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9. ABSTRACT

In a field experiment soil samples buried at the warmer temperature regime nitrified added ammonium faster than soils buried at the cooler temperature regime. Nitrification occurred more rapidly under both regimes in a soil which had developed in a warm climate zone than in two other soils developed under cooler conditions. The rate of nitrification of added ammonium in soils incubated at 5, 15, 25, and 40°C. in the laboratory increased with increase in the temperature up to 25°C. in three out of four soils. In the fourth soil, nitrification was as active at 40°C. as at 25°C.. The temperature range for appreciable nitrification to occur in a soil was related to the environmental conditions under which the soil was formed. Mineralization of organic nitrogen occurred to a greater extent at 40°C. than at three lower incubating temperatures of 5, 15, and 25°C.. Rapid and active mineralization was associated with high organic matter and C/N ratio in soils.

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EFFECT OF TEMPERATURE ON NITROGEN TRANSFORMATION IN HAWAIIAN SOILS

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SUMMARY

In a field experiment soil samples buried at the warmer temperature regime nitrified added ammonium faster than soils buried at the cooler temperature regime. Nitrification occurred more rapidly under both regimes in a soil which had developed in a warm climatic zone than in two other soils developed under cooler conditions.

The rate of nitrification of added ammonium in soils incubated at 5, 15, 25 and 40 C in the laboratory increased with increase in the temperature up to 25 C in three out of four soils. In the fourth soil nitrification was as active at 40 C as at 25 C. The temperature range for appreciable nitrification to occur in a soil was related to the environmental conditions where the soil was formed.

Mineralization of organic nitrogen occurred to a greater extent at 40 C than at three lower incubating temperatures of 5, 15, and 25 C. Rapid and active mineralization was associated with high organic matter and C/N ratio in soils.

INTRODUCTION

Nitrogen transformation in soil is governed by a number of environmental factors, such as pH, status of mineral nutrients, temperature, aeration, and moisture. The climatic patterns associated with land areas have a marked influence upon the formation and loss process that regulates the quantity of mineral nitrogen in the soil. Temperature is one of the most important environmental factors that affect nitrogen transformation. It is known to affect biological changes in the soil and these changes in the field are

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closely related with the season. Therefore, seasonal influence on biological changes is often explained in terms of temperature effect. Studies by Anderson and Purvis [2], Broadbent *et al.*[5], Frederick [9], Sabey *et al.*[15], Tyler and Broadbent [19], have shown that nitrification of applied ammonia occurs in some soils at a temperature lower than was previously thought necessary for nitrification. As examples, Broadbent *et al.*[5] found that nitrification was greatly reduced but still measurable at 3°C, while Sabey *et al.* [15] found measurable nitrification at 8°C. Sabey *et al.*[16], Frederick [10], and Anderson and Purvis [2] concluded that differences among soils in regard to the temperature range over which nitrate formation from ammonium salts occur appear to be due to difference in the initial population of nitrifiers. Waksman and Madhock [20], and Frederic [9] reported that a temperature of 27 to 37°C to be the most favorable for the activities of nitrifying bacteria. Meiklejohn [13] estimated that 30 to 36°C is the optimum nitrification range but also stated that this optimum is not sharply defined.

It is generally acknowledged that the relatively high temperature in the tropics will tend to increase most microbiological activities. There is, however, a tendency to dismiss the effect of temperature on nitrogen transformation in the tropics because of the small fluctuation in seasonal temperatures. Consequently, comparatively few studies have been carried out on the relationship between temperature and N changes in tropical soils. There is an especial scarcity of data on these changes occurring under field conditions. The present study was therefore undertaken to observe the effect of temperature on nitrogen transformation in tropical Hawaiian soils under field and laboratory conditions. The primary objective was to study the effect of temperature on nitrification of added ammonium. Additional information was also obtained on the mineralization of native nitrogen.

