³
	paste	%	%	%		1/3 bar	15 bars	millimhos per cm.
Elfrida sandy clay loam	7.7	1.1	0.6	0.063	9.8	18.4	11.4	0.80
Walla Walla silt loam	6.1	2.8	1.7	0.122	13.6	23.1	7.8	0.29
Willamette silt loam	5.6	2.0	1.2	0.105	11.1	25.5	11.7	0.41
Yolo loam	6.8	1.4	0.8	0.081	10.3	19.2	10.2	0.92

^aDetermined at Utah State University

mounted and connected to a supersensitive relay for accurate temperature control. A water pump was mounted on one end of each tank to circulate the water for uniform temperature throughout the bath.

Continuous aeration with moisture control

Maintenance of a desired moisture content during the course of incubation had, heretofore, been accomplished generally by periodic additions of water, the amount added in each case being determined by weighing the samples and containers. Since the incubation vessels were generally covered only with a glass plate or a cotton plug, aeration was considered optimum. Another method used with varying success was an incubation chamber which was humidified by setting pans of water in the controlled temperature chamber to insure that loss of moisture from the samples would be at a minimum. This method proved quite unsatisfactory since the loss of moisture was so high. Fitts (1955) found that after 1 week in this type of humidifying chamber nitrate production was seriously impaired and by the end of 3 weeks the loss of moisture was so great that nitrification had essentially stopped. An improvement over this method was to insert the vessel containing the sample into a larger gallon jar with a small amount of water in the bottom. The hole in the sample vessel was made smaller and the size of the sample was made larger to cut down on the amount of moisture lost by surface evaporation. Although moisture control was adequate, the degree of aeration was probably inadequate.

A more desirable method which insures both moisture control and adequate aeration is that of controlling the rate of flow and humidity of an air stream passing continuously over the samples (Bartholomew and Broadbent, 1949). In the study conducted by Justice and Smith (1960)

it was found that moisture could be quite adequately controlled by this method over a broad range of soil moisture tensions.

Moisture and aeration of the soil samples of the present study were controlled by passing an air stream of controlled relative humidity continuously over the soil samples. The flow of air was controlled by the use of capillary tubes enclosed in a protective casing and calibrated to allow 4 cc of air to pass into the flask each minute under a constant pressure of 15 cm of water. The relative humidity of the air was controlled by this differential in pressure. Prior to passing over the soil samples, the air was bubbled through large bottles containing distilled water, then passed through rubber tubing and into large cylindrical plastic tubes which were held permanently below the surface of the water. The tubes were half filled with distilled water and an atmosphere of air saturated with water vapor was in equilibrium with the solution. The air stream passed over this solution to insure complete saturation at the given temperature. Since the air stream was held under a constant pressure of 15 cm of water, the humidity of the air stream decreased to a calculated 98.5 percent upon release to the atmospheric pressure of the flasks. The air stream then passed over the soil sample and from the flask through a glass tube extending above the surface of the water.

After the soil in each flask had been treated with the proper proportions of nitrogen solution and distilled water to bring the moisture up to the desired level, the flask was fitted with a rubber stopper with dual air conduits for incoming and outgoing air and placed in the tank. The flasks were secured to the bottom of the tanks with rubber bands and paper clips which fastened the bands to the wire mesh bottom of the tank.

Each soil was incubated in this apparatus for a period of time

depending upon the moisture level and the calculated time required for the applied ammonium sulfate to be completely oxidized.

Incubation at 2° C

Preparation of soil

One hundred gram soil samples ground fine enough to pass a 2 mm sieve were weighed into pint fruit jars. Ammonium sulfate solution was added to each sample along with enough distilled water to bring the N level up to 150 ppm N and the moisture level of each sample up to the predetermined value to give 0.3 bar tension. The soil and moisture were mixed immediately by hand with a spatula. A fine mist of water was sprayed around the top of the container to provide a partially saturated atmosphere in the jar to prevent initial loss of moisture from the samples. The jar was sealed with a lid and the samples were then placed immediately in a controlled temperature room.

Aeration and moisture control

To insure an adequate supply of air in the container, the lid of each sample was removed twice weekly and the air completely exchanged within the container by use of a squeeze bulb. Justice and Smith (1960) in preliminary studies found that aeration twice weekly was sufficient for the highest rate of microbial activity at this temperature in Millville loam soil which has a nitrification capacity similar to or exceeding that of the soils studied. Before the container was resealed after aeration, another fine mist of moisture was sprayed around the top of the jar to compensate for any moisture lost in the exchange of the air. Each of the soils was incubated for approximately 5 months.

Chemical Analysis

Each week, in the case of the controlled temperature tanks, and

every 2 weeks in the controlled temperature room, 3 replications of each soil were sampled and analyzed for ammonium nitrogen, nitrate nitrogen, nitrite nitrogen and moisture. Samples were weighed out for moisture determination immediately upon removal from the incubation treatment. The samples were then treated with toluene to stop any microbial activity, sealed and placed in a refrigerator until the other analyses could be made. All analyses were made on the same week that they were sampled. Ammonium nitrogen determination was made on a neutral normal potassium chloride extract of the soils by the Nessler method, using alkaline tartrate and gum acacia in a modification of the method given by Jackson (1958). Nitrate was determined from a saturated calcium hydroxide extract by the phenodisulfonic acid method, after first destroying nitrites by use of ammonium sulfamate. Nitrite nitrogen was determined from the same saturated calcium hydroxide extract using the method given by Shinn (1941) which employs sulfanilamide and a coupling reagent. Total inorganic nitrogen recovered was taken as the total of the three previous determinations.

RESULTS AND DISCUSSION

Moisture Control

Control of moisture at a constant level has been established as a critical component in nitrification studies, so it was important to maintain the desired moisture level throughout the entire experiment regardless of length of incubation. Results of the determinations from all studies are shown with variance and coefficient of variation in table 2.

The best overall moisture control was observed in the Willamette silt loam soil with the greatest variation occurring in the Walla Walla silt loam. At the higher temperatures of 25 and 35° C the best control tended to be at 0.3 bar moisture tension. There was no trend toward loss of moisture with time, and samples incubated at 35° C maintained the desired moisture level as well as those incubated at 25° C. The major part of the variation observed within samples is probably due to the method of adding the moisture and hand mixing of the samples. The fact that the moisture values were more consistent at 0.3 bar than at 15 bars moisture tension can probably be explained as being due to the relative humidity of the air stream. A constant pressure of 15 cm of water was maintained on the air lines regardless of the soil moisture or temperature at which the soil was being incubated. The humidity of the released air stream was a calculated 98.5 percent using 910 cm of water as the average barometric pressure for this area. This humidity is probably more closely associated with moisture values approaching field capacity than permanent wilting point.

