EFFECT OF TEMPERATURE AND BIOLOGICAL CONTROL CHEMICALS ON NITROGEN TRANSFORMATION IN HAWAIIAN SOILS

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INTRODUCTION

Nitrogen transformation in soil is governed by a number of environmental factors, such as mineral nutrients, temperature, aeration and moisture content. These environmental factors, alone or in combination, may affect nitrogen transformation by acting upon the initial population of bacteria (Frederic, 1957). These transformations in soil are also affected by modern practices of pest and disease control, which entail the use of chemicals in many cases.

In agriculture and biology the formation of nitrate by biological agencies has long been of interest among scientists. There have been many attempts to determine the season or the stage of growth in which nitrogen is needed by plants and to control nitrogen release after application. Nitrogen transformation is an important process in agriculture because the microbial formation of nitrate is the major means whereby plants are supplied with this anion. If the nitrate is not utilized by the crop or immobilized by the microflora, it may be lost through leaching (Greenland, 1958), denitrification (Wagner and Smith, 1958; Soulides and Clark, 1958) and volatilization (Martin and Chapman, 1951; Ernst and Massey, 1960). Thus in modern practices inhibitors are often used to conserve soil nitrogen by keeping it in the reduced form. How these chemicals affect nitrogen transformation and how in

turn they interact with the environmental factors in producing such changes is a subject that calls for more research.

The climatic patterns associated with land areas have a marked influence upon the formation and loss process regulating the quantity of mineral nitrogen in the soil. The high temperatures in tropical areas will tend to increase all microbiological activities. Soils in Hawaii have developed under varying tropical and sub-tropical climatic conditions and thus have physical and chemical properties which are unusual as compared to the soils found in other parts of the world. Here in Hawaii, aside of the investigations of Tam (1945) and Koike (1961), very little work has been done on fumigants in relation to nitrification. Furthermore, the temperature-fumigant interaction in the nitrification process has not been investigated. The specific objectives of the present study were to determine:

1. Nitrification of added ammonium nitrogen in soil samples contained in plastic bags in the field.
2. Effect of temperature on nitrification of added ammonium nitrogen and mineralization of native nitrogen.
3. Effect of temperature and biological control chemicals on nitrification of added ammonium nitrogen in Hawaiian soils.

REVIEW OF LITERATURE

Effect of temperature on nitrogen transformation

Temperature is one of the most important environmental factor which affects nitrogen transformation. Temperature is known to affect the biological changes in the soil and this effect in field conditions goes very closely with the season, so that whatever influence the season of the year has on these biological changes it is often explained in terms of temperature effect. It has been generally stated that increasing the temperature stimulates microbial activities, but raising the temperature above a certain optimum level will retard microbial activity (Waksman and Madhook, 1937; Frederic, 1956).

When the effect of temperature on mineralization of organic nitrogen is studied, a differentiation should be made between ammonification and nitrification, because ammonification can take place at high temperatures whereas nitrification is inhibited. Panganiban (1925), in his studies, found that ammonification took place between 15 and 60°C and at higher temperatures the rate is faster. Similar reports were given by Jenny (1941), Patak and Shrikande (1953), Sabey et al. (1956) and McIntosh and Frederic (1958).

Recent studies by Anderson and Purvis (1955), Broadbent, et al. (1958), Frederic (1956), Sabey et al. (1956), Tyler and

Broadbent (1960), Stojonovic and Broadbent (1956) have shown that nitrification of applied ammonium occours in some soils at temperatures lower than was previously thought necessary for nitrification, and that there are considerable differences among soils in their nitrifying characteristics at low temperatures.

Anderson and Boswell (1964) noted that accumulation of nitrates at 3 - 8°C varied widely among several cultivated acid soils to which 100 ppm NH_4NO_3 was added. Nitrification was either completely suppressed or delayed for several weeks in a sandy soil at 6°C. But in a clay loam, though nitrate accumulation was severely retarded at 3°C, rapid accumulation occurred at 8°C after a six-week delay period. The effect of soil differences is further emphasized by the fact that nitrate accumulation in clay loam at 8°C was much more rapid than in a loamy sand at 32°C. Thus the importance of obtaining information on different soils is indicated. Anderson (1960) and Frederic (1956) reported considerable nitrification occurred when the temperature exceeded 7°C; however, vigorous nitrification at temperatures as low as 3°C has been reported by Tyler _et al_. (1959). Most investigators agree that at low temperatures nitrification is inhibited. Tyler _et al_. (1959) said that low temperature and alkaline soil reaction appear to favor nitrite accumulation from ammonical fertilizers, even at low levels of addition. This finding suggests that bacteria which convert nitrite to nitrate are more sensitive to low

temperature than the other group which convert ammonium to nitrite. Previous studies have indicated that low temperatures did not severely limit the oxidation of ammonia, but that temperatures above 40°C were inhibitive (Meiklejohn, 1953; Warrington, 1879).

In Iowa, Sabey et al. (1959) found nitrification under field conditions was more rapid at low temperatures than the rate found under constant temperatures in the laboratory. In the field soils the temperature fluctuated both diurnally and over a longer period of time. Frederic (1956) has shown that more rapid nitrification in field soils at low temperatures was a result of fluctuating temperatures. He reported that in some soils 50 lb/A of ammonium nitrogen could be nitrified in two months, even when the average temperature was near freezing (0-2°C).

Parker and Larson (1962) found that in the range of 16-20°C, a 2°C difference had a measurable effect upon the oxidation of ammonium nitrogen to nitrate nitrogen in soil materials in the laboratory. The total amount of nitrate produced increased with increasing temperature, indicating that the nitrification of the residual soil nitrogen was temperature dependent and was the determining factor in the total production of nitrate.

Sabey et al. (1959), Frederic (1957), and Anderson and Purvis (1955), concluded that differences among soils in regard to the temperature range over which nitrate formation from ammonium salts occurs appear to be due to differences in the initial

population of nitrifiers. Waksman and Madhock (1937) and Fraderic (1956) have reported that a temperature of 27-37°C was the most favorable for nitrifying bacteria activity. Above and below this temperature range, the rate of nitrification was reduced. Meiklejohn (1954) reported that nitrification did not proceed at 40°C in a tropical Uganda soil.

Dhar (1935) and Dhar and Plant (1944) have said that the high mineralization rate of nitrogen under dry conditions is due to sunlight, and, therefore, the process is photo-chemical. However, this work could not be confirmed by other workers (Joshi and Biswas, 1948; Meiklejohn, 1953a). Furthermore, Fraps and Stergers (1935) reported that photonitrification is of little importance, and they said that sunlight actually hinders the bacteria concerned in nitrate formation, whereas nitrite-forming organisms are more resistant to sunlight. The foregoing examination of review of literature on temperature influences on nitrification leads one to conclude that there is no optimum temperature, although several values have been suggested, because the type of soils formed under varying climatic conditions are different in their nitrifying characteristics with difference in population.

Effect of biological control chemicals on nitrogen transformation

In many regions of the world, selective chemicals are used to control or eliminate agricultural pests. When these chemicals are applied to destroy non-beneficial organisms, they may also

destroy beneficial microorganisms. Among the more important of these latter organisms are the nitrifying bacteria which are essential for the conversion of ammonium nitrogen to nitrate nitrogen. The key position of the nitrifiers in the nitrogen cycle, their role in plant nutrition and nitrogen losses (by leaching, denitrification, etc.), and the great sensitivity of the autotrophs to the environmental change requires an examination of the possible harmful action of these chemicals upon nitrate production.

Soil fumigation is recognized as an essential production practice for certain crops in many parts of the world. The inhibition of nitrification and subsequent increase or accumulation of ammonium nitrogen which results from soil fumigation has been reported by a number of investigators. Stark et al. (1939) showed that high concentrations of chloropicrin and formaldehyde inhibited nitrification for a long time. Carter (1953) reported that 1-3 dichloropropane and 1-2 dichloropropane (DD) controlled nematodes in Hawaii when applied at 150 lbs/A, but pineapples failed to grow well as in soils treated with 200 lbs/A of chloropicrin. Later, Tam and Clark (1945) reported that pineapples grew well in the presence of high amounts of ammonium nitrogen resulting from soil applications of 400 lbs/A of DD which delayed nitrification. Tam (1944) also reported that 200 lbs/A DD suppressed nitrification for eight weeks but that 200 lbs. of chloropicrin was inhibiting for at least 24 weeks, showing that chloropicrin is more effective

than DD.

Aldrich and Martin (1952) studied the effects of fumigants and found that the accumulation of ammonium nitrogen following treatments began earlier in a sandy alkaline soil than in an acid mountain soil high in organic matter. Nitrification began earlier in ethylene dibromide-treated soils than in soils treated with DD or chloropicrin. The degree of inhibition of nitrification is correlated with the type of soil and the fumigant used. Newhall (1955) observed that chemical and biological changes are caused by heat and chemical treatments which caused inhibition for a certain time because of the reduction of the nitrifying population. Since ammonifiers are not affected by such treatments, there is an increase in ammonia.

The effects of methyl bromide fumigation on four groups of bacteria (ammonifiers, nitrifiers, denitrifiers and cellulose decomposers) were studied by Wensly (1953) who found that nitrifiers and cellulose decomposers are sensitive to methyl bromide fumigation, while ammonifiers and denitrifiers showed resistance to the chemical. He also studied the concentration and time of exposure of the fumigant and found that methyl bromide was a more active and effective fumigant than ethylene dibromide or DD. A soil with a high organic matter content and water holding capacity required a greater exposure period at a given concentration to produce 90 percent reduction in numbers than did a sandy

loam soil. He showed that with exposure periods of less than 24 hours or less than 12 hours, the destruction of the nitrifying bacteria was not as severe as with longer exposure periods.

Methyl bromide fumigation resulted in high ammonia levels and suppressed nitrification for a longer period of time than ethylene dibromide (Thiegs, 1955). He reported that the inhibition period with methyl bromide was 4-8 weeks. McCants et al. (1959) found that methyl bromide and DD mixture reduced nitrification to a greater extent than ethylene dibromide. Winfree and Cox (1948) studied the comparative effects of fumigation with chloropicrin and methyl bromide on mineralization of nitrogen in a peat soil. Both caused accumulation of ammonium nitrogen which was converted into nitrates after two months.

Kincaid and Volk (1949) found a prolonged retention of ammonium nitrogen in Florida soils fumigated with DD mixture or ethylene dibromide. The ammonium nitrogen level remained for a longer period in soil treated with DD mixture than in soil treated with ethylene dibromide.

In Hawaii Koike (1961) found that 1-2 dibromo-3 chloropropane at 3 gals/A was less toxic to nitrifying bacteria than ethylene dibromide at 8 gals/A or DD at 40 gals/A and inhibited nitrification for 4-8 weeks.