MATERIALS AND METHODS

Description of soils used

Hawaiian surface soils (approximately 0–15 cm) representing four subgroups were used for this study. These soils, developed under a wide range of climatic conditions, differ in physical, chemical and mineralogical properties and are described by Cline [7]. They are: (1) Wahiawa silty clay (Tropeptic Eutrorthox) which is derived from basalt and developed under an annual

rainfall of 1200 mm. and at an elevation of 250 m. The average air temperature for this soil site for the months of January and July are about 69°F (20.6°C) and 73°F (22.8°C), respectively. Mean annual temperature is about 71° F (21.7°C). The soil has a cation exchange capacity of 15 me/100 grams and a base saturation of 40%. The clays are predominantly kaoline with some iron oxide and the buffering capacity is low. (2) Paaloa silty clay (Humoxic Tropohumult) is formed from basalt under an annual rainfall of 2000 mm and at an elevation of 400 m. The average air temperature for this soil site for the months of January and July are about 67°F (19.4°C) and 73°F (22.8°C), respectively. Mean annual temperature is about 70°F (21.1°C). The clay minerals present in the soil are kaolin, illite, iron oxides and gibbsite. The soil has a cation exchange capacity of 35 me/100 grams and a base saturation of 5%. Its buffering capacity is moderately high. (3) Lualualei clay (Typic Chromustert) is formed from deep alluvium under an annual rainfall of 500 mm and at an elevation of 5 m. The average air temperature for this soil site for the months of January and July are about 71°F (21.7°C) and 78°F (25.6°C), respectively. Mean annual temperature is about 75°F (23.9°C). The dominant clay mineral is montmorillonite. The soil has a cation exchange of 56 me/100 grams and is 100% base saturated due to the presence of free gypsum and carbonates. Its buffering capacity is high. (4) Maile silt loam (Hydric Dystrandept) is derived from volcanic ash, under an annual rainfall of 1600 mm at an elevation of 850 m. The average air temperature for this soil site for the months of January and July are about 54°F (12.2°C) and 63°F (17.2°C), respectively. Mean annual temperature is about 58°F (14.4°C). The soil has a cation exchange capacity of 85 me/100 grams and a base saturation of about 40%. The clay is dominated by allophane and hydrated oxides of iron and aluminum, and the buffering capacity is high. Additional properties of these four soils are given in Table 1.

All sampled soils were kept moist in plastic bags in their original field condition and were passed through an 8-mesh sieve before use.

TABLE 1

Some properties of the experimental soils

Soil	Moisture equivalent* %	pH	Total N* %	Organic C* %	C:N
Wahiawa silty clay	32.6	5.4	0.174	1.44	8.2
Paaloa silty clay	31.1	5.8	0.252	3.26	12.9
Lualualei clay	41.8	7.7	0.099	1.06	10.7
Maile silt loam	105.5	5.6	0.775	11.94	15.4

* Values expressed on oven-dry weight basis.

Field studies

Nitrification of added ammonium sulphate was studied in the field under a 'cool' and a 'warm' temperature regime. Two locations and two seasons of the year were chosen to give these two regimes. For the cool condition, a location at Helemano on the Island of Oahu and the winter months of January and February were selected, while for the warm condition, a location at Lualualei on the Island of Oahu and the summer months of July and August were selected. These two sites, Helemano and Lualualei, were also where the Paaloa silty clay and the Lualualei clay, respectively, were sampled for this study. Air temperature, rainfall, and elevation data are as given for the Paaloa silty clay and the Lualualei clay in the foregoing section on 'Description of Soils Used.'

The method used for incubation in the field was similar to that of Eno [8], who used polyethylene bags *. This technique allows diurnal changes in the soil temperature to be taken into consideration in microbial processes, such as nitrification.

Fifty g (oven-dry basis) of Wahiawa, Paaloa and Lualualei soils were placed in a 8 × 10 × 33 cm polyethylene bag and ammonium sulphate was added at a 200 ppm N rate. Soil moisture was adjusted to approximately moisture equivalent of the soil. The bags were closed to essentially the volume of the soil and secured with rubber bands. The ammonium sulphate-treated soils were buried at a soil depth of 22 mm along with a maximum-minimum thermometer to record the soil temperature. Samples were removed at weekly intervals for a period of six weeks. Weekly maximum and minimum temperature readings of the buried theremometer were recorded when the soil samples were removed.