Table 2. A comparison of various mean moistures at all incubation temperatures

Soil	Tem- perature	Days incubated	Moisture tension	Original	Mean	Variance	CV ^a
	°C		bars	%	%		%
Elfrida sandy clay loam	35	16	0.3	18.37	18.31	0.31	3.10
	35	56	15.0	11.36	11.04	0.33	5.16
	25	16	0.3	18.37	18.86	0.52	3.82
	25	56	15.0	11.36	11.51	0.73	7.38
	2	133	0.3	18.37	16.23	0.80	5.50
Yolo loam	35	42	0.3	19.24	19.43	0.90	4.88
	35	56	15.0	10.24	10.52	0.87	8.84
	25	42	0.3	19.24	20.12	0.66	4.02
	25	56	15.0	10.24	11.14	0.37	5.48
	2	161	0.3	19.24	18.52	0.45	3.62
Willamette silt loam	35	42	0.3	25.48	25.26	0.45	2.66
	35	56	15.0	11.74	11.70	0.79	7.61
	25	42	0.3	25.48	25.77	0.53	2.79
	25	56	15.0	11.74	12.61	0.51	5.63
	2	161	0.3	25.48	25.37	0.35	2.32
Walla Walla silt loam	35	42	0.3	23.05	22.76	0.42	2.83
	35	56	15.0	7.75	7.89	0.59	9.63
	25	42	0.3	23.05	23.13	0.43	2.81
	25	56	15.0	7.75	7.82	0.67	10.36
	2	133	0.3	23.33	20.33	0.66	3.98

^aCoefficient of variation

It is worth noting that at the 20° C incubation of Elfrida sandy clay loam and Walla Walla silt loam soils the actual moisture values were not duplicated too closely. This was due to improper aeration techniques at the beginning of the incubation period. When the lids of the containers were removed for aeration during the first two weeks, no attempt was made to compensate for the loss of moisture in the exchange of air and a drop of moisture was noted in the soil samples. In all later aeration of these samples as well as the subsequent soils, a fine mist was sprayed around the mouth of the jar and on the lid to compensate for the moisture removed when the air was forced into the jar and the moisture levels remained constant.

As a whole, moisture control for both phases of the experiment was considered to be very adequate and little or no variation is believed to have evolved from moisture fluctuation.

Nitrate production of soils studied

In all soils used there was present initially various quantities of nitrate and ammonia nitrogen as determined by the phenoldisulfonic acid method and Nesslerization. The quantities in the air dry soil are presented in table 3.

Table 3. Initial quantities of ammonium nitrogen, nitrite nitrogen and nitrate nitrogen present in the soils on an air dry basis

Soil	NH ₃ -N ppm	NO ₂ -N ppm	NO ₃ -N ppm
Elfrida sandy clay loam	11	-	2
Walla Walla silt loam	14	-	4
Willamette silt loam	45	-	44
Yolo loam	6	-	36

Figures tabulated in the tables which follow represent an average of the 3 replications sampled. They are the totals of the nitrogen added to the soil plus that which was present initially. However, the data presented in graphical form are given as nitrate produced and the amount of nitrate given in the last column of table 3 has been subtracted from the averages obtained from the 3 samples analyzed.

Elfrida sandy clay loam

Results obtained from the Elfrida soil for the studies at all temperatures and moisture tensions are presented in table 4. Nitrate production is presented for the studies at all temperatures and moisture tensions in figure 1. This was the first soil studied and accounts for the short incubation period at the 0.3 bar moisture tension. It was intended to incubate the soils only until the applied ammonium sulfate had been completely oxidized. The 2 weeks allotted for incubation proved satisfactory at the 35° C temperature, but was insufficient time for complete oxidation of the applied ammonium sulfate at 25° C. Nitrate production was so intense at 35° C and 0.3 bar moisture that samples were taken every 4 days so that a more accurate account of nitrate production could be obtained. At this temperature and moisture, 16 days was a sufficient amount of time to allow complete oxidation of the applied nitrogen. When incubating at the same temperature and 15 bars tension, it took 49 days for complete oxidation. Nitrates produced at 25° C amounted to approximately one-eighth of the amount produced at 35° C at both levels showing the effect of temperature on the nitrifying population of the soil.

The 2° C temperature resulted in a definite inhibitory effect on the nitrifying population and after an incubation period of 133 days

Table 4. Changes in ammonium nitrogen, nitrite nitrogen and nitrate nitrogen during incubation of 150 ppm nitrogen added as ammonium sulfate in Elfrida sandy clay loam soil at various moistures and temperatures

days	25° C						35° C						2° C			
	0.3 bar			15 bars			0.3 bar			15 bars			0.3 bar			
	NH ₃ -N	NO ₂ -N	NO ₃ -N	NH ₃ -N	NO ₂ -N	NO ₃ -N	NH ₃ -N	NO ₃ -N	NO ₂ -N	NH ₃ -N	NO ₂ -N	NO ₃ -N	NH ₃ -N	NO ₂ -N	NO ₃ -N	
	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	
4	150	4	4				145	8	12				7	168	-	2
7				154	-	4				152	13	16	21	171	-	1
8	139	12	12				74	25	47				35	140	-	3
12	134	-	18				11	-	136				49	153	1	4
14				150	-	10				130	-	35	63	158	1	3
16	135	-	21				1	-	156				77	148	2	3
21				152	-	15				118	-	33	91	163	3	4
28				129	-	11				71	-	73	105	158	5	4
35				122	-	17				62	-	82	119	154	8	4
42				114	-	15				38	-	135	103	146	9	5
49				112	-	24				5	-	147				
56				107	-	40				2	-	147				

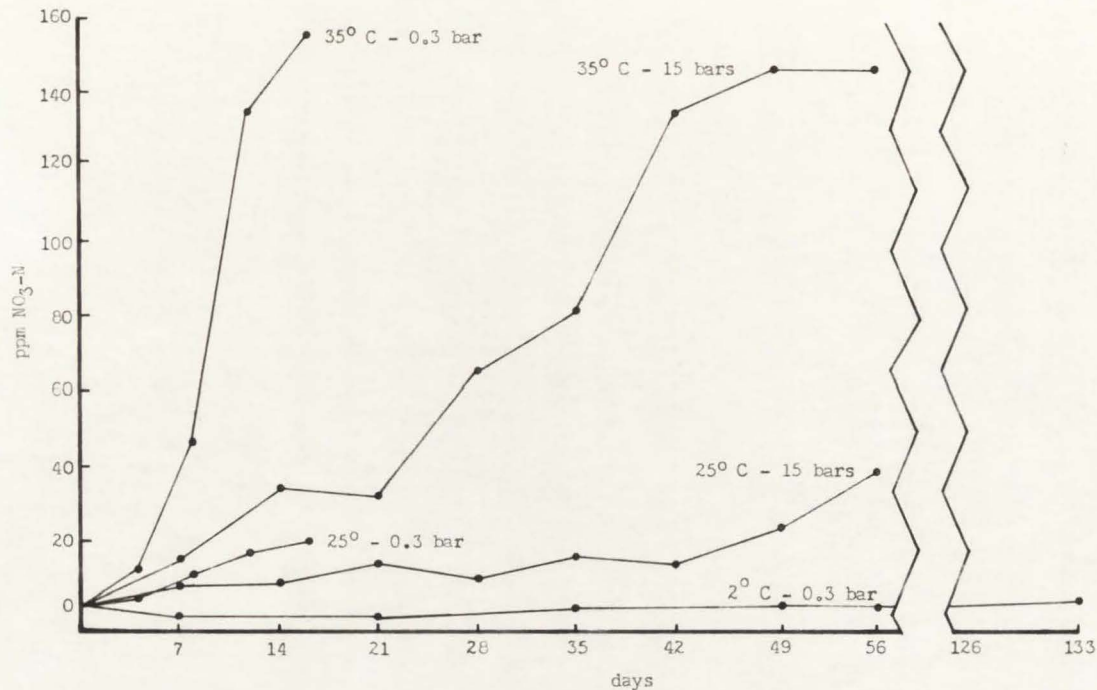


Figure 1. A comparison of changes in nitrate nitrogen during incubation of ammonium sulfate added to Elfrida sandy clay loam at the rate of 150 ppm nitrogen as influenced by moisture level (0.3 and 15 bars) and temperature (2, 25 and 35° C)

there was no appreciable increase of nitrates.