Goring (1962) reported that the chemical 2-chloro-6 (trichloromethyl) Pyridine (N-serve)[1/] inhibited nitrification. Complete control of nitrification by this chemical was obtained at 10°C and 21°C for 24 weeks, even at the lowest concentration of the chemical used (1 ppm). However, at 32°C there was no comparable control. He stated that within the normal range of soil temperatures, the rate of decomposition of the chemical will probably increase with increasing temperature as will the rate of recovery of the surviving nitrifying organisms. Turner et al. (1962) showed that nitrification was generally reduced as the level of the chemical was increased. Partial control of nitrification in these soils was obtained at rates varying from 0.5 to 2 percent of the ammonium nitrogen in the fertilizer.

In a preliminary incubation study with 1-3-dichloropropane incubated at 22 and 35°C, Wolcott et al. (1960) found that nitrification was suppressed for 7-8 weeks. At 5°C there was an indefinite lag in nitrification. During the period of retarded nitrification at the two higher temperatures, ammonium nitrogen accumulated to high levels and total mineralised nitrogen (NH_4+NO_3)-N was increased over that in unfumigated soil. Nitrification was delayed for about 8 weeks at soil temperatures above 16°C and for longer periods at lower temperatures. During the period of

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[1/] N-serve is the trade name given to this chemical by the Dow Chemical Company.

retarded nitrification, ammonium nitrogen accumulated. The level of accumulated ammonium nitrogen in soil high in organic matter increased with the soil temperature during this period. McClellan et al. (1949) reported that the fumigants, ethylene dibromide, DD and chloropicrin were most effective in retention in wet soils, and they were retained for a longer time in wet, low temperature soils than in dry, high temperature soils. Gasser and Peachey (1964) reported that in field soils, methyl bromide increased the mineralization of soil organic nitrogen more than other sterilants.

Thus, from the above review of literature, it is evident that the permanency and inhibition of nitrification depends not only on one factor but on many factors, such as the fumigant and dosage, soil type, pH and temperature.

MATERIALS AND METHODS

Description of the soils used

Surface soils representing four great soil groups were collected for this study. These soils, developed under a great range of climatic conditions, differ in physical, chemical and mineralogical properties, and are described by Cline et al. (1959). The four soils studied and the classification are given in Table 1.

Table 1. Classification of the Experimental Soils

Series	Great Soil Group[1/]	Sub-Group[2/]
Wahiawa	Low Humic Latosol	Tropeptic Eutrustox
Paaloa	Humic Latosol	Humoxic Trophohumults
Lualualei	Dark Magnesium Clay	Typic Chromusterts
Maile	Latosolic Brown Forest intergraded to Humic Latosol	Hydric Dystrandepts

1/ Great Soil Group as classified by Cline et al. (1955).

2/ Sub-Group as presently classified by the U.S. Soil Survey Staff of the Soil Conservation Service.

A brief description of each of these four soils are as follows:

Wahiawa silty clay is a low humic latosol belonging to the Wahiawa family. The soil is derived from basaltic lavas, under an annual rainfall of 30-40 inches at elevations ranging from 250-

1200 feet above sea level. It has a low to moderate base saturation with a low buffering capacity. The soil has a high clay content but shows the physical properties of a silty clay loam. The clays are predominantly kaolinitic.

Paaloa silty clay is a humic latosol belonging to the Honolua family. This soil is formed from basalt weathered in place under an annual rainfall of 70-100 inches at elevations ranging from 500-1200 feet above sea level. The clay minerals present in this soil are kaolin, illite, iron oxides and gibbsite. Roots are numerous at the surface. Cation saturation is low and the buffering capacity is moderately high.

Lualualei clay belongs to the Lualualei family, which is the only family in the dark magnesium clay group. The soil has developed from deep alluvium under an annual rainfall of 15-20 inches at an elevation of 20 feet above sea level. The dominant mineral present in the clay is montmorillonite, and the soil has sticky and plastic properties that are associated with such clays. Gypsum crystals are commonly found at subsoil depths. The pH and the buffering capacities are high.

Maile silt loam is a latosolic brown forest, intergraded to humic latosol belonging to the Maile family. The soil is derived from volcanic ash under an annual rainfall of 65-90 inches at an elevation of 2860 feet. The clay is dominated with allophane, iron and aluminum oxides. The cation saturation, organic matter and

the total nitrogen content are high.

The moisture equivalent, pH, total nitrogen and organic carbon data are given in Table 2.

Table 2. Some Physical and Chemical Properties[1/] of Experimental Soils

Soil	Moisture Equivalent %	pH	Total N %	Organic C %	C:N
Wahiawa	32.60	5.4	0.714	1.44	8.25
Paaloa	31.06	5.8	0.252	3.26	12.91
Lualualei	41.75	7.7	0.099	1.06	10.73
Maile	105.5	5.6	0.97	26	26.80

1/ Nitrogen and carbon data are those reported by Agarwal (1967) and Tamimi (1966).

Soil preparation

The Wahiawa, Paaloa and Lualualei soils were collected from the Island of Oahu. The Maile soil was collected from the Mealani Experimental Farm, Island of Hawaii. All soil samples were passed through an 8-mesh steel sieve, and the samples were stored in polyethylene bags for use throughout the study.

Experiment 1. Nitrification of Added Ammonium Nitrogen in Soil Samples Contained in Plastic Bags in the Field

Two locations were chosen for this study, one at an elevation of 1200 feet (Helemano, Oahu) above sea level during the winter season of 1965-66 and the other at an elevation of 20 feet

(Lualualei, Oahu) above sea level during the summer season of 1966. The method used for inoubation was similar to that of Eno (1960), who used polyethylene bags.[2/] This technique allows natural diurnal changes in the soil temperature to be taken into consideration in the study of microbial processes, such as nitrification.

Fifty grams each of Wahiawa, Paaloa and Lualualei soil (O.D. basis) were placed in 4x3x13 inch polyethylene bags and ammonium sulphate was added at 200 ppm N rate. Soil moisture was adjusted to approximately moisture equivalent level. The bags were closed to essentially the volume of the soil and secured with rubber bands. The soils were buried at a depth of 9 inches along with a maximum-minimum thermometer to record the soil temperature. Samples were removed at weekly intervals for a period of six weeks.

Ammonia-nitrogen and nitrate nitrogen were extracted by KCl extraction, distilled in a micro-Kjeldahl distillation unit and titrated with standard acid.

Experiment 2. Effect of Temperature on Nitrification of Added Ammonium-Nitrogen and Mineralization of Native Nitrogen

In this experiment the Wahiawa, Paaloa, Lualualei and Maile

[2/] Polyethylene is generally unaffected by moisture, soil and chemicals. It is very slightly permeable to water. Its O_2 and CO_2 diffusion rates are very high. A study for 24 weeks showed that no nitrate diffused through the film by Eno (1960).

soils were used to study the effect of temperature on the mineralization of native soil without any treatment and nitrification of added ammonium. The soils were incubated at 5, 15, 25 and 40°C in the laboratory for six weeks.

Fifty grams of soil (oven-dry basis) were placed in 4x3x13 inch polyethylene bags for incubation at 5, 15, and 25°C and in 250 ml Erlenmeyer flasks for the 40°C incubation. All soils were treated with 0 and 200 ppm of ammonium nitrogen (added as ammonium sulphate). Soil moisture was adjusted to approximately moisture equivalent. Samples were analyzed at weekly intervals as previously for a period of six weeks.

Experiment 3.

Effect of Temperature and Biological Control Chemicals on Nitrification of Added Ammonium Nitrogen in Hawaiian Soils

Two fumigants, methyl bromide and ethylene dibromide, added at rates of 1 lb/100 sq. ft. (0.314 ml/2500 g soil) and 8 gals/A (0.00167 ml/50 g of soil), respectively, and a chemical, N-serve (2-chloro-6 [trichloromethyl] Pyridine), added at 10 ppm rate were treated in Wahiawa, Paaloa and Lualualei soils. Maclean applicator was used for the methyl bromide fumigation. The soils were incubated at 5, 25 and 40°C. An aromatic oil,

55AR[3/], was used as a diluent in the case of ethylene dibromide to facilitate measuring of the fumigant. Aromatic oil also was tested for its effect on nitrification.

Fifty grams of soil (oven-dry basis) were incubated in 250 ml Erlenmeyer flasks covered with polyethylene sheets to allow aeration but limit moisture loss. The treatments consisted of soil alone, soil plus 200 ppm ammonium nitrogen and soil plus fumigants plus 200 ppm ammonium nitrogen. Soil moisture was adjusted to approximately moisture equivalent. The samples were analyzed for ammonium-nitrogen and nitrate-nitrogen at 2, 4, 8 and 12-week intervals, using the previous extraction and distillation method.

Analytical procedure for soil analysis

Exchangeable ammonium and nitrate determinations were carried out by the method of Bremner (1965) with minor modifications. Details regarding the suitability and use of this method have been discussed by Bremner and Keeney (1966).

Samples were extracted with 2N KCl (soil to KCl solution ratio was 1:6 - a test between 1:6 and 1:10 gave comparable results for the soils under study), shaken for 1 1/2 hours on an end-to-end shaker, allowed to settle for 45 minutes, and then 50 ml of the supernatant liquid were drawn off for determination of

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[3/] 55AR is an herbicide oil developed by Dr. F. E. Hance of the Experiment Station, Hawaiian Sugar Planters' Association, and contains 55 percent of aromatic constituents.

NH_4^+ and NO_3^- with a micro-distillation unit.

Ammonium was determined first; 0.5 g ignited heavy MgO was added to the extract and the distilled NH_4^+ was received in 10 ml of mixed boric acid-indicator solution. After this distillation, 1 g of less-than-100 mesh Devarda's alloy was added to reduce NO_3^- to NH_4^+ (this also includes NO_2^-) and the contents were redistilled into a separate receiving flask. The rate of distillation was set at 7-8 ml per minute. Approximately 4-5 minutes were required for either NH_4^+ or NO_3^- distillation. All analyses were carried out in duplicate.

Statistical analysis

Statistical analyses were done on portions of the field and laboratory data to determine the level of experimental error.

RESULTS AND DISCUSSION

Experiment 1. Nitrification of Added Ammonium Nitrogen in Soil Samples Contained in Plastic Bags in the Field

Many workers have studied the effect of temperature on nitrate production in the laboratory; however, not much work has been done in the field under fluctuating temperature conditions. Eno (1960) used polyethylene bags to study the effect of temperature on soils buried in the field. No work of this nature has been done in Hawaii. The present study was initiated to determine the effect of temperature on nitrate production in soils buried at two different temperature regimes under Hawaiian conditions.