The weekly removed soil samples were brought back to the laboratory where they were analyzed for NH_4- and NO_3-N by the extraction-distillation procedure of Bremner [4].

Laboratory studies

Effect of temperature on nitrification of added ammonium and mineralization of native nitrogen was studied in the Wahiawa, Paaloa, Lualualei, and Maile soils. The soils were incubated at 5, 15, 25 and 40 C in the laboratory.

Fifty g soil (oven-dry basis) was placed in 8 × 10 × 33 cm polythylene bags for incubation at 5, 15, 25 C and in 250-ml Erelenmeyer 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 and maintained at approximately moisture equivalent. Samples were analysed at weekly intervals for ammonium and nitrate nitrogen by the previously mentioned method of Bremner [4] for a period of six weeks.

* 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 high. A study for 24 weeks showed that no nitrate diffused through the film [8].

RESULTS AND DISCUSSION

Field studies

The effect of temperature on nitrification of added ammonium was studied in three soils buried at 'cool and 'warm" climatic regimes. Weekly maximum and minimum temperature readings for the cool (Helemano site) and warm (Lualualei site) regimes and NO_3 -N data for the three soils are given in Fig. 1.

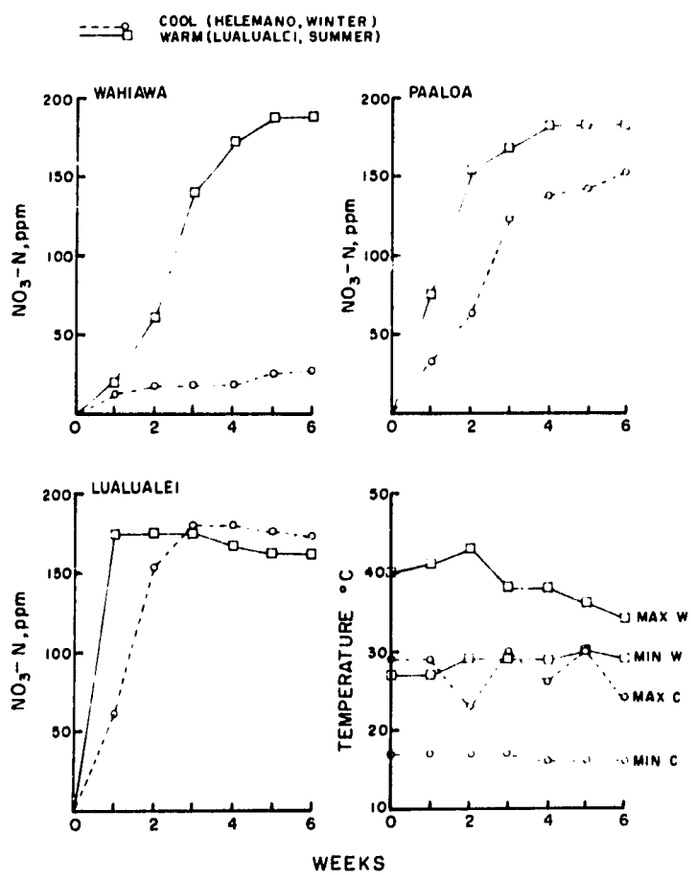

Fig. 1. Nitrification of added ammonium in three buried soils and the maximum-minimum temperatures in cool (c) and warm (w) climatic regimes in the field.

An examination of the temperature data will indicate as expected that the warm regime showed higher maximum and minimum temperatures than the cool regime. There was very little fluctuation in the minimum temperature for both cool (range: 16–17°C) and warm (range: 27–30°C) environments. With the maximum temperature, however, there was slightly greater fluctuation from week to week. There was a range of 23–30°C for the cool and 34–41°C for the warm regime.