Elfrida sandy clay loam was the only soil studied which accumulated any appreciable amounts of nitrites during the incubation period (table 4). Nitrites accumulated at all temperatures and moisture tensions except at 25° C and 15 bars tension. Incubation at 35° C and at 0.3 and 15 bars moisture tension produced considerable amounts of nitrite during the early stages of incubation but it had disappeared completely at the 14-day sampling. At 2° C nitrites began to appear after 49 days and continued to increase slowly throughout the remainder of the incubation.

Walla Walla silt loam

The data obtained from incubation of Walla Walla silt loam soil are presented in table 5 and figure 2.

One of the most interesting aspects of this soil is the apparent peak of nitrate production in the soils incubated at 15 bars tension and 25° C which was reached at the 42-day sampling with a subsequent loss or decrease of nitrates the last 2 weeks of the incubation. Nitrate production may have stopped with subsequent denitrification of that nitrate produced. This would not be considered uncommon if some logical explanation for the apparent inhibition of nitrification could be made. Denitrification under apparent aerobic conditions does exist (Corbet and Wooldridge, 1940; Marshall et al., 1953; Broadbent, 1951; Meiklejohn, 1940), but under the present conditions of continuous aeration during incubation it is unlikely. A possible explanation for the abrupt halt in nitrate production could be that the critical moisture for nitrification had been reached. Nitrification has been found to cease at moisture levels slightly below the permanent wilting point (Justice and Smith, 1960; Robinson, 1957) and perhaps this critical point had been reached. Moisture data do not support this view, however, since close

Table 5. Changes in ammonium nitrogen, nitrite nitrogen and nitrate nitrogen during incubation of 150 ppm nitrogen added as ammonium sulfate in Walla Walla silt loam soil at various moistures and temperatures

25° C							35° C						2° C			
0.3 bar			15 bars				0.3 bar			15 bars			0.3 bar			
	NH ₃ -N	NO ₂ -N	NO ₃ -N	NH ₃ -N	NO ₂ -N	NO ₃ -N	NH ₃ -N	NO ₂ -N	NO ₃ -N	NH ₃ -N	NO ₂ -N	NO ₃ -N		NH ₃ -N	NO ₂ -N	NO ₃ -N
days	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	days	ppm	ppm	ppm
7		-	20	156	-	13		-	18	120	-	12	7	168	-	9
14	140	-	46	177	-	16	168	-	25	179	-	12	21	152	-	9
21	107	-	81	169	-	22	165	-	37	173	-	16	35	147	-	12
28	78	-	119	151	-	23	152	-	47	173	-	13	49	152	-	23
35	43	-	149	138	-	43	138	-	65	179	-	12	63	147	-	32
42	44	-	161	122	-	58	143	-	69	146	-	19	77	133	-	47
49				136	-	50				196	-	17	91	100	-	81
56				152	-	31				215	-	18	105	88	-	98
													119	80	-	107
													133	76	-	109

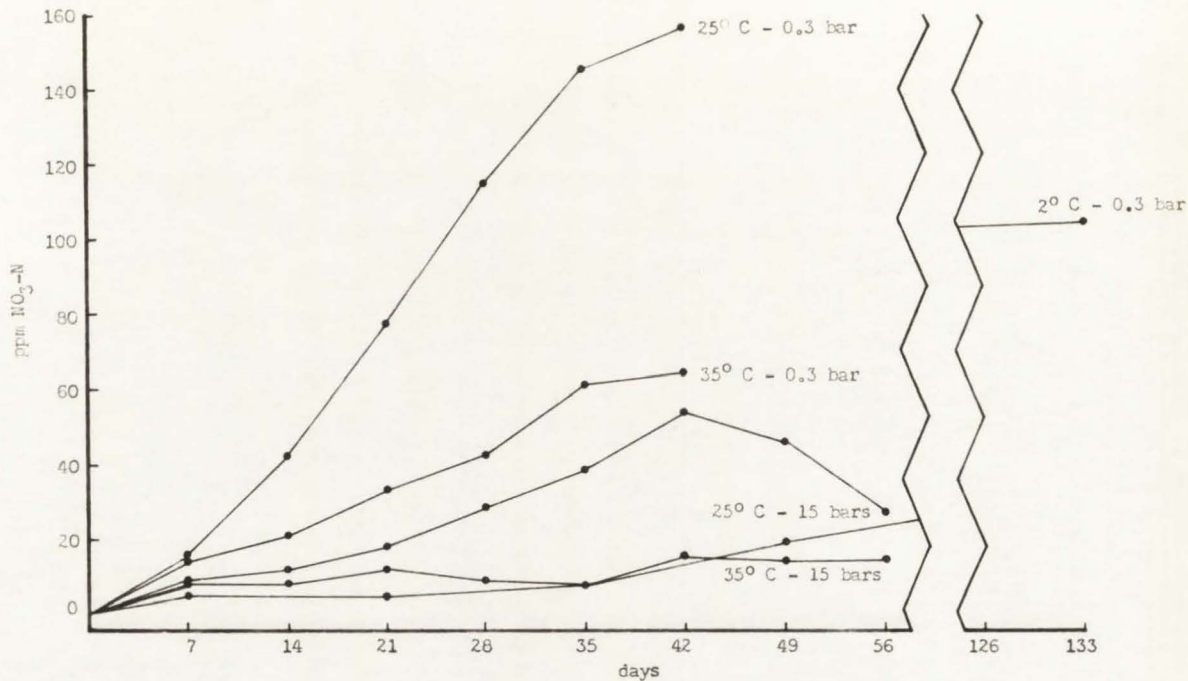


Figure 2. A comparison of changes in nitrate nitrogen during incubation of ammonium sulfate added to Walla Walla silt loam at the rate of 150 ppm nitrogen as influenced by moisture level (0.3 and 15 bars) and temperature (2, 25 and 35° C).

appraisal of the original data showed a slight increase in percent moisture during the 56 days. At 25° C and 0.3 bar moisture, strong nitrification is noted and no peak of nitrate production was noticed during the entire 42 days. The increase of ammonia which accompanied the nitrate loss (table 5) can be logically explained as coming from ammonification of the natural soil organic matter. This soil had the highest organic matter content of any studied (table 1). This organic matter would be necessary for denitrification, as postulated above, to occur.

It is interesting to note that at the 49-day sampling, nitrate production at 2° C and 0.3 bar moisture was greater than at 35° C and 15 bars moisture, demonstrating the inhibitory effect on the nitrifying population of the high temperature and low moisture. At 2° C, an initial lag of about 35 days was noticed before the buildup in the population of nitrifiers was high enough to produce appreciable amounts of nitrate nitrogen. Once the initial inhibition of temperature had been overcome, the production of nitrates proceeded at a rapid rate even at this low temperature. The initial lag is probably caused by the low temperature since at 25 and 35° C and 0.3 bar moisture no lag period can be detected.