The data on nitrate accumulation in soils treated with 200 ppm of NH_4-N as ammonium sulphate under the two temperature regimes are shown in Table 3 and Figure 1. Triplicate determinations showed the experimental error to be very small, generally less than ± 1 to 2 ppm. The coefficient of variation calculated from the error variance for all three soils (results for 6th week warm temperature regime) was only 0.07%. A more thorough statistical analysis of the data by analysis of variance procedures was not considered necessary due to the large differences between means and the extremely low experimental error.

The Paaloa and Lualualei soils showed nitrification at both temperature regimes, while the Wahiawa soil showed very little

Table 3. Nitrification of Added N (200 ppm N as Ammonium Sulphate) in Soil Samples Buried in the Field

Soil	Field Temperature Regime	Elapsed Time, Weeks					
		1	2	3	4	5	6
		NO_3-N, ppm[1/]					
Wahiawa	Cool	13	17	18	19	26	27
	Warm	20	62	140	172	185	187
Paaloa	Cool	34	69	124	138	144	154
	Warm	76	154	168	183	184	183
Lualualei	Cool	62	154	180	180	176	174
	Warm	174	175	175	167	163	164

1/ Oxidation of added NH_4-N was estimated by subtracting the amount of NO_3-N produced by soil alone from that produced by soil plus added NH_4-N. Initial values are shown in Table 2 of the Appendix.

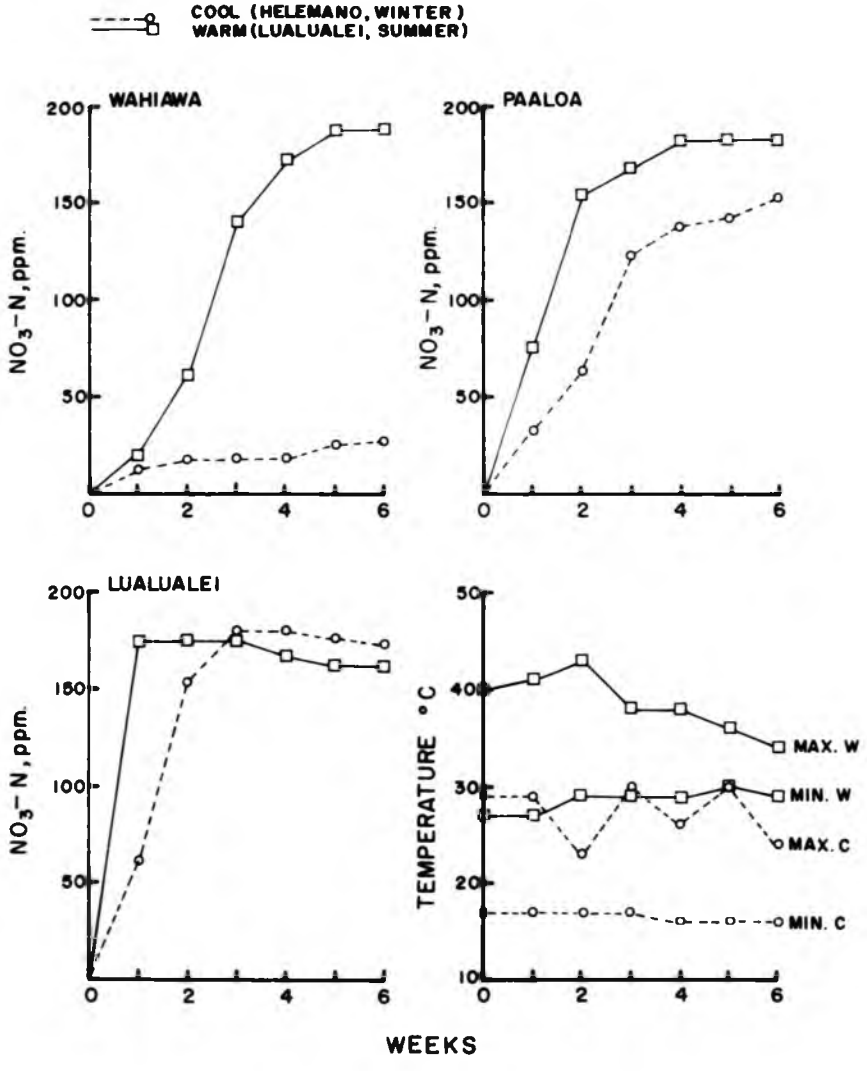

Fig. 1. Nitrification of Added Ammonium Nitrogen to Soils Buried in the Field and the Maximum-Minimum Temperature Regime at Helemano (Winter) and Lualualei (Summer).

nitrification with the cool temperature regime. Nitrification of added nitrogen during the warm temperature regime in the Paaloa and Wahiawa soils was observed to be higher than that of the cool temperature regime. This difference is especially prominent in the case of the Wahiawa soil (Fig. 1). This may be due to the differences in the initial population of nitrifiers and temperature. In the case of the Lualualei soil the amount nitrified is about the same at both temperatures, but there was a difference in the rate of nitrification. All the added NH_4-N was nitrified in one week during the warm temperature regime, whereas the same amount of NO_3-N accumulated after three weeks had elapsed for the cool temperature regime. This finding agrees very well with the work of Mahendrappa et al. (1966) who found that soils which were developed in a warmer climatic zone required less time to nitrify the added NH_4-N than soils developed in cooler climatic zones. Thus the Lualualei, a soil developed in a warm climatic zone, took less time to nitrify the added NH_4-N when incubated under a warm temperature regime than the other two soils which have developed under a less warm climatic zone. In the Paaloa soil, four weeks were needed to nitrify the same amount during the warmer temperature regime as compared to the Lualualei soil. Furthermore, nitrification was not complete under the cool temperature regime. The Wahiawa soil took five weeks to complete its nitrification during the warm temperature regime, and very little

nitrate was produced under the cool temperature regime. The Lualualei, Paaloa and Wahiawa soils required one, four and five weeks, respectively, to complete nitrification during the warm temperature regime, and more time was required during the cool temperature regime. There are considerable differences among soils in their nitrifying characteristics. This agrees with the findings of Anderson and Purvis (1955), Broadbent et al. (1958), Frederic (1956) and Sabey et al. (1956).

Incomplete recovery of the added mineral nitrogen at the end of six weeks in the case of Lualualei at both temperature regimes is thought to be partly due to ammonium fixation (Tamimi, 1964; Mikami, 1966). It was found that there was a loss of nitrogen after the three-week period in the case of the Lualualei (Table 3) at both temperature regimes, with the loss being greater at the warmer site. This agrees well with the work of Acquaye and Cunningham (1962), Volk (1961) and Wahhab et al. (1957), who found that volatilization increased when soil pH and temperature increased. In the case of the Paaloa and Wahiawa soils, there was no noticeable loss of nitrogen for the length of the experiment.

In summary, under the two temperature regimes existing at the field sites utilized, all three soils showed higher rates of nitrification at the higher temperature, and this effect was greatest on the Wahiawa soil and least on the Lualualei soil.

Experiment 2. Effect of Temperature on Nitrification of Added Ammonium Nitrogen and Mineralization of Native Nitrogen

Nitrification

Results of nitrate accumulation in soils treated with 200 ppm of NH_4-N as ammonium sulphate and incubated under four temperatures in the laboratory are presented in Figure 2. Duplicate determinations showed the experimental error to be very small, generally less than ± 1 to 2 ppm. The coefficient of variation calculated from the error variance for all four soils (results of 6th week at 25°C) was only 0.1%. The low temperature effect was especially noticeable in all the soils where nitrate accumulation either failed to occur or was delayed for several weeks. Total nitrate accumulation in Wahiawa, Paaloa, Lualualei and Maile soils never exceeded 1, 6, 26 and 98 ppm (Table 4), respectively, in six weeks at 5°C. At any one temperature, the rate of oxidation among the four soils was different. Recent investigations (Anderson and Purvis, 1955; Broadbent *et al.*, 1958; Frederic, 1956; Sabey *et al.*, 1959; Stojonovic and Broadbent, 1956; Justice and Smith, 1962) showed that the nitrification of applied ammonium occurred in some soils at temperatures considerably lower than was previously thought, and that there are differences among soils in their nitrifying characteristics at low temperatures. These studies agree with the present study in that at 5°C all the four soils nitrified differently.

Table 4. Effect of Temperature on Nitrification of Added N (200 ppm N as Ammonium Sulphate) in Four Soils

Soil	Temperature °C	Incubation Period, Weeks					
		1	2	3	4	5	6
		NO_3-N, ppm[1/]					
Wahiawa	5	- 1	2	1	1	1	1
	15	1	1	9	10	29	40
	25	3	11	38	41	75	129
	40	8	14	28	31	31	33
Paaloa	5	2	3	4	5	5	6
	15	2	12	63	130	177	177
	25	10	45	130	178	178	180
	40	3	8	14	11	16	17
Lualualei	5	3	7	9	19	22	26
	15	48	127	143	141	142	142
	25	124	143	141	141	140	138
	40	131	140	136	119	110	111
Maile	5	5	9	22	43	61	98
	15	23	79	153	154	153	152
	25	56	154	168	165	165	163
	40	4	26	26	27	35	37

[1/] See footnote for Table 3 for calculation of NO_3-N.