The Paaloa and Lualualei soils showed appreciable nitrification at both temperature regimes while the Wahiawa showed very little nitrification in the cool regime (Fig. 1). Nitrification of added nitrogen during the warm regime in the Paaloa and Wahiawa soils was observed to proceed at a higher rate than in the cool regime. This difference was especially prominent in the case of the Wahiawa soil in which only 27 ppm NO_3-N was found at the end of 6 weeks under cool conditions. This may be due to the initial population of nitrifiers. In case of the Lualualei soil the magnitude of nitrification was about the same at both temperatures but there was a difference in the rate of nitrification. The Lualualei, Paaloa, and Wahiawa soils, needed one, four and five weeks, respectively, to complete nitrification during the warm regime. In the cool regime, maximum nitrification was reached at 3 weeks in the Lualualei soil, whereas there was no indication that maximum nitrification has been attained in the Wahiawa and Paaloa soils. The extremely low NO_3-N values for the Wahiawa soil for the cool condition was unexpected because this soil is developed under drier and slightly higher temperature conditions than the Paaloa soil which showed appreciable nitrification. These findings must be associated with the population of nitrifiers in these soils. Anderson and Purvis[2], Broadbent *et al.*[5], Frederick[9], and Sabey *et al.*[15] have noted considerable differences among temperate region soils in their nitrifying characteristics. These findings also agree with the work of Mahendrappa *et al.*[12] who found that soils which are 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 in Hawaii took less time to nitrify the added NH_4-N when incubated under both regimes than the other two soils which have developed under a cooler climatic zone.

In spite of the rapid nitrification occurring under both warm and

cool conditions in the Lualualei soil, recovery as NO_3-N was lowest among the three soils under warm temperatures. This relatively low recovery is believed to be due to ammonium fixation (this soil is montmorillonitic) and to loss of gaseous ammonia at the high soil pH. Both of these phenomena are known to occur to a greater extent at high than at low temperatures.

Laboratory studies

a. Nitrification of added ammonium. Weekly NO_3-N values for six weeks in the Wahiawa, Paaloa, Lualualei, and Maile soils treated with 200 ppm NH_4-N applied as ammonium sulphate, and under incubation temperatures of 5, 15, 25, and 40°C are shown in Fig. 2. The Wahiawa soil showed practically no nitrification at 5°C but nitrification increased with increasing temperature up to 25°C. Furthermore, the lag in nitrification decreased with increasing temperature up to 25°C. At 15°C, 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 6-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-week lag period, nitrification increased sharply and was completed in five weeks. The 25°C incubation treatment showed only a week delay in this soil and the added ammonium was completely nitrified in four weeks. At 40°C only 17 ppm of nitrate was produced, indicating that nitrification inhibition at this temperature was greatest in this soil of the four soils tested.

It should be noted that in the warm regime of the previous field experiment, the maximum temperature stood at or exceeded 40°C for the first two weeks of incubation (Fig. 1); yet considerable nitrification of added ammonium occurred in both Wahiawa and Paaloa soils for this warm regime. These apparent contradictory nitrification results at 40°C between the field and laboratory studies for these two soils are most probably due to the fact that there is a fluctuation in temperature of at least five degrees between the maximum and minimum temperatures in the warm regime of the field experiment which provided conditions conducive to rapid nitrification. In the laboratory experiment, on the other hand, the