Willamette silt loam

This particular soil was taken from under timber growth in Western Oregon which accounts for its relatively low pH (5.6). This is apparently a soil with very low initial population of nitrifiers which is probably due to the low pH and the vegetation under which the soil has formed. Information on nitrification in the soil is given in table 6 and figure 3.

Very little nitrification was measured during the course of incubation at any of the 3 temperatures or either of the 2 moisture tensions

Table 6. Changes in ammonium nitrogen, nitrite nitrogen and nitrate nitrogen during incubation of 150 ppm nitrogen added as ammonium sulfate in Willamette silt loam soil at various moistures and temperatures

days	25° C						35° C						2° C			
	0.3 bar			15 bars			0.3 bar			15 bars			0.3 bar			
	NH ₃ -N	NO ₂ -N	NO ₃ -N	NH ₃ -N	NO ₂ -N	NO ₃ -N	NH ₃ -N	NO ₂ -N	NO ₃ -N	NH ₃ -N	NO ₂ -N	NO ₃ -N	NH ₃ -N	NO ₂ -N	NO ₃ -N	
	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
7		-	39	209	-	43		-	39	216	-	44	7	216	-	42
14	201	-	46	213	-	46	218	-	42	211	-	43	21	217	-	38
21	211	-	48	224	-	40	223	-	41	230	-	43	35	242	-	39
28	212	-	51	215	-	43	257	-	45	218	-	42	49	203	-	40
35	191	-	59	225	-	43	251	-	41	233	-	40	63	211	-	40
42	188	-	67	218	-	45	254	-	38	222	-	41	77	209	-	44
49				217	-	42				220	-	41	91	203	-	46
56				214	-	44				221	-	42	105	220	-	43
													119	213	-	40
													133	211	-	49
													147	185	-	53
													161	175	-	65

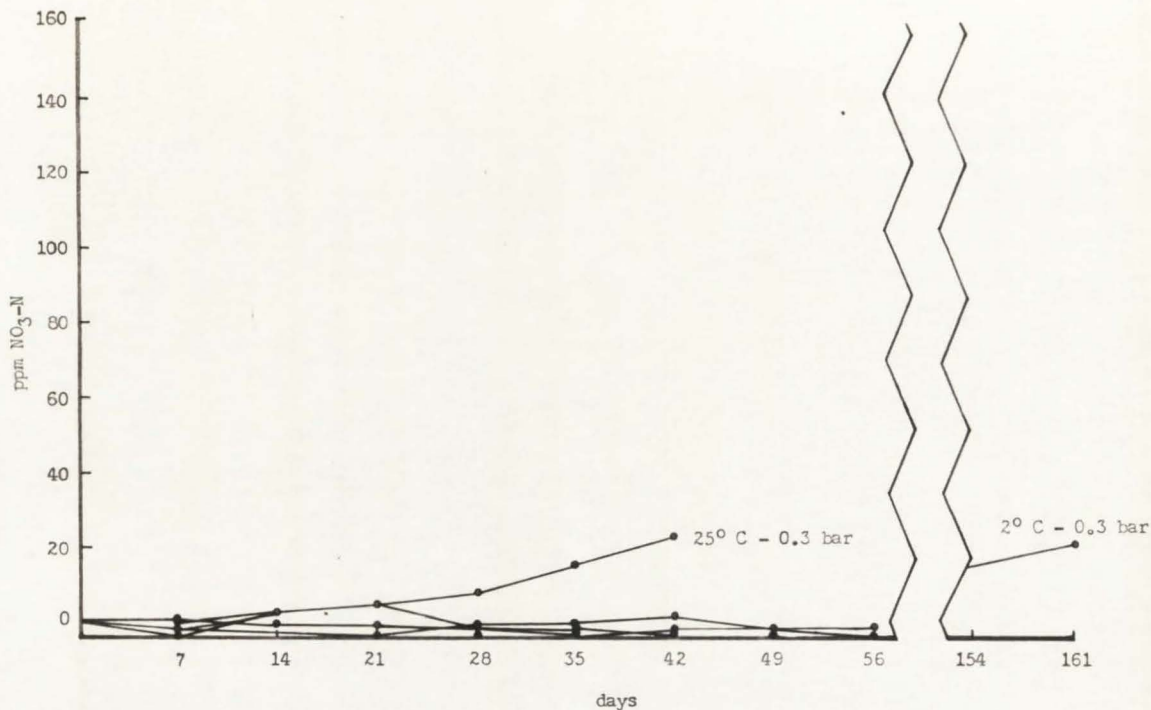


Figure 3. A comparison of changes in nitrate nitrogen during incubation of ammonium sulfate added to Willamette silt loam at the rate of 150 ppm nitrogen as influenced by moisture level (0.3 and 15 bars) and temperature (2, 25 and 35° C). Shows only those moisture tensions and temperatures where nitrates accumulated.

used. An initial lag period of at least 3 weeks was noticed at all temperatures and moisture tensions. No appreciable nitrification occurred at either moisture level at 35° C, or at 15 bars tension at 25° C during the entire course of incubation. Some nitrification was evident at 25° C and 0.3 bar moisture after an initial lag phase of 21 days. Nitrate production was also noticeable after 133 days at 2° C and 0.3 bar moisture. Slow nitrification in all cases with this soil is attributed to the low pH.

It should be noted that although the soil lacked the capability to produce nitrates in any great quantity at any temperature initially, the optimum temperature for this soil would have to be taken as 25° C with a definite inhibition at 35° C at least for the length of the incubation period used.

Yolo loam

Of the 4 soils studied, Yolo loam had the greatest capacity to produce nitrate nitrogen at a faster rate under the variety of conditions. Of the temperatures used, 25° C was the optimum for nitrate production. Appreciable nitrate production was observed at all temperatures and moisture tensions. No lag was observed at any of the increased temperatures and only a short initial lag period was noticed at 21 days at the 2° C temperature. Nitrate production at 2° C and 0.3 bar moisture was greater after 35 days than at 35° C and 15 bars moisture tension.

At 2° C there seemed to be some indication of nitrite production in the early stages of incubation (table 7) but after 49 days all traces had disappeared and were not detectable throughout the remainder of the incubation study. Results from the study of this soil are presented in table 7 and figure 4.