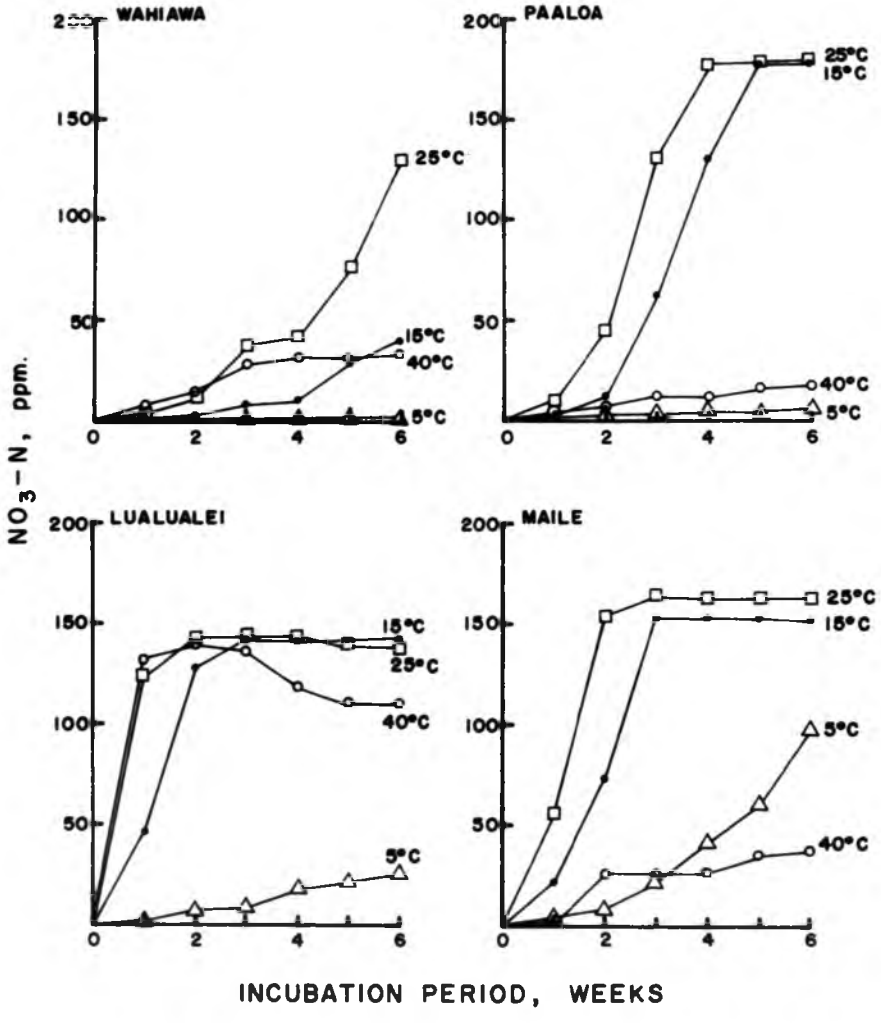

Fig. 2. Nitrification of the Ammonium Nitrogen Added to the Four Soils Incubated at 5, 15, 25 and 40°C in the Laboratory.

The Wahiewa soil showed practically no nitrification at 5°C. But nitrification increased with increasing temperature up to 25°C. Furthermore, the delay period in nitrification decreased with increasing temperature up to 25°C. At 15°C only 40 ppm of NO_3-N was produced after a six-week period, whereas at 25°C 129 ppm of NO_3-N was accumulated during this corresponding period. There was some nitrate production at 40°C, but there was no steady increase during the six-week period. The Paaloa soil behaved the same as the Wahiawa soil for the 5°C treatment, but for the 15°C treatment, after a two-weeks delay period, nitrification in the Paaloa soil increased steadily and was completed in five weeks. The 25°C incubation treatment showed only a week's delay in this soil, and the added ammonium was nitrified in four weeks. At 40°C only 17 ppm (Table 4) of nitrate was produced showing greater inhibition than other soils at this temperature.

The Lualualei had a slight increase in nitrification at 5°C (26 ppm in six weeks) as compared to the Wahiawa and Paaloa soils. At 15, 25 and 40°C the added ammonium nitrogen was nitrified in 3, 2 and 2 weeks, respectively, showing that with the increase in temperature, the rate of nitrification was faster in this soil. No delay period was observed at 25 and 40°C.

The Maile soil at 5°C after a delay period of two weeks nitrified slowly, and the rate increased with time. At the end of

six weeks, 50 percent of the added ammonium nitrogen was nitrified, showing that this soil can nitrify at lower nitrification temperatures than the other soils. This finding agrees well with the work of Mahendrappa et al. (1966) in which they found that a soil from a cool climatic region incubated at the cooler temperatures (20 and 25°C) nitrified faster than at higher temperatures (35 and 40°C). In the present study at 15 and 25°C nitrification was complete in 3 and 2 weeks, respectively. Like the other two soils, Wahiawa and Paaloa, this soil showed very little nitrification at 40°C.

The Lualualei and Maile soils at 15°C nitrification was completed in three weeks. The only difference between the two is in the amount of nitrate produced in the first and second week, where the Lualualei accumulated 48, 127 ppm and the Maile accumulated 23, 79 ppm, respectively. The Paaloa showed a greater increase in nitrification at 15° than the Wahiawa, especially at incubation periods of 3 weeks and longer.

This study shows that even if the temperature is the same for all incubating soils, there is a difference in nitrate production which can be attributed to the difference in the nitrifying capacities of the soils. Frederic (1957) has also shown that differences in temperature range of nitrification in soils are also due to variations in population of nitrifiers, and the delay phase appears to be correlated to the difference in the temperature ranges for nitrification

found in different soils.

Rapid nitrification took place in all the soils at 25°C, and the only difference among the soils was in the delay period. The Lualualei and Maile behaved in a similar way, in that nitrification of the added ammonia was complete in two weeks. The only difference between these two soils was the amount of nitrate produced in the first week (Table 4). The Paaloa and Wahiawa differed in their delay periods. The Paaloa completed its nitrification of added ammonia in four weeks, whereas in Wahiawa nitrification was almost complete in six weeks. The foregoing results of rapid nitrification at 25°C agree well with the report of Sabey et al. (1959), but it is also seen that all the soils showed nitrification at 15°C. Furthermore, three of the four soils showed complete nitrification at this temperature with differences only in their delay period.

Meiklejohn (1954) reported that nitrification did not proceed at 40°C in a tropical Uganda soil, and Warrington (1879) found a similar effect with an English garden soil. This study shows nitrification at 40°C in all soils at the end of six weeks. The Wahiawa, Paaloa, Lualualei and Maile accumulated 33, 17, 140 and 37 ppm of NO_3-N, respectively. The production of nitrate at this temperature is contrary to the findings of the above investigations but agrees very well with the recent report by Mahendrappa et al. (1966). They found that the soils from a

warmer climatic zone incubated at 35° and 40°C nitrified faster than at 20 and 25°C temperatures. In this study the Lualualei, which is a soil developed under a warm climatic zone, nitrified all the added ammonium nitrogen in two weeks at 40°C and required more time to nitrify the same amount at lower temperatures of 15 and 25°C. This rapid nitrification in the Lualualei at 40°C suggests that the optimum nitrification temperature range 27-35°C reported by Waksman and Madhock (1937) and Frederic (1957) may be too low for a soil like the Lualualei.

There was a loss of nitrate at high temperatures with increase of time. This is very clear in the case of the Lualualei soil. Furthermore, all the added ammonium was not recovered as nitrate. This low recovery is most probably due to ammonium fixation and volatilization because of high pH and increase in temperature. Ammonium fixation in soils containing montmorillonite, illite, and vermiculite has been shown by Allison et al. (1953), Barshad (1948), Page and Baver (1940). The Lualualei soil is a 2:1 clay and has been shown to fix ammonium (Tamimi, 1964; Mikami, 1966). This soil has a high pH and some loss may be due to volatilization at high temperatures as reported by Jewitt (1942), Martin and Chapman (1951), Jackson and Chang (1947), Ernst and Massey (1960), Wahhab et al. (1957) and Acquaye and Cunningham (1962). There was a lag in nitrification in the laboratory studies, but there was no comparable delay in the field

studies. This might be attributed to the diurnal temperature changes in the field.

Mineralization of native nitrogen

Mineralization is defined as the conversion of an element from an organic to an inorganic form as a result of microbial decomposition (S.S.S.A.P., 1965). When the effect of temperature on the mineralization of organic nitrogen is studied, a distinction should be made between ammonification and nitrification, because ammonification can take place at relatively high temperatures, whereas nitrification is inhibited. Both ammonification and nitrification are limited by low temperatures (Panganiban, 1925; Sabey et al., 1956). In the four soils studied maximum ammonium and nitrate nitrogen was found at 40°C (Fig. 3). At temperatures below 40°C, less available nitrogen was found. Panganiban (1925) found an increase in ammonification as the temperature was raised to 40°C and even up to 60°C.

As in the nitrification study, it was observed that there is a difference among soils in their mineralizing capacities. At 40°C the Paaloa and Maile soils accumulated 116 and 155 ppm of (NH_4+NO_3)-N in six weeks as compared to the Wahiawa and Lualualei which accumulated only 26 and 36 ppm (Table 5). This difference in mineralization may be attributed to the amount of organic matter present. Since the organic matter content of the Paaloa (Agarwal, 1967) and Maile (Tamimi, 1966) are

Table 5. Effect of Temperature on Mineralization of Native Nitrogen in Four Soils

Soil	Temperature °C	Incubation Period, Weeks					
		1	2	3	4	5	6
		NH_4+NO_3 N, ppm					
Wahiawa	5	1	1	1	4	5	7
	15	1	2	10	12	12	15
	25	1	6	8	13	17	17
	40	10	19	20	25	25	26
Paaloa	5	1	8	10	17	20	24
	15	5	6	8	12	15	16
	25	3	5	10	11	20	22
	40	62	68	96	110	115	116
Lualualei	5	1	2	5	4	5	6
	15	3	3	4	4	3	4
	25	1	4	7	8	7	10
	40	21	25	26	27	34	36
Maile	5	5	5	6	6	5	7
	15	4	5	5	7	7	7
	25	4	6	14	16	19	20
	40	30	71	81	138	145	155

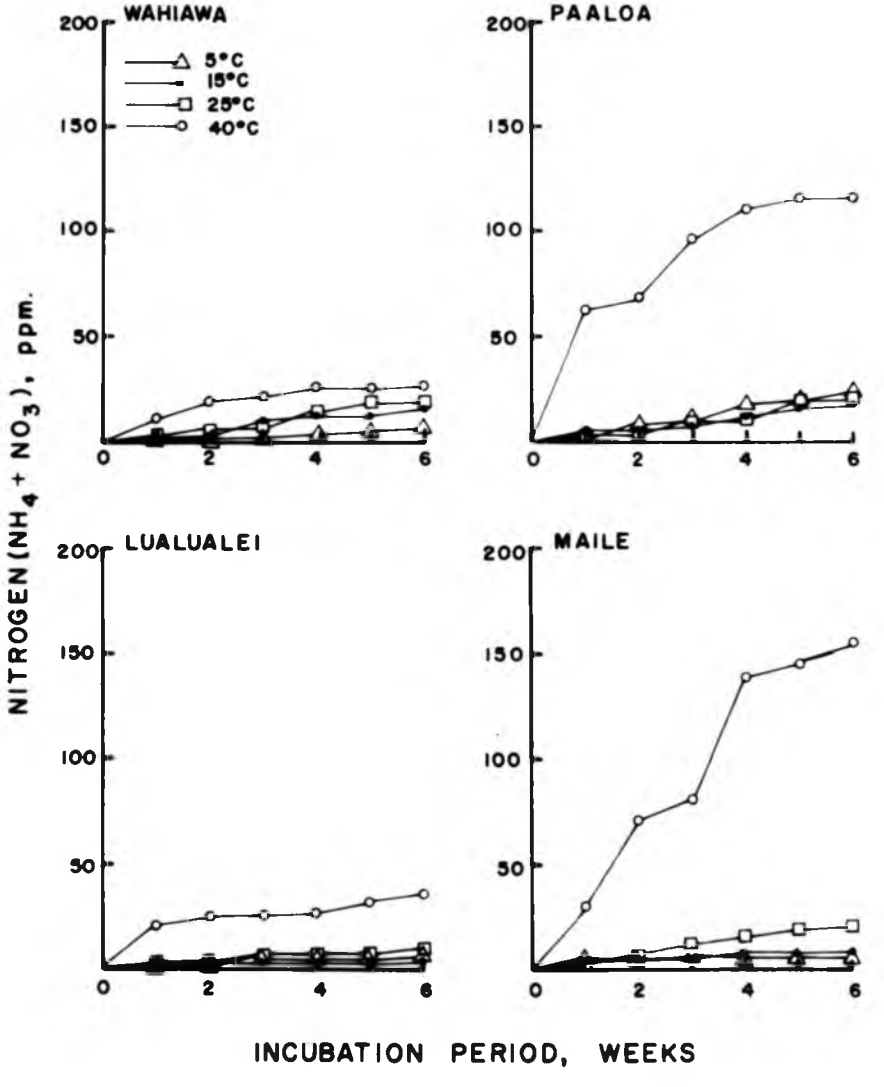

Fig. 3. Mineralization of Native Nitrogen in Four Hawaiian Soils Incubated in the Laboratory at 5, 15, 25 and 40°C.

higher than that of Wahiawa or Lualualei, the high mineralization is undoubtedly due to the high organic matter decomposition. Thus mineralization is not only governed by temperature but by other factors, such as the moisture content, amount of organic matter, etc.