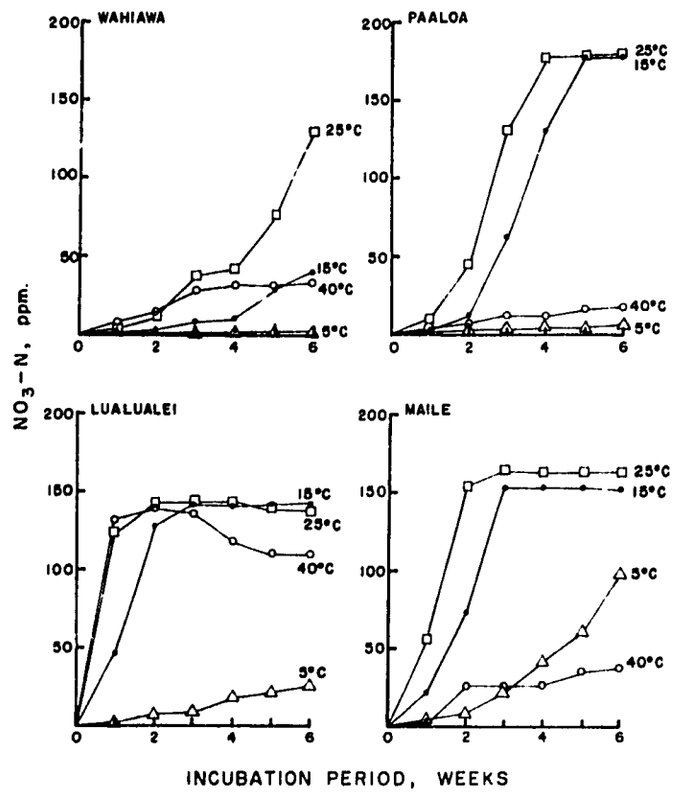

Fig. 2. Nitrification of added ammonium in four Hawaiian soils incubated at 5, 15, 25 and 40°C in the laboratory.

incubation temperature was held at a constant 40°C for the entire six week duration.

There was a slight increase in nitrification at 5°C in the Lualualei soil as compared to Wahiawa and Paaloa soils. At 15, 25, and 40°C, the added ammonium nitrogen was nitrified in 3, 2, 2 weeks respectively showing that with an increase in temperature, the rate of nitrification was faster in this soil, than in the former two soils. No lag period was observed at 25°C and 40°C, as was true with the Wahiawa and the Paaloa soils. This phase of the laboratory study corroborates the field study findings in that nitrification proceeded more rapidly under all temperature conditions in the Lualualei soil than in the Wahiawa and Paaloa soils. It is also noteworthy to

point out that as in the field study, recovery of NO_3-N at the highest temperature (40°C in the laboratory study) dropped to lower values with longer periods of incubation after maximum nitrification was reached in two weeks, whereas at 15 and 25°C NO_3-N values remained almost constant throughout the corresponding periods of incubation. Here again, ammonium fixation and gaseous loss of ammonia are believed to have occurred to a greater extent in this Lualualei at 40°C than at the lower temperatures.

In the Maile soil at 5°C after a lag period of 2 weeks, nitrification took place slowly and the rate steadily increased with time. At the end of six weeks, 50 per cent of the added NH_4-N was nitrified, showing that in this soil nitrification can occur at lower temperatures than in the other three soils. At 15°C and 25°C nitrification was complete in 3 and 2 weeks respectively. Very little nitrification occurred at 40°C as was true with the Wahiawa and Paaloa soils. It is significant to note that the mean annual air temperature for this 'cool' Maile soil is 14°C as contrasted to the 'warm' Lualualei soil with a corresponding mean temperature of 24°C.

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. Frederick[10] has also shown that differences in the temperature range of nitrification in soils are due to variations in population of nitrifiers, and the lag 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 lag period. The Lualualei and Maile soils behaved in a similar way in that nitrification of added ammonia was essentially complete in two weeks. The Paaloa and Wahiawa soils differed in their lag periods. The Paaloa completed its nitrification of added ammonia in four weeks, whereas in the Wahiawa, there was no indication that nitrification was complete in six weeks.