Table 7. Changes in ammonium nitrogen, nitrite nitrogen and nitrate nitrogen during incubation of 150 ppm nitrogen added as ammonium sulfate in Yolo loam soil at various moistures and temperatures

days	25° C						35° C						2° C			
	0.3 bar			15 bars			0.3 bar			15 bars			0.3 bar			
	NH ₃ -N	NO ₂ -N	NO ₃ -N	NH ₃ -N	NO ₂ -N	NO ₃ -N	NH ₃ -N	NO ₂ -N	NO ₃ -N	NH ₃ -N	NO ₂ -N	NO ₃ -N	NH ₃ -N	NO ₂ -N	NO ₃ -N	
	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	
7	128	-	65	125	-	46	114	-	50	126	-	50	7	131	1	41
14	116	-	76	113	-	55	109	-	71	108	-	60	21	130	1	46
21	105	-	86	115	-	65	106	-	79	109	-	60	35	111	1	67
28	49	-	124	112	-	69	94	-	92	108	-	61	49	96	-	84
35	33	-	147	95	-	76	74	-	107	104	-	63	63	88	-	91
42	9	-	198	93	-	84	65	-	123	99	1	66	77	76	-	86
49				58	-	120				90	-	74	91	53	-	107
56				39	-	136				92	-	80	105	73	-	126
													119	36	-	141
													133	25	-	155
													147	25	-	164
													161	32	-	174

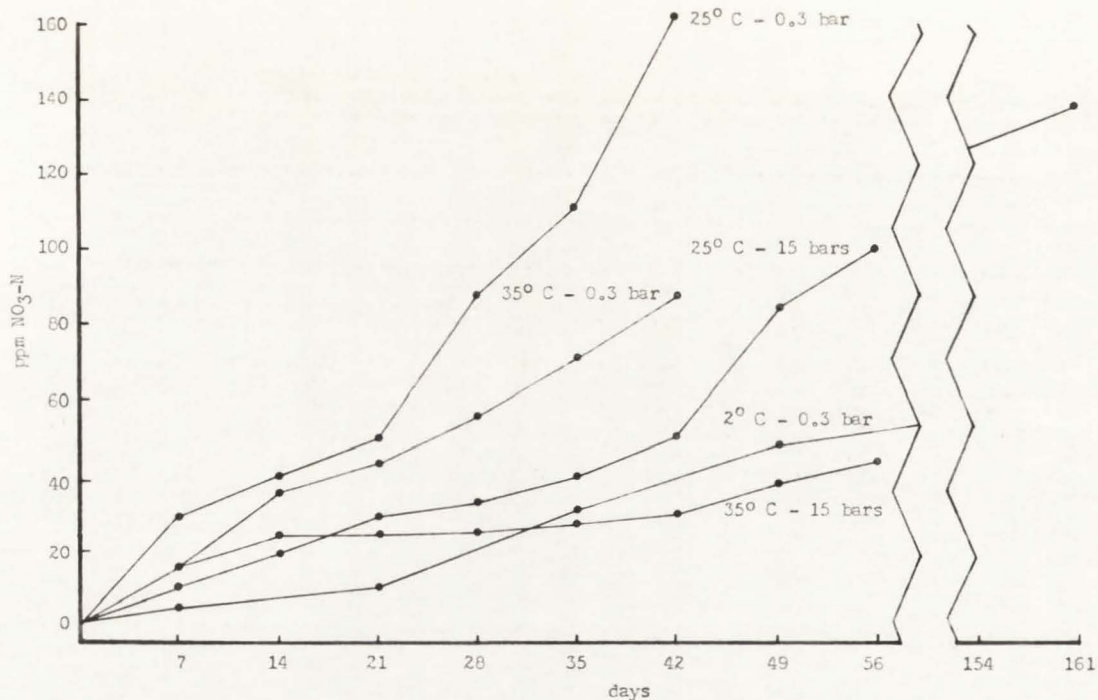


Figure 4. A comparison of changes in nitrate nitrogen during incubation of ammonium sulfate added to Yolo loam at the rate of 150 ppm nitrogen as influenced by moisture level (0.3 and 15 bars) and temperature (2, 25 and 35°C)

Comparison of soils studied

Incubation at 25 and 35° C and 0.3 bar.--Comparison of nitrate production at 25 and 35° C and 0.3 bar moisture tension in figures 5 and 6 is designed to show the maximum amount of nitrates produced under the optimum condition of moisture used in the study. This is not to say that 25 or 35° C is the optimum temperature for nitrate production of that soil. Elfrida sandy clay loam produced a maximum of nitrates at 35° C while the other 3 soils used produced more nitrates at 25° C.

At 25° C Yolo loam and Walla Walla silt loam produced more nitrate than either the Willamette silt loam or the Elfrida sandy clay loam soils. A definite lag of approximately 21 days was noticed in the Willamette soil while the lag period in Elfrida sandy clay loam soil seemed to be only about 4 days. Yolo loam and Walla Walla silt loam exhibited no lag and nitrate production proceeded at a high rate throughout the nitrification period. At 25° C the Yolo and Walla Walla soils exhibited comparable nitrification rates.

At 35° C the situation was almost entirely reversed, in which case Elfrida sandy clay loam had a very high maximum rate of production. The Elfrida soil exhibited the highest rate of nitrification in the shortest period of time of any of the soils studied regardless of temperature or moisture tension. Yolo loam and Walla Walla silt loam, although they did not exhibit any lag period, did not compare with the Elfrida soil at this higher temperature. Willamette silt loam produced no nitrates during the 6-week incubation period and a general decrease in total nitrates is noted.

Incubation at 25 and 35° C and 15 bars.--Figures 7 and 8 compare all soils at 25 and 35° C and 15 bars moisture tension as to their relative nitrate producing ability. Nitrification, to some extent, occurred

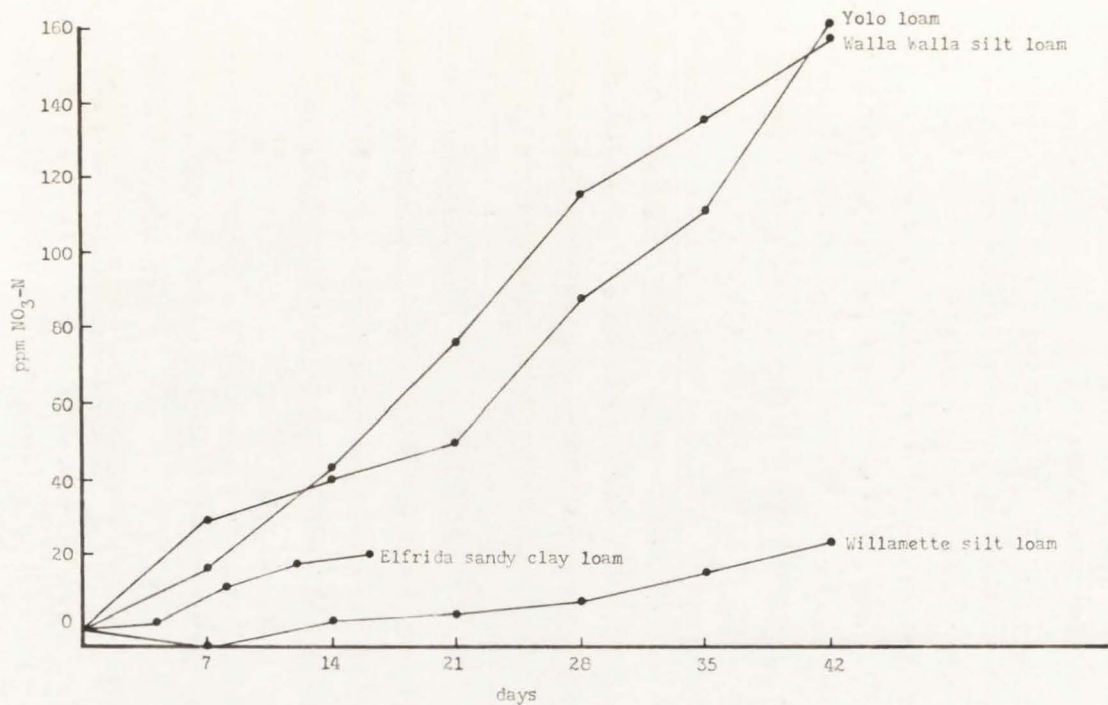


Figure 5. A comparison of changes in nitrate nitrogen in all soils studied with 150 ppm nitrogen added as ammonium sulfate at 0.3 bar moisture tension and 25° C