Experiment 3.

Effect of Temperature and Biological Control Chemicals on Nitrification of Added Ammonium Nitrogen in Hawaiian Soils

All three soils, Paaloa, Wahiawa and Lualualei, behaved differently in their nitrifying capacities with methyl bromide (M.B.), ethylene dibromide (E.D.B.) and 2-chloro-6(trichlomethyl)pyridine (N-serve) when incubated at 5, 25 and 40°C. It was found that fumigants and chemicals temporarily inhibit nitrification in soils. Ammonium nitrogen added in the form of $(NH_4)_2SO_4$ at the rate of 200 ppm was rapidly nitrified at 25°C in all soils without any chemical treatment.

Incubation at 5°C

Methyl bromide, ethylene dibromide and N-serve added with the nitrogen source completely inhibited nitrification at 5° in all three soils for 12 weeks (Figs. 4, 5, and 6). The soils treated with ammonium sulphate alone contained more nitrogen than the soils treated with chemicals + $(NH_4)_2SO_4$. Wolcott *et al.* (1960) and McClellan *et al.* (1949) have reported that an indefinite lag in

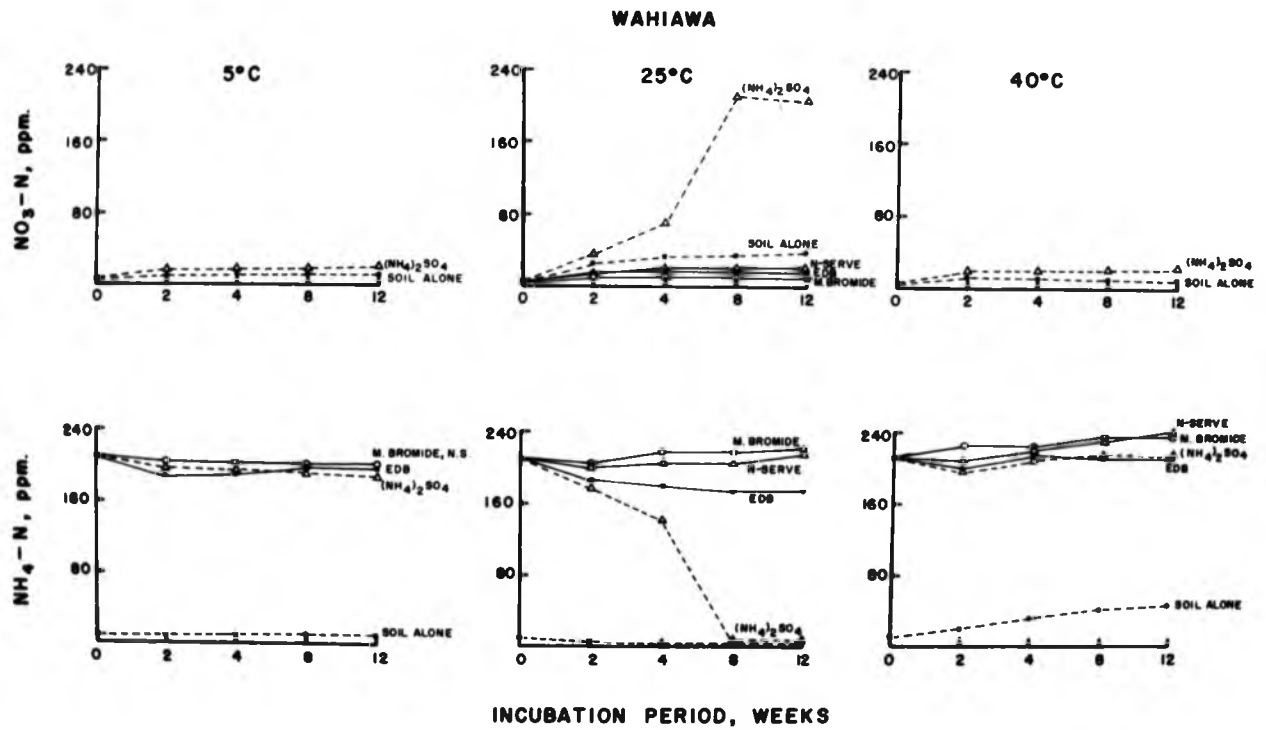

Fig. 4. Effect of Temperature and Biological Control Chemicals on Nitrogen Transformation in Wahiawa Soil.

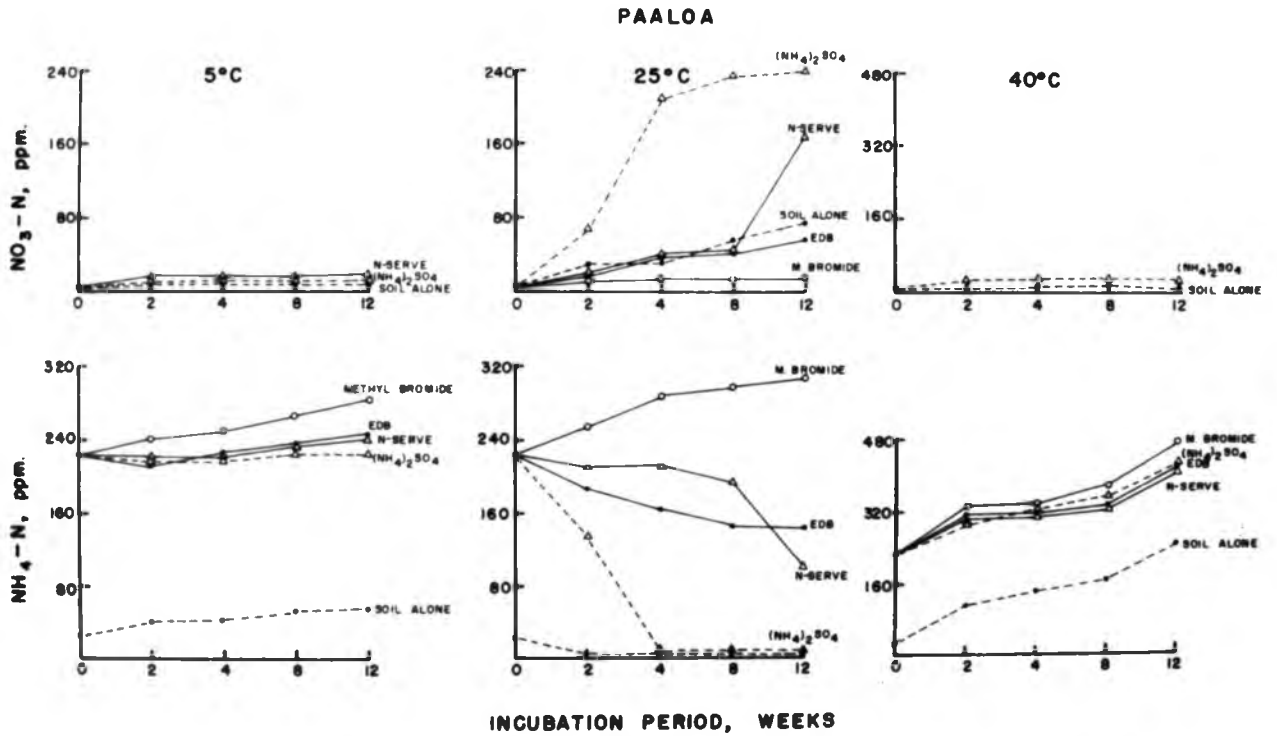

Fig. 5. Effect of Temperature and Biological Control Chemicals on Nitrogen Transformation in Paaloa Soil.

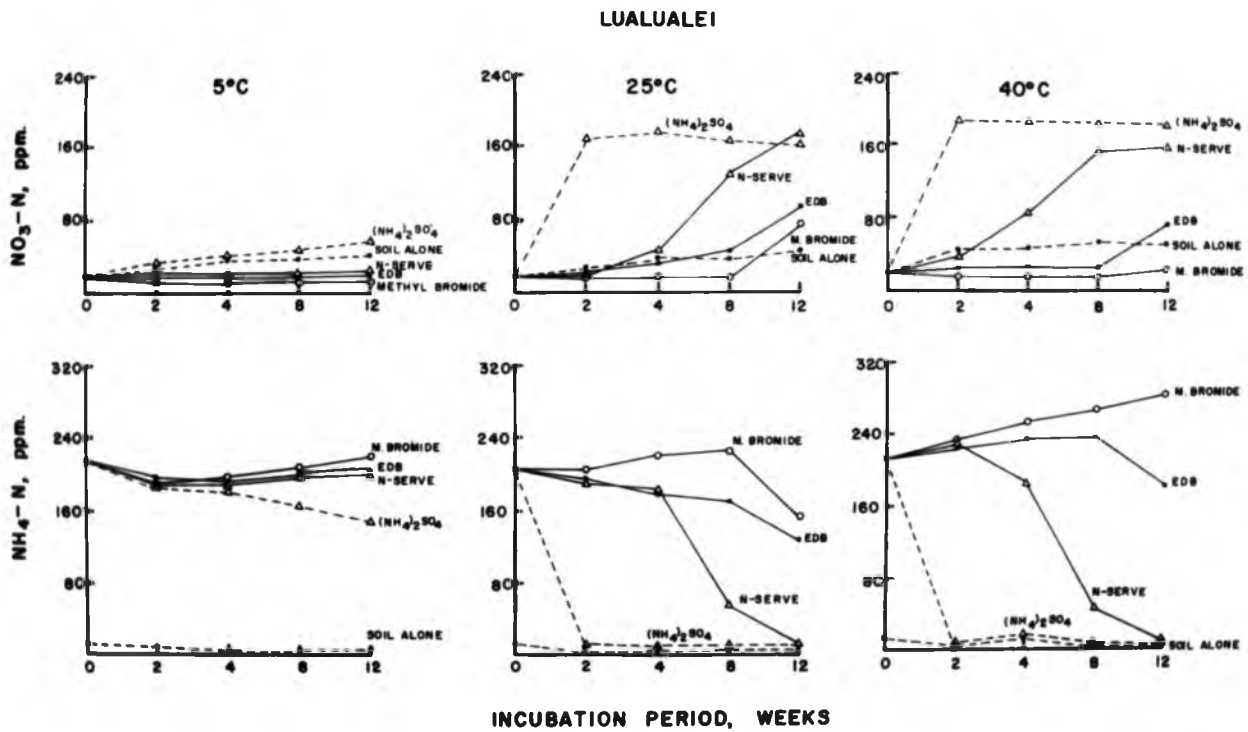

Fig. 6. Effect of Temperature and Biological Control Chemicals on Nitrogen Transformation in Lualualei Soil.