The foregoing results of rapid nitrification at 25°C agree well with the results of Sabey *et al.*[16], 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 difference only in their lag period. Meiklejohn[13] reported that nitrification did not proceed at 40°C in a tropical Uganda soil but this study shows

nitrification took place at this temperature in all soils at the end of six weeks. The Wahiawa, Paaloa, Lualualei and Maile accumulated 33, 17, 140 and 37 ppm NO_3–N, respectively. Mahendrappa *et al.*[12] found that soils from a warmer climatic zone incubated at 35 and 40°C nitrified faster than at 20 and 25°C. In this study, the Lualualei, which is a soil developed under the warmest climatic zone of the four soils, nitrified all the added ammonium nitrogen in two weeks at 40°C and required more time to nitrify the same amount at a lower temperature of 15°C. The other three soils, developed under cooler climatic conditions than the Lualualei, all showed less than 25 per cent nitrification of added ammonium at 40°C. In an earlier paper, Thiagalingam and Kanehiro [18] reported that nitrification at 40°C was not stopped in the Lualualei soil even in the presence of a commercially prepared nitrification inhibitor (2-chloro-6-trichloromethyl pyridine), whereas inhibition of nitrification was complete in the Wahiawa and Paaloa soils for 12 weeks under comparable conditions. This rapid and complete nitrification in the Lualualei soil at 40°C suggests that the optimum nitrification temperature range of 27 to 35 °C reported by Waksman and Madhock [20] and Frederick [10] may be too low for a soil like the Lualualei.

b. Mineralization of native N. Mineralization of organic N was determined in the control treatment (no ammonium sulphate added) of the Wahiawa, Paaloa, Lualualei and Maile soils by analyzing for NH_4– and NO_3–N at the same temperature (5, 15, 25 and 40°C) and at the same weekly intervals as the previous nitrification study in the laboratory. The cumulative amounts of NH_4– and NO_3–N are given in Fig. 3. Maximum ammonium and nitrate nitrogen were found at 40°C in all four soils. As difference on nitrifiability among these four soils was noted in the earlier section on nitrification, it is also noted here that there was 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, respectively, in 6 weeks as compared to the Wahiawa and Lualualei soils which accumulated only 26 and 36 ppm, respectively. Panganiban [14] reported ammonification to occur between 15 and 60°C and observed a faster rate at higher temperatures. Substantial release of NH_4–N in a high organic matter Hawaiian soil by long-term oven-drying (90°C) was also reported by Singh and Kanehiro [17].

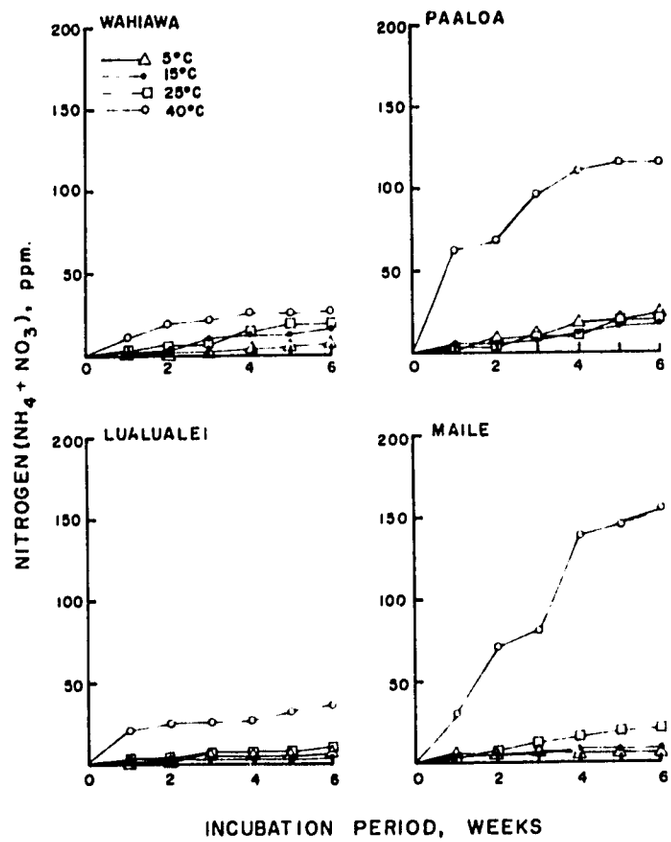

Fig. 3. Mineralization of native N in four Hawaiian soils incubated at 5, 15, 25, and 40°C in the laboratory. Ammonium plus nitrate values are cumulative.