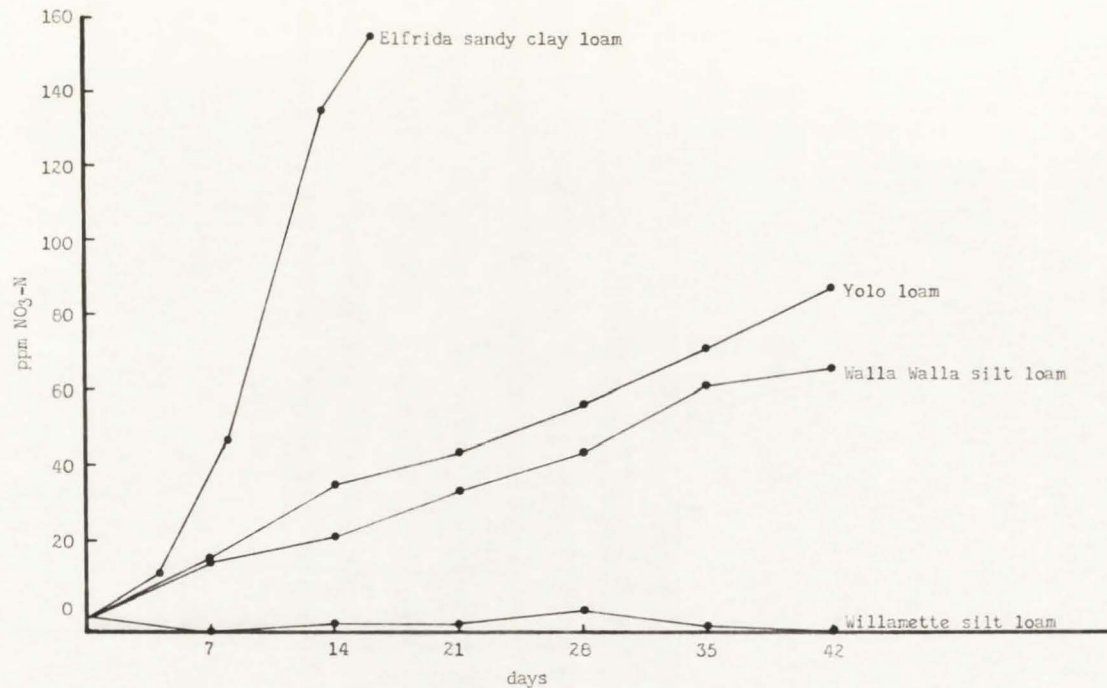


Figure 6. A comparison of changes in nitrate nitrogen in all soils studied with 150 ppm nitrogen added as ammonium sulfate at 0.3 bar moisture tension and 35° C

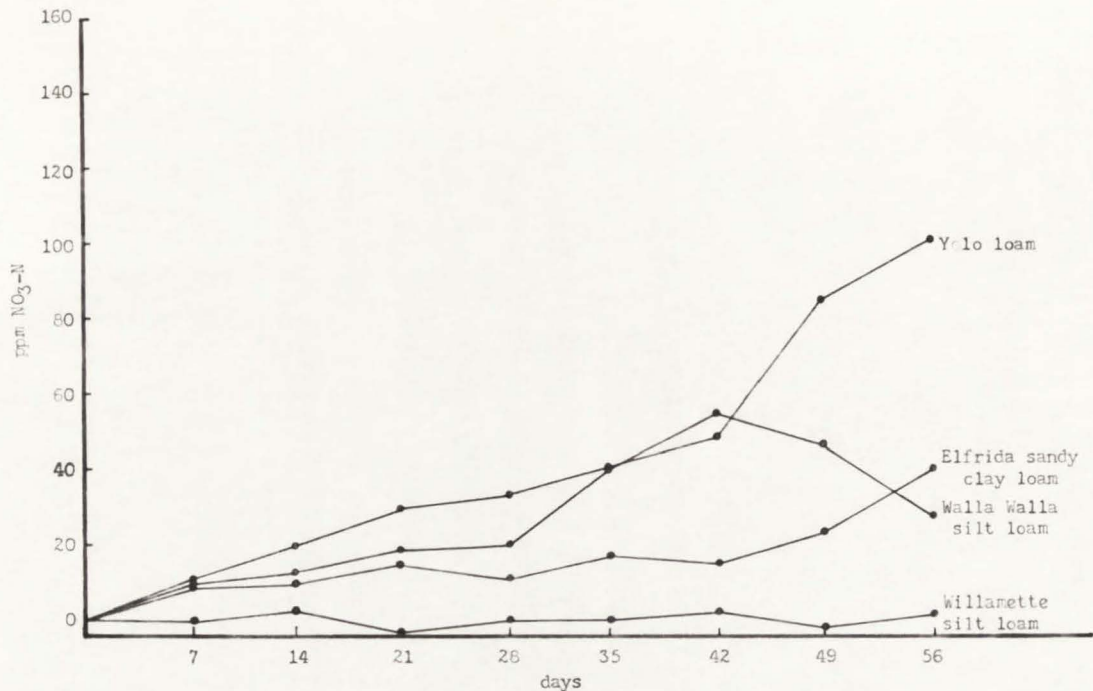


Figure 7. A comparison of changes in nitrate nitrogen in all soils studied with 150 ppm nitrogen added as ammonium sulfate at 15 bars moisture tension and 25° C

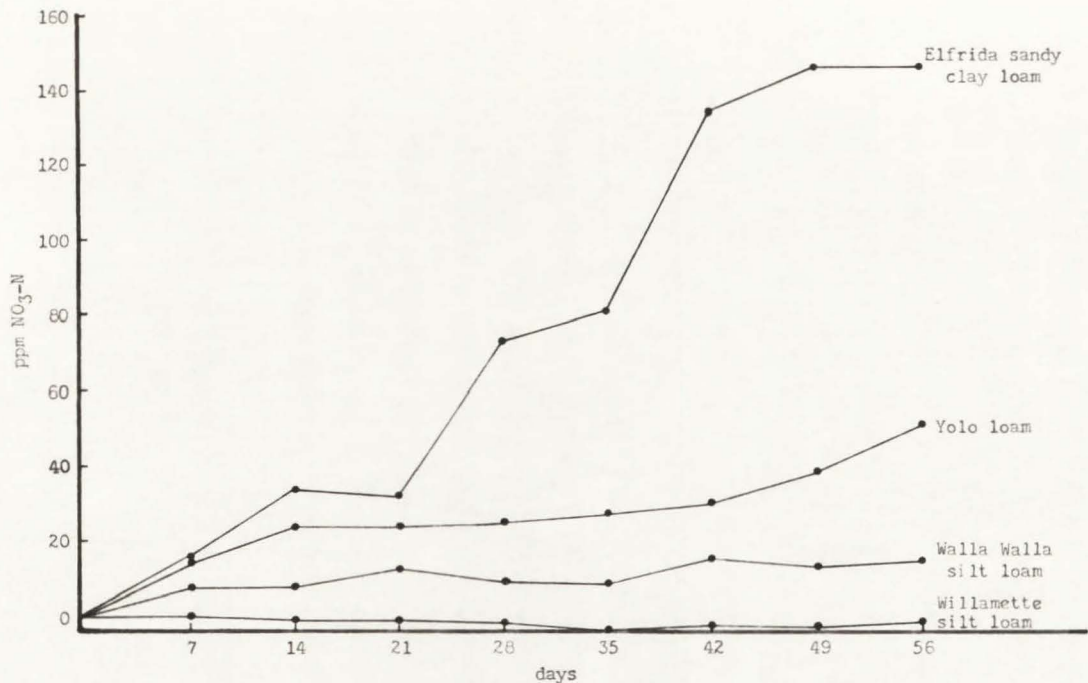


Figure 8. A comparison of changes in nitrate nitrogen in all soils studied with 150 ppm nitrogen added as ammonium sulfate at 15 bars moisture tension and 35° C