nitrification was found at low temperatures, where the fumigants are retained for a long period. Thus their work agrees with the findings in this study at 5°C. In the case of the Lualualei 56 ppm (Table 8) was produced with the ammonium sulphate treatment followed by soil alone, N-serve, EDB and methyl bromide treatments in which 40, 20, 18 and 11 ppm, respectively, of nitrate nitrogen was obtained at the end of twelve weeks. In the Wahiawa and Paaloa the amount of nitrate produced ranged from 5-20 ppm for the comparable treatments and period.

The level of ammonium nitrogen increased in all soils, with the greatest increase found in soils treated with methyl bromide, followed by EDB and N-serve. The amount of ammonium accumulation varied from soil to soil. The Lualualei and Wahiawa soils did not show any increase, but Paaloa showed an increase up to 280 ppm (Table 7) with methyl bromide followed by EDB and N-serve which had 245 and 240 ppm, respectively. In the Lualualei and Wahiawa soils the amount never exceeded 220 ppm (Tables 6 and 8) in the 12-week period. Since the Paaloa is a soil higher in organic matter than the other two soils, the chemicals might have stimulated the decomposition of organic matter, resulting in a high accumulation of ammonium nitrogen.

Incubation at 25°C

At 25°C, nitrification was completely suppressed for a 12-week period in the Wahiawa soil, when the samples were treated

Table 6. Effect of Temperature and Biological Control Chemicals on Ammonium and Nitrate Changes in Wahiawa Soil

Treatments	0 Weeks	2 Weeks			4 Weeks			8 Weeks			
		5°C	25°C	40°C	5°C	25°C	40°C	5°C	25°C	40°C	5°C
					NH_4-N, ppm						
Soil Alone	10	8	8	21	10	4	33	11	2	42	9
Soil + 200 ppm NH_4-N	210	198	179	198	194	142	219	190	4	228	187
Soil + 200 ppm NH_4-N + MB[1/]	210	203	204	224	201	219	224	200	219	239	200
Soil + 200 ppm NH_4-N + EDB[2/]	210	188	188	199	192	180	223	200	176	225	197
Soil + 200 ppm NH_4-N + N-serve[3/]	210	199	205	209	198	208	221	201	209	237	200
					NO_3-N, ppm						
Soil Alone	5	11	26	12	12	32	12	12	34	10	13
Soil + 200 ppm NH_4-N	5	15	36	19	17	70	19	18	210	21	20
Soil + 200 ppm NH_4-N + MB	5	15	11	12	13	13	13	13	12	14	12
Soil + 200 ppm NH_4-N + EDB	5	11	15	10	12	15	10	13	14	12	16
Soil + 200 ppm NH_4-N + N-serve	5	13	13	13	13	18	14	18	18	13	19

[1/] Methyl bromide

[2/] Ethylene dibromide

[3/] 2-chloro-6(trichloromethyl)pyridine

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8 Weeks		12 Weeks		
25°C	40°C	5°C	25°C	40°C
2	42	9	2	48
4	228	187	4	228
219	239	200	221	238
176	225	197	175	227
209	237	200	219	243
34	10	13	39	10
210	21	20	207	23
12	14	12	12	13
14	12	16	16	11
18	13	19	20	13

Table 7. Effect of Temperature and Biological Control Chemicals on Ammonium and Nitrate Changes in Paaloa Soil

Treatments	0 Weeks	2 Weeks			4 Weeks			8 Weeks			12 Weeks	
		5°C	25°C	40°C	5°C	25°C	40°C	5°C	25°C	40°C	5°C	25°C
					NH_4-N, ppm							
Soil Alone	22	42	4	115	43	4	144	53	2	168	54	4
Soil + 200 ppm NH_4-N	222	218	134	296	217	4	321	225	3	355	226	4
Soil + 200 ppm NH_4-N + MB[1/]	222	240	251	328	249	288	335	265	295	370	280	308
Soil + 200 ppm NH_4-N + EDB[2/]	222	214	186	315	228	162	318	238	146	335	245	142
Soil + 200 ppm NH_4-N + N-serve[3/]	222	217	210	300	219	210	305	237	203	329	240	100
					NO_3-N, ppm							
Soil Alone	5	9	30	9	9	29	6	9	57	7	5	73
Soil + 200 ppm NH_4-N	5	11	69	12	12	209	13	12	234	14	14	249
Soil + 200 ppm NH_4-N + MB	5	8	11	11	9	13	13	9	13	11	6	14
Soil + 200 ppm NH_4-N + EDB	5	11	13	10	10	32	12	10	39	11	11	53
Soil + 200 ppm NH_4-N + N-serve	5	16	13	9	16	35	9	17	40	8	19	169

1/ Methyl bromide

2/ Ethylene dibromide

3/ 2-chloro-6(trichloromethyl)pyridine

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8 Weeks			12 Weeks		
5°C	25°C	40°C	5°C	25°C	40°C
53	2	168	54	4	242
225	3	355	226	4	423
265	295	370	280	308	467
238	146	335	245	142	423
237	203	329	240	100	418
9	57	7	5	73	2
12	234	14	14	249	11
9	13	11	6	14	10
10	39	11	11	53	11
17	40	8	19	169	12

Table 8. Effect of Temperature and Biological Control Chemicals on Ammonium and Nitrate Changes in Lualualei Soil

Treatments	0 Weeks	2 Weeks			4 Weeks			8 Weeks			12 Wee	
		5°C	25°C	40°C	5°C	25°C	40°C	5°C	25°C	40°C	5°C	25°C
					NH_4-N, ppm							
Soil Alone	11	9	2	4	3	3	12	3	2	2	3	4
Soil + 200 ppm NH_4-N	211	185	6	4	180	4	15	163	4	4	149	3
Soil + 200 ppm NH_4-N + MB[1/]	211	190	203	233	198	220	252	207	225	269	219	151
Soil + 200 ppm NH_4-N + EDB[2/]	211	193	193	222	194	179	233	205	174	236	204	126
Soil + 200 ppm NH_4-N + N-serve[3/]	211	190	190	230	194	181	187	200	52	44	203	5
					NO_3-N, ppm							
Soil Alone	19	23	27	43	32	35	45	36	34	51	40	45
Soil + 200 ppm NH_4-N	19	31	169	187	40	177	187	46	168	186	56	163
Soil + 200 ppm NH_4-N + MB	19	11	15	14	10	17	14	10	18	13	11	79
Soil + 200 ppm NH_4-N + EDB	19	15	26	22	16	31	25	16	44	23	18	92
Soil + 200 ppm NH_4-N + N-serve	19	21	24	38	22	44	83	22	130	154	20	172

1/ Methyl bromide

2/ Ethylene dibromide

3/ 2-chloro-6-(trichloromethyl)pyridine

12 Weeks		
5°C	25°C	40°C
3	4	2
149	3	4
219	151	281
204	126	181
203	5	8
40	45	52
56	163	188
11	79	22
18	92	75
20	172	160

with chemicals. The amount of nitrate in soil alone was greater than in the chemically-treated soils (Fig. 4). Complete nitrification was observed in the sample treated with $(NH_4)_2SO_4$ alone at the 8-week period. The difference in nitrate accumulation is not great. Among the chemicals, the N-serve treated sample had 20 ppm followed by EDB and MB with 16 and 12 ppm, respectively (Table 6). The ammonium nitrogen in the Wahiawa treated with MB, EDB and N-serve remained in that state for 12 weeks, with a slight increase of NH_4-N in methyl bromide-treated samples. At the same time a decrease in ammonium nitrogen level was noticed in EDB-treated samples even though no nitrification was observed.

The Paaloa soil at this same temperature of 25°C showed a delay in nitrification at 8-12 weeks. Nitrification was delayed for 12 weeks in samples treated with methyl bromide, followed by EDB and N-serve where the inhibiting period was only 8 weeks. The level of ammonium nitrogen increased with increase in time from 222 to 308 ppm in samples treated with methyl bromide, whereas in the samples treated with EDB and N-serve there was a decline in the ammonia level after 8 weeks (Table 7), showing that the nitrification rate had increased in these samples. The level of nitrate increased from 5 to 53 ppm in the case of the EDB-treated soil and from 5 to 169 ppm in the N-serve treatment, showing the re-establishment of the nitrifying population in the soil

samples. The soil alone produced 73 ppm of nitrate nitrogen as compared to 53 ppm with EDB-treated samples.

The Lualualei soil behaved differently than the other two soils with all the chemicals. Complete control of nitrification was observed for 8 weeks in samples treated with MB and EDB, and nitrification was resumed after 4 weeks in N-serve treated samples, whereas in the other two soils complete inhibition with MB was observed for 12 weeks and EDB and N-serve showed inhibition for 8 weeks in the Paaloa soil. This shows that N-serve is less effective in the Lualualei soil at the 25°C temperature than EDB or MB. The amount nitrified in 12 weeks differed with different chemicals. Samples treated with methyl bromide, EDB and N-serve obtained 79, 92 and 172 ppm of nitrate nitrogen, respectively (Table 8), which are also greater than the Wahiawa where 12, 16, 20 ppm and 14, 53, 169 ppm in Paaloa were obtained for the comparable treatment and period (Tables 6 and 7). The level of ammonium nitrogen remained in that state for 4 weeks in N-serve treated samples and for 8 weeks in MB and EDB-treated samples and declined with time.