The difference in mineralization capacity is attributed to the amount of organic matter present. It is significant to note also that the amount of available N release at 40°C is correlated better with C/N ratio than with per cent total N in the soils (see properties in Table 1). Agarwal *et al.*[1] also noted a significant correlation to exist between C/N ratios of the soils and N released during drying and rewetting treatments.

In addition to the well-known fact that ammonifiers survive high temperature better than nitrifiers, a number of other reasons have been advanced to explain increased NH_4–N release with high tem-

perature. Some of these reasons are: 1. increased solubility of organic matter in aqueous solution with heating due to chemical alteration [3], 2. killing off of micro-organisms because their death and subsequent lysis would be accompanied by an increase in water-soluble organic matter [11], 3. chemical oxidation of humus or the release of not-readily exchangeable NH_4^+ [6], and 4. splitting off of NH_4^+ from N-bearing organic compounds, especially proteins and amino acids [17].

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Plant and Soil

Contents Volume 38, No. 1, February 1973

S. A. Visser, M. Witkamp and *R. C. Dahlman*, Flow of microbially fixed nitrogen in a model ecosystem 1

M. Bakheit Said, Ammonium fixation in the Sudan Gezira soils . . . 9

C. O. Othieno, The effect of organic mulches on yields and phosphorus utilization by plants in acid soils 17

S. P. Jaiswal, N. K. Saini and *S. K. Sharma*, The effect of some pesticides in conjunction with nitrogen sources on growth and carbohydrate and nitrogen constituents of sugarcane during formative phase 33

A. Wallace, A. M. Abou-Zamzam and *J. W. Cha*, Effect of calcium on transport of cations to the xylem exudate of tobacco 41

E. I. Newman and *Rosalie E. Andrews*, Uptake of phosphorus and potassium in relation to root growth and root density 49

S. R. Poonia and *D. R. Bhumbla*, Effect of gypsum and calcium carbonate on plant yield and chemical composition and calcium availability in a non-saline sodic soil 71

Zvi Plaut, The effect of soil moisture tension and nitrogen supply on nitrate reduction and accumulation in wheat seedlings. 81

Arthur Troughton, Mineral nutrition and the reaction of *Lolium perenne* to defoliation 95

Y. Wong You Cheong and *P. Y. Chan*, Incorporation of P^{32} in phosphate esters of the sugar cane plant and the effect of Si and Al on the distribution of these esters 113

Norman A. Richards, Albert L. Leaf and *Donald H. Bickelhaupt*, Growth and nutrient uptake of coniferous seedlings: comparison among 10 species at various seedbed densities. 125

D. R. Lungley, The growth of root systems – a numerical computer simulation model 145

K. K. S. Bhat and *P. H. Nye*, Diffusion of phosphate to plant roots in soil. I. Quantitative autoradiography of the depletion zone. 161

K. Thiagalingham and *Y. Kanehiro*, Effect of temperature on nitrogen transformation in Hawaiian soils 177

J. R. Boyle and *G. K. Voigt*, Biological weathering of silicate minerals. Implications for tree nutrition and soil genesis 191

Ibrahim Ahmed Nasr, Survival of *Alternaria cheiranthi* in soil 203

Short Communications

R. C. Stutz and *L. C. Bliss*, Acetylene reduction assay for nitrogen fixation under field conditions in remote areas 209

W. H. Eppendorfer and *S. W. Bille*, Decomposition in presence of nitrate of amino acids, especially tyrosine, during acid hydrolysis of plant material and standard amino acid solution 215

K. R. Middleton and *M. R. J. Toxopeus*, Diagnosis and measurement of multiple soil deficiencies by a substractive technique 219

A. K. Varma and *N. S. Subba Rao*, Sucrose and application of Rhizobium on seed 227

Instructions to Authors – 1973 231