in all soils except Willamette silt loam. Organisms from Yolo loam were possibly better adapted, through genetic selection, to this moisture level and 25° C than were the organisms from the other 3 soils studied. Elfrida sandy clay loam produced more nitrates at 35° C than it did at 25° C. It is interesting to note that at the final sampling at 25° C the Elfrida soil contained higher amounts of nitrate than did the Walla Walla soil which had better initial nitrate production (0 to 42 days). There remains, however, the unexplained drop in nitrates in the Walla Walla soil after 6 weeks of incubation. Nitrate production of Walla Walla silt loam at 35° C did not exhibit the pronounced drop in nitrates that was noticed at 25° C, yet only a very small amount of nitrates were produced over the 56-day period. With the evidence available, no correlation could be drawn between the 2 temperatures in relation to this drop in nitrates in this soil.

Willamette silt loam did not compare favorably with any of the other soils at this temperature and no nitrates were produced during the entire 56 days of incubation.

Low temperature and 0.3 bar.—In an attempt to evaluate the nitrate producing capacity of the soils at 2° C and 0.3 bar moisture, which is assumed to approximate field conditions in the winter time in many areas, the soils were incubated in a 2° C controlled temperature room for an extended period of time. The soils were prepared for incubation at 2 different times and accounts for the variation in the length of time incubated. The first incubation at this temperature included the Walla Walla silt loam and the Elfrida sandy clay loam soils and they were incubated for 133 days. When the Yolo loam and Willamette silt loam soils were prepared for incubation in the controlled temperature

room, additional samples were prepared and the study was conducted for a total of 161 days. This turned out to be quite interesting as far as the Willamette soil was concerned. At 133 days no appreciable nitrification had occurred, yet at 161 days the nitrates produced had risen to 21 ppm. It can be concluded that this soil under the conditions of incubation would produce nitrates from added ammonium sulfate, but only after an initial lag period of 133 days. By this time a sufficient population of nitrifiers, which is hardy enough to withstand the environment imposed upon the soil, had developed.

Willamette silt loam and Elfrida sandy clay loam experienced prolonged lag periods before any nitrification was detected, but in the case of the Elfrida soil, as has already been stated, some nitrites began to appear at the 49-day sampling and continued to increase slightly through the rest of the incubation period. This was probably a result of the inability of the nitrite oxidizing organisms to tolerate the lowered temperature as well as the ammonia oxidizing bacteria. Some nitrites were also evident at the beginning of the incubation period at this temperature in Yolo loam soil but disappeared after 35 days.

Yolo loam did very well at this temperature and no lag period was noticed. Nitrate production proceeded at a high rate through the entire incubation period and nearly all of the added ammonium sulfate was oxidized at the end of 161 days. Some lag was noted in the Walla Walla silt loam soil. This can be seen in figure 9.

From the data obtained, it can be concluded that fall application of ammonium fertilizers would not be practical for soils similar to Yolo loam and Walla Walla silt loam. This type of fertilization on a soil similar to Willamette silt loam or Elfrida sandy clay loam would be of possible economical importance. It should be observed, however,

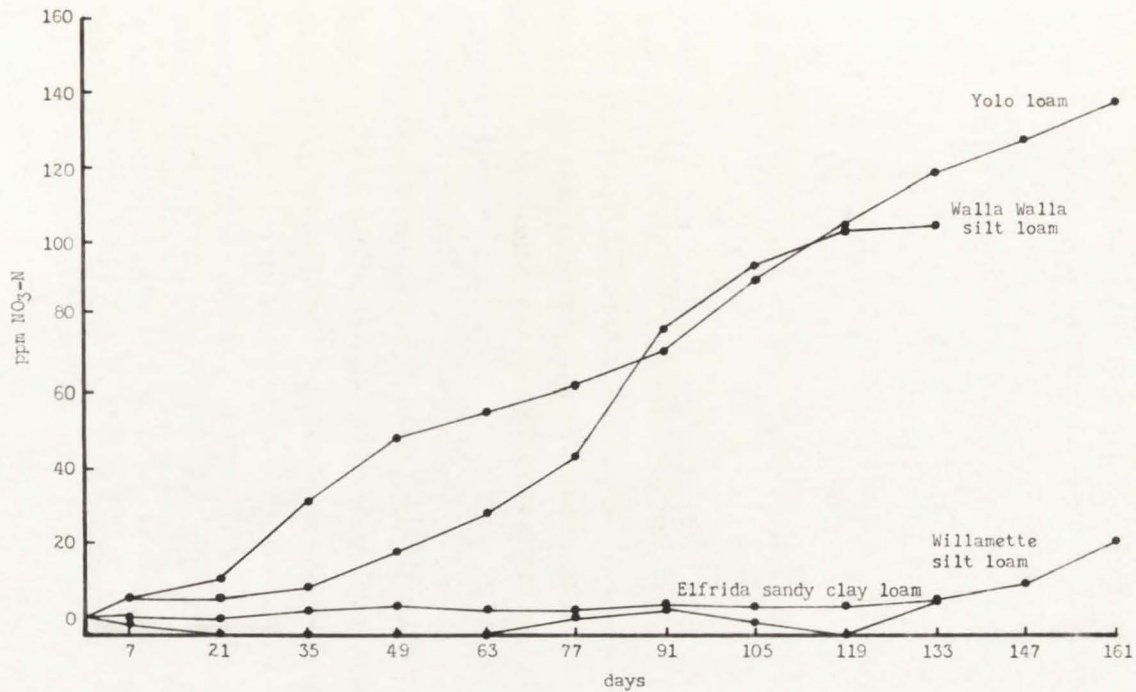


Figure 9. A comparison of changes in nitrate nitrogen in all soils studied with 150 ppm nitrogen added as ammonium sulfate at 0.3 bar moisture tension and 20° C

that the region from which Elfrida sandy clay loam soil was taken would rarely encounter such a low temperature and then only for a short period of time. For the Willamette silt loam it could be said that ammonium fertilizers applied in the fall would not be oxidized to the nitrate form in any appreciable amount by spring. At 25° C and 0.3 bar moisture, which were the optimum conditions for nitrate production for this soil (table 8), there was still a 21-day lag before any nitrates were produced. Therefore, no great quantity of nitrate would accumulate regardless of moisture tension and temperature of the soil.