Incubation at 40°C

Results at 40°C showed a complete inhibition of nitrification for 12 weeks in Wahiawa and Paaloa soils with all chemicals. In these two soils where only $(NH_4)_2SO_4$ was added, a slight increase in nitrification was shown over that of chemically-treated

samples. The Lualualei soil showed complete nitrification in the ammonium-treated sample in two weeks. In N-serve treated samples the inhibitory effect was only for two weeks and after that nitrification resumed as reflected by nitrate figures of 38, 83, 154 and 160 ppm at 2, 4, 8 and 12 weeks interval, showing that this chemical decomposes quickly with an increase in temperature. Goring (1962) found that with the same amount of this chemical added at 32°C, nitrification resumed only after 16 weeks. In the present study the temperature was higher than that of Goring's and also the type of soil may have influenced the rate of nitrification, as seen in the other two soils, where nitrification was inhibited for 12 weeks. The MB-treated Lualualei sample showed complete inhibition for 12 weeks, and the same soil treated with EDB showed inhibition for 8 weeks at 40°C.

The ammonium nitrogen levels in all the soils at this temperature in samples treated with methyl bromide were generally higher than in other treatments. In 12 weeks, 238, 281 and 467 ppm of ammonium nitrogen were found in Wahiawa, Lualualei and Paaloa soils, respectively, when treated with MB and $(NH_4)_2SO_4$. The Paaloa soil had 423, 423 and 467 ppm (Table 7) of ammonium nitrogen at the end of 12 weeks with EDB, N-serve and methyl bromide treatments, showing a greater increase in ammonification in this soil than the other two soils. This agrees with the reports of other workers (Wolcott et al., 1960; Gasser and Peachey,

1964) who stated that the accumulation of ammonium nitrogen in soils high in organic matter increases with increase in temperature, and this also agrees with the findings in Experiment 2 of the present study. This accumulation is probably enhanced in organic soils by a partial sterilization effect of the chemicals which results in more rapid ammonification of organic forms of nitrogen.

Discussion

Nitrification of ammonium added as $(NH_4)_2SO_4$ was delayed for different lengths of time, depending upon the severity of action of various chemicals upon the nitrifying organisms in the soil. Methyl bromide was found to be the most effective fumigant, inhibiting nitrification for 12 weeks in all soils at all the temperatures, except in the Lualualei which at 25°C after a delay period of 8 weeks resumed nitrification. The present study agrees with the reports of Thiegs (1955), Wolcott *et al.* (1960) who found that fumigation with methyl bromide resulted in high ammonia levels and suppressed nitrification for a long period of time.

The chemical N-serve gave complete control of nitrification at 5°C in all the soils and at 40°C in Wahiawa and Paaloa. This effect may be mainly due to temperature, because soils treated with $(NH_4)_2SO_4$ alone inhibited nitrification at 5°C. The chemical-temperature interaction might have caused the greater delay period. At 25°C after an 8-week delay in Paaloa and 4-week delay in

Lualualei, nitrification was resumed. The difference may have been due to the difference in nitrifying characteristics among soils as reported by recent investigators (Anderson and Purvis, 1955; Broadbent et al., 1958; Sabey et al., 1956). Goring (1962) reported that this chemical is highly sorbed by organic matter in moist condition. Since the Paaloa is high in organic matter the chemical is sorbed and retained for a longer time in this soil than in Lualualei, which is low in organic matter content. At 40°C, the Lualualei with this chemical showed only a two-week delay period. This increase in temperature probably caused rapid decomposition of the chemical.

Goring (1962) found that at 10 and 21°C nitrification was delayed for 24 weeks at 10 ppm level of chemical, whereas at the same level when the temperature was increased to 32°C, the delay was found to be 16 weeks. The time of resumption is quicker in the present study than Goring's. This may be attributed to a higher temperature and the type of soil. Thus this chemical may not be effective in a tropical climate where the soil temperature often goes above 40°C, and in certain types of soil. At the same time it may be effective in delaying nitrification in the tropics with soil types like the Wahiawa and Paaloa at the same temperature of 40°C.

The effect of chemical EDB was intermediate between N-serve and methyl bromide. It behaved in the same way as N-serve

in the Paaloa and Wahiawa soils at all temperatures, but, in the Lualualei, it was inhibitory for 12 weeks at 5°C and for 8 weeks at 25 and 40°C.

Thus the effect of temperature and chemicals may be the same for a given soil, but different soils behaved differently in their nitrifying capacities. Complete control of nitrification is obtained in the Wahiawa with all the chemicals and at all temperatures, whereas in the Paaloa nitrification was delayed for 12 weeks with methyl bromide and EDB at all temperatures. The N-serve treated Paaloa at 25°C resumed nitrification after 8 weeks but delayed nitrification for 12 weeks at 5 and 40°C. In the Lualualei soil only at 5°C was complete control obtained.

Martin and Aldrich (1952) observed that nitrification occurred earlier in an alkaline sandy soil than in an acid mountain soil. This agrees with the present study where nitrification started earlier with N-serve at 25°C in the Lualualei than in the Paaloa. The present results were also in agreement with those of Koike (1961), Thiegs (1955), Wensly (1953) and Goring (1962) who reported that chemicals which are used as sterilants to control nematodes prevent nitrification of ammonium nitrogen in the soil. The amount of ammonium nitrogen accumulating during a treatment period will depend both on soils and chemicals. The present study shows that temperature is also equally important as soil type and chemical. It was further found that when these chemicals

were used as soil sterilants they may increase the mineralization of soil nitrogen, depending upon the type of soil, chemical and temperature. It was seen that methyl bromide increased mineralization to a greater extent than EDB or N-serve. This agrees with the reports of Gasser and Peachy (1964).

SUMMARY AND CONCLUSIONS

The effect of temperature and biological control chemicals on nitrogen transformation was studied in three Hawaiian (Paaloa, Wahiawa and Lualualei) soils. In a field experiment soils buried at the warmer temperature regime nitrified added ammonium faster than soils buried at the cooler temperature regime, showing a difference in the effect of temperature. This difference was most prominently seen in the case of the Wahiawa. It was also found that there is a considerable difference among soils in their nitrifying capacities at both temperature regimes.

Nitrification of ammonium sulphate as influenced by temperature was studied in four Hawaiian (Paaloa, Wahiawa, Lualualei and Maile) soils in the laboratory, at temperatures ranging from 5 to 40°C. At a given temperature, the ammonia oxidation rates differed among soils studied. Nitrification of ammonium sulphate proceeded more rapidly in Lualualei at 40°C than at 25°C or 15°C. In other soils it was most rapid at 25°C. Some nitrification at 5°C occurred in six weeks in all soils tested, but only in the Maile and the Lualualei was nitrification appreciable. Rates of nitrification varied among soils. Nitrification began sooner in the Lualualei than in the other three soils. These differences tended to decrease with increase in time at 25°C.

The formation of nitrates took place at all temperatures studied between 5 and 40°C when all other factors were favorable. The rate of nitrification increased with rise in temperature up to 25°C in all the soils studied, and this rate increased up to 40°C in the case of the Lualualei. The greatest increase in nitrification rate occurred between 15 and 25°C as shown by the amount of nitrate formed at different temperatures (Fig. 2). The temperature range for nitrification in these soils were quite different. Maile showed appreciable nitrification between 5-25°C, whereas Lualualei showed this maximum nitrification between 15-40°C and for Paaloa and Wahiawa it was between 15-25°C. This difference in temperature for optimum nitrification can be correlated to the difference in the environment where the soil is formed. The Lualualei was formed under a very warm climatic region, thus the microbial population can survive better at 40°C than at 5°C. On the other hand, at the other extreme, the Maile soil was formed under relatively cool, moist conditions and nitrified faster at 25, 15 and 5°C than at 40°C. In the case of the Lualualei, it was speculated that the loss of nitrate was influenced by pH, volatilization and ammonium fixation.

Mineralization of the untreated soil was found to be highest at 40°C in all the soils, with Paaloa and Maile showing the maximum increase.

In the chemical-temperature study, MB, EDB and N-serve

showed complete control of nitrification at 5°C in all soils (Paaloa, Wahiawa and Lualualei) used. Nitrification in Paaloa and Wahiawa soils were suppressed for 12 weeks at 40°C with all the chemicals, whereas in the Lualualei only methyl bromide suppressed nitrification for 12 weeks at this temperature followed by EDB and N-serve. At 25°C nitrification the Wahiawa soil showed complete inhibition with all chemicals, but nitrification in Paaloa soil showed inhibition with methyl bromide for 12 weeks and with N-serve and EDB for 8 weeks. In Lualualei nitrification was resumed in the MB- and EDB-treated soils after 8 weeks and after 4 weeks in the N-serve treated soils.

Tam (1945) reported that pineapples grew well in NH_4-N nutrition obtained by application of 400 lbs/A of DD mixture which controlled nitrification for 24 weeks. Our present study shows that methyl bromide is the most effective fumigant in controlling nitrification with increase of NH_4-N through mineralization which might increase the availability of soil nitrogen in these soils. The release of ammonia at 40°C with these three chemicals suggests that the application of fumigants during the summer time will give greater NH_4-N nutrition to the plants which are tolerant to ammonium nitrogen.

Methyl bromide was found to be the most effective fumigant for inhibiting nitrification of added ammonium at all temperatures, irrespective of the type of soil. N-serve was the least effective

chemical at higher temperatures and EDB was intermediate between them.

Appendix Table 1
Soil and Air Temperatures (°C) at Field Locations of Nitrification Studies

Location of Test	Weekly Temperature						
	0	1	2	3	4	5	6
Helemano[1/]							
Weekly Maximum (Soil)	29	29	23	30	26	30	24
Weekly Minimum (Soil)	17	17	17	17	16	16	16
Soil Temp. 2 p.m.	23	22	20	20	20	20	19
Air Temp. 2 p.m.	30	29	27	24	24	27	24
Lualualei[2/]							
Weekly Maximum (Soil)	40	41	43	38	38	36	34
Weekly Minimum (Soil)	27	27	29	29	29	30	29
Soil Temp. 2 p.m.	36	39	42	41	37	39	36
Air Temp. 2 p.m.	33	33	39	39	34	30	30

1/ Measurement period: 27th December 1965 to 7th February 1966 (cool site).

2/ Measurement period: 27th June 1966 to 8th August 1966 (warm site).

Appendix Table 2
Initial Ammonium and Nitrate Levels in Three Soils for the Two Temperature Regimes

Soil	Cool		Warm	
	NH_4	NO_3	NH_4	NO_3
	ppm			
Wahiawa	8	6	7	10
Paaloa	9	5	9	19
Lualualei	28	12	8	12

LITERATURE CITED

Anderson, O. E. 1960. The effect of low temperature on nitrification of ammonia in Cecil sandy loam. Soil Sci. Soc. Amer. Proc. 24:286-289.