Nitrogen recovery

Total recoverable inorganic nitrogen in this study was taken as the sum of the nitrate, nitrite and ammonia nitrogen. The totals obtained from each week's sampling is presented in table 9.

Variation in total nitrogen recovered is probably due to variation in the ammonia nitrogen determination. It was found to be extremely difficult to duplicate ammonia nitrogen results closely due to the sensitivity of the method used. The results of nitrate and nitrite nitrogen recovered were quite consistent.

Two facts seem to stand out from the results tabulated in table 9. First, the total nitrogen tended to decrease with length of incubation in the Elfrida sandy clay loam. This was most noticeable at 15 bars moisture tension and at 25 and 35° C. There is no apparent loss of nitrogen from the system at 0.3 bar and 2° C during the 133-day incubation period. Loss of nitrogen is probably a result of volatilization of nitrogen gases. The results obtained from the Elfrida soil are in general agreement with the work done by Martin and Chapman (1951) who found that from 1 to 27 percent of the nitrogen added as ammonium

Table 8. Maximum rate of nitrate nitrogen production under the various conditions of temperature and moisture

Temperature	Soil	Moisture tension, bars			
		0.3		15	
		Time interval	Maximum rate	Time interval	Maximum rate
°C		days	ppm/day	days	ppm/day
25	Elfrida sandy clay loam	4-16	1.5	42-56	1.8
	Walla Walla silt loam	0-42	3.7	28-42	2.6
	Willamette silt loam	14-42	0.8	0	0
	Yolo loam	0-42	3.9	42-56	3.7
35	Elfrida sandy clay loam	0-16	9.8	21-49	4.1
	Walla Walla silt loam	0-42	1.3	0	0
	Willamette silt loam	0	0	0	0
	Yolo loam	0-42	2.1	42-56	1.5
2	Elfrida sandy clay loam	0	0		
	Walla Walla silt loam	63-119	1.2		
	Willamette silt loam	119-161	0.6		
	Yolo loam	21-161	0.9		

Table 9. Mean total inorganic nitrogen recovered at each sampling from all soils studied at all temperatures (2, 25 and 35° C) and all moisture tensions (0.3 and 15 bars)

Temperature	Soil	Moisture tension	Days											
			4	7	8	12	14	16	21	28	35	42	49	56
0C		bars												
35	Elfrida scl	0.3	158		163	152		156						
		15		163			160		167	140	139	129	136	147
	Walla Walla sil	0.3		195			193		202	199	203	212		
		15		132			191		189	186	191	165	213	233
	Willamette sil	0.3					290		254	302	292	292		
		15		260			254		273	260	273	263	261	263
	Yolo 1	0.3		194			180		185	186	181	188		
		15		176			168		169	169	167	166	164	172
25	Elfrida scl	0.3	165		146	147		157						
		15		181			165		161	144	144	173	152	149
	Walla Walla sil	0.3		187			186		188	197	192	205		
		15		169			193		191	174	181	180	186	183
	Willamette sil	0.3					247		259	263	250	255		
		15		252			259		264	258	268	263	259	258
	Yolo 1	0.3		193			192		191	173	180	207		
		15		171			168		180	181	171	177	178	175

Table 9. Continued

Temperature	Soil	Moisture tension	Days											
			7	21	35	49	63	77	91	105	119	133	147	161
°C		bars												
	Elfrida scl	0.3	170	172	143	158	162	153	170	167	166	160		
	Walla Walla sil	0.3	177	161	159	175	179	180	181	186	187	185		
2	Willamette sil	0.3	258	255	281	243	251	253	249	263	253	260	238	240
	Yolo 1	0.3	173	177	179	180	179	162	160	199	177	180	190	206

sulfate was lost, presumably through volatilization. The greatest losses came from soils with a pH higher than 7.2. The Elfrida soil has a pH of 7.7. They also found that moisture content of the soils had little effect except that evaporation of water was necessary for appreciable volatilization of ammonia from the soil.

The second observation is that total inorganic nitrogen in the Walla Walla silt loam increased at all temperatures and moisture tensions during the incubation. This is probably due to the high organic content of the soil and subsequent ammonification of this organic matter.

Fluctuation of total inorganic nitrogen in the Willamette soil was so great that no conclusions can be drawn as to whether there was a loss or gain in total nitrogen. Generally, there seems to be no loss or gain of nitrogen over the incubation period.

Yolo loam showed no nitrogen loss at 25 and 35° C but there was a tendency for the total inorganic nitrogen to increase slightly at 2° C during the incubation.

SUMMARY AND CONCLUSIONS

Incubation studies designed to measure the nitrifying capacity of several soils, differing in their physical and chemical properties, were conducted under conditions of controlled temperature and moisture. The soils used were collected from the Western region and included Elfrida sandy clay loam, Walla Walla silt loam, Willamette silt loam and Yolo loam.

The first phase of the study was conducted at 25 and 35° C in controlled temperature baths with continuous aeration of the samples. In this part of the experiment 33.3 grams of soil was incubated in 250 ml Erlenmeyer flasks at 0.3 and 15 bars moisture tension. These moisture tensions and temperatures were designed to approximate extremes in field conditions during the summer months. The second phase of the study was conducted in a controlled temperature room where 100 gram samples of each soil were incubated at 2° C with intermittent aeration for an extended period of time and was designed to approximate field conditions during the winter time. In both phases of the experiment, 150 ppm N as ammonium sulfate on a dry soil basis was added to the samples along with adequate distilled water to bring the moisture up to the desired level.

The following observations and conclusions were noted:

1. Different soils vary greatly in their ability to oxidize applied ammonium sulfate at various temperatures and moisture tensions. The difference is apparently due to genetic selection of the biological

population due to the environmental conditions under which it has evolved.

2. At low temperatures, Elfrida sandy clay loam soil tended to accumulate nitrites throughout the entire incubation period. At 25° C and 15 bars moisture tension no nitrites accumulated but they were noticed initially at 0.3 bar and this temperature. Strong nitrite accumulation was noticed for the first week in this soil at 35° C and both moisture tensions used but had disappeared by the 14-day sampling in all cases. Strong inhibition of the nitrite oxidizing organisms is therefore apparent at the 2° C temperature.

3. Fall application of ammonium fertilizers would not be practical in soils with nitrifying capacities comparable to Walla Walla silt loam and Yolo loam. More economical use could be made of this method of fertilization in soils similar to Elfrida sandy clay loam and Willamette silt loam if these soils existed in regions of low winter temperature.

4. Active nitrification occurred in the soils studied at moisture tensions approximating the permanent wilting point except in Willamette silt loam where the soil pH was the limiting factor.

5. Loss of total recoverable inorganic nitrogen was definitely noticeable only in the Elfrida sandy clay loam with the greatest loss at 15 bars moisture tension and 35° C. Walla Walla silt loam soil showed an increase in total inorganic nitrogen recovered at all moisture tensions and temperatures studied. This was concluded to be a result of the ammonification of natural soil organic matter.

6. To aid in future nitrification studies and fertilizer practices dealing with ammoniacal fertilizers, more complete data must be compiled on different soil types as to their relative ability to oxidize applied

ammonium fertilizers under a variety of moisture tensions and soil temperatures.

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