Anderson, O. E. and E. R. Purvis. 1955. Effects of low temperatures on nitrification of ammonia in soils. Soil Sci. 80:313-318.

Anderson, O. E. and F. C. Boswell. 1964. The influence of low temperature and various concentrations of ammonium nitrate on nitrification in acid soils. Soil Sci. Soc. Amer. Proc. 28:525-529.

Aldrich, D. G. and J. P. Martin. 1952. Effect of fumigation on some chemical properties of soils. Soil Sci. 73:149-159.

Allison, F. E., M. Kefauver and E. M. Roller. 1953. Ammonium fixation in soils. Soil Sci. Soc. Amer. Proc. 17:107-110.

Acquaque, D. K. and R. K. Cunningham. 1962. Losses of nitrogen by ammonia volatilization from surface fertilized tropical forest soils. Trop. Agric., Trin. 42:281-292.

Agarwal, A. S. 1967. Immobilization and mineralization of nitrogen in Hawaiian soils. Ph.D. Dissertation, Univ. of Hawaii Library, Honolulu, Hawaii.

Barshad, I. 1948. Vermiculite and its relationship to biotite as revealed by base exchange reactions, X-ray analysis, differential thermal curves and water content. Am. Mineral. 33:655-678.

Bremner, J. M. 1965. Inorganic forms of nitrogen. In C. A. Black (ed.) Methods of Soil Analysis, Part 2. ASA No. 9 pp. 1179-1237.

Bremner, J. M. and D. R. Keeney. 1966. Determination of isotope-ratio analysis of different forms of nitrogen in soils. 3. Exchangeable ammonium, nitrate and nitrite by extraction-distillation methods. Soil Sci. Soc. Amer. Proc. 30:577-582.

Broadbent, F. E., K. E. Tyler and G. N. Hill. 1958. Low temperature effects on nitrification in four Californian soils. Soil Sci. 87:123-129.

Carter, W. 1943. A promising new amendment and disinfectant. Science 97:383-384.

Cline, M. G. et al. 1955. Soil Survey of the Territory of Hawaii. USDA Soil Survey Series 1939, No. 25.

Dhar, N. R. 1935. Influence of light on some biochemical processes. Soc. Biol. Chem. (Indian Inst. Sci., Bangalore) pp. 77.

Dhar, N. R. and N. N. Plant. 1944. Nitrogen losses from soils and oxide surfaces. Nature 153:115-116.

Eno, C. F. 1960. Nitrate production in the field by incubating the soil in polyethylene bags. Soil Sci. Soc. Amer. Proc. 24:277-278.

Ernst, J. W. and H. F. Massey. 1960. The effects of several factors on volatilization of ammonia formed from urea in the soil. Soil Sci. Soc. Amer. Proc. 24:87-90.

Frederic, L. R. 1956. The formation of nitrate from ammonium nitrogen in soils. I. Effect of temperature. Soil Sci. Soc. Amer. Proc. 20:496-500.

Frederic, L. R. 1957. Formation of nitrate from ammonium nitrogen in soils. II. Effect of population of nitrifiers. Soil Sci. 83:481-485.

Fraps, G. S. and A. J. Stergers. 1935. Effect of sunlight on the nitrification of ammonium salts in soils. Soil Sci. 39: 85-94.

Gasser, J. K. and J. E. Peachey. 1964. A note on the effects of soil sterilents on the mineralization and nitrification of soil nitrogen. J. Sci. Food. Agr. 15:142-146.

Goring, A. I. 1962. Control of nitrification by 2-chloro-6 (trichloromethyl)pyridine. Soil Sci. 93:211-218.

Greenland, D. J. 1958. Nitrate fluctuations in tropical soils. J. Agr. Sci. 50:82-92.

Jenny, H. 1941. Factors of Soil Formation. McGraw-Hill, New York. pp. 278.

Jackson, M. L. and S. C. Chang. 1947. Anhydrous ammonia retention by soils as influenced by depth of application, soil texture, moisture content, pH value and tilth. J. Am. Soc. Agron. 39:623-633.

Jewitt, T. N. 1942. Loss of ammonia from ammonium sulphate applied to alkaline soils. Soil Sci. 54:401-409.

Justice, J. K. and Smith, R. L. 1962. Nitrification of ammonium sulphate in a calcareous soil as influenced by combinations of moisture, temperature and levels of added nitrogen. Soil Sci. Soc. Amer. Proc. 26:246-250.

Kincaid, R. R. and G. M. Volk. 1949. Soil fumigation for cigar wrapper tobacco in Florida. (Abstr.) Phytopathology 39:11.

Koike, H. 1961. The effects of fumigants on nitrate production in soil. Soil Sci. Soc. Amer. Proc. 25:204-206.

Martin, J. B. and H. D. Chapman. 1951. Volatilization of ammonia from surface fertilized soils. Soil Sci. 71:25-34.

Mahendrappa, M. K., R. L. Smith and A. T. Christiansen. 1966. Nitrifying organisms affected by climatic region of Western United States. Soil Sci. Soc. Amer. Proc. 30: 60-63.

McCants, C. B., E. O. Skogley and W. G. Woltz. 1959. Influence of certain soil fumigation treatments on the response of tobacco to ammonium and nitrate forms of nitrogen. Soil Sci. Soc. Amer. Proc. 23:466-469.

McIntosh, T. H. and L. R. Frederic. 1958. Distribution and nitrification of anhydrous ammonia in a nicolet sandy loam. Soil Sci. Soc. Amer. Proc. 22:402-405.

Meiklejohn, J. 1954. Some aspects of physiology of the nitrifying bacteria. (In Autotropic Microorganisms, pp. 68-83, Cambridge University Press, Cambridge.)

Meiklejohn, J. 1953. The nitrifying bacteria. A review. J. Soil Sci. 4:59-68.

Meiklejohn, J. 1953a. The microbial aspects of soil nitrification with special reference to Kawanda Expt. on nitrate accumulation. East Afr. Agr. J. 19:54-56.

Mikami, D. T. 1966. Native fixed ammonium and fixation of applied ammonium in Hawaiian soils. M. S. Thesis, Univ. of Hawaii Library, Honolulu, Hawaii.

McClellan, W. D., J. R. Christie and N. L. Horn. 1949. Efficacy of soil fumigants as affected by soil temperature and moisture. Phytopathology 39:272-283.

Newhall, A. G. 1955. Disinfestation of soil by heat, flooding and fumigation. Bot. Rev. 21:189-250.

Page, J. B. and L. D. Baver. 1940. Ionic size in relation to fixation by cations by colloidal clay. Soil Sci. Soc. Amer. Proc. 4:150-155.

Panganiban, H. E. 1925. Temperature as a factor in nitrogen changes in the soil. J. Am. Soc. Agron. 17:1-32.

Pathak, A. N. and J. G. Shrikande. 1953. Optimum temperature for nitrification and nitrogen fixation. J. Indian Soc. Soil Sci. 1:131-136.

Parker, D. T. and W. E. Larson. 1962. Nitrification as affected by temperature and moisture content in mulched soil. Soil Sci. 44:361-375.

Sabey, B. R., W. V. Bartholomew, R. Shaw, and J. Pesak. 1956. Influence of temperature on nitrification in soils. Soil Sci. Soc. Amer. Proc. 20:357-360.

Sabey, B. R., L. R. Frederic, and W. V. Bartholomew. 1959. The formation of nitrate from ammonium in soils. III. Influence of temperature and initial population of nitrifying organisms on the maximum rate and delay period. Soil Sci. Soc. Amer. Proc. 23:462-465.

Soil Science Society of America. 1965. Glossary of Soil Science Terms. Soil Sci. Soc. Amer. Proc. 29:330-351.

Soulides, D. A. and F. E. Clark. 1958. Nitrification in grassland soils. Soil Sci. Soc. Amer. Proc. 22:308-311.

Stojanovic, B. J. and F. E. Broadbent. 1956. Influence of low temperature on nitrogen transformation in Honeoye silt loam. Soil Sci. 84:243-248.

Stark, F. L., J. B. Smith and F. L. Howard. 1939. Effect of chloropicrin fumigation on nitrification and ammonification in soil. Soil Sci. 48:433-442.

Tam, E. K. and C. B. Clark. 1943. Effect of chloropicrin and other soil disinfectants on the nitrogen nutrition of pineapple plant. Soil Sci. 56:245-261.

Tam, E. K. 1945. The comparative effects of a 50:50 mixture of 1-3-dichloropropane and 1-2-dichloropropane and of chloropicrin on nitrification in soils and on the growth of pineapple plant. Soil Sci. 59:191-205.

Thiegs, B. J. 1955. Effect of soil fumigation on nitrification. Down to Earth 11(1):14-15.

Turner, O. E. et al. 1962. Effect of 2-chloro-6(trichloromethyl)pyridine on nitrification of ammonia fertilizers in field soils. Soil Sci. 94:270.

Tamimi, Y. N. 1964. Ammonium fixation in Hawaiian soils. Ph.D. Dissertation, Univ. of Hawaii Library, Honolulu, Hawaii.

Tyler, K. B., F. E. Broadbent and G. N. Hill. 1959. Low temperature effects on four Californian soils. Soil Sci. 87: 123-129.

Tyler, K. B. and F. E. Broadbent. 1960. Nitrate transformation in Californian soils. Soil Sci. Soc. Amer. Proc. 24:279-282.

Volk, G. M. 1961. Gaseous loss of ammonia from surface applied nitrogenous fertilizers. J. Agr. Food. Chem. 9:280-283.

Waksman, S. A. and M. R. Madhock. 1937. Influence of light and heat upon the formation of nitrates in soil. Soil Sci. 44:361-375.

Wolcott, A. R., F. Maciak, L. N. Shepherd and R. E. Lucus. 1960. Effects of telone on nitrogen transformations and on growth of celery in organic soil. Down to Earth 16(1): 10-14.

Wahhab, A., M. S. Randhawa and S. Q. Alam. 1957. Loss of ammonia from ammonium sulphate under different conditions when applied to soils. Soil Sci. 84:249-255.

Wensly, R. N. 1953. Microbiological studies of the action of selected soil fumigants. Canad. Jour. Bot. 31:277-308.

Winfree, J. P. and R. S. Cox. 1958. Comparative effects of fumigation with chloropicrin and methyl bromide on mineralization of nitrogen in Everglade peat. Plant Disease Reporter 42:807-810.

Wagner, G. H. and C. E. Smith. 1958. Nitrogen losses from soils fertilized with different nitrogen carriers. Soil Sci. 85:125-129.

Warrington, R. 1879. On nitrification. Part 2. Jour. Chem. Soc. (Trans.) 35:429-456.