

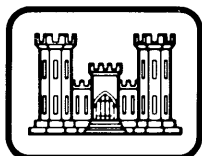
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EFFECT OF SOIL TEMPERATURE AND pH ON NITRIFICATION KINETICS IN SOILS RECEIVING A LOW LEVEL OF AMMONIUM ENRICHMENT

L.V. Parker, I.K. Iskandar and D.C. Leggett

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20. Abstract (cont'd).

as expected. While nitrite-plus-nitrate production appeared logarithmic, suggesting a growing nitrifier population, the MPN counts of the nitrifiers did not exhibit logarithmic growth. To study the effect of soil pH on nitrification kinetics, soil samples from field plots having the same soil type but different pHs (4.5, 5.5, and 7.0) were spiked with low levels of ammonium and the rate of nitrite-plus-nitrate production was measured. The maximum rate of nitrification was greater at pH 5.5 than at 4.5. Unexpectedly rapid disappearance of ammonium, nitrite and nitrate, caused by immobilization, obscured the expected effects of pH on the nitrification rate at the highest pH.

PREFACE

This report was prepared by L.V. Parker, Microbiologist, Dr. I.K. Iskandar, Research Chemist and D.C. Leggett, Research Chemist, all of the Earth Sciences Branch, Research Division, U.S. Army Cold Regions Research and Engineering Laboratory.

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This report was technically reviewed by Dr. D.R. Keeney and Dr. E. Schmidt.

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EFFECT OF SOIL TEMPERATURE AND pH ON
NITRIFICATION KINETICS IN SOILS RECEIVING
A LOW LEVEL OF AMMONIUM ENRICHMENT

L.V. Parker, I.K. Iskandar and D.C. Leggett

INTRODUCTION

All land treatment systems for wastewater must strive towards the removal of N (nitrogen) in the percolate and minimization of the NO_3^- (nitrate) leached into groundwater. The concentration of N in domestic wastewater applied to land rarely exceeds 50 $\mu\text{g N/mL}$, of which approximately 85-90% is in the NH_4^+ (ammonium) form (Iskandar et al. 1976). Once in contact with the soil, NH_4^+ is sorbed on the soil exchange sites and taken up by plants or microorganisms (immobilization) or oxidized by soil microorganisms (nitrification). In contrast NO_3^- is only slightly sorbed by most soils and thus moves down the soil profile with the water unless it is first taken up by the plants or reduced to N_2 or N_2O (nitrogen, nitrous oxide) gases by soil microorganisms (denitrification).

It was first demonstrated a century ago that nitrification is mediated by microorganisms when Schloesing and Muntz (1877) discovered that the production of NO_2^- (nitrite) and NO_3^- from NH_4^+ in sewage percolating through soil could be terminated by the addition of chloroform. Nitrification is generally believed to be mediated by autotrophic nitrifiers. These organisms derive the energy necessary for growth from the oxidation of either NH_4^+ or NO_2^- and do not require organic growth factors. Nitrosomonas, Nitrosococcus, Nitrosospira, Nitrosolobus, and Nitrosovibrio are the terrestrial NH_4^+ oxidizers. However, only Nitrosomonas, Nitrosospira, and Nitrosolobus are widely distributed in soils (Belser 1979). The other two genera have been only occasionally isolated from the soils. Nitrobacter is believed to be the only terrestrial NO_2^- oxidizer.

Nitrifying bacteria are in the highest numbers at the soil surface (Ardakani et al. 1974), consistent with the highest levels of organic matter, total N, O_2 , and cation exchange capacity. Ardakani et al. (1974) also found that the number of NH_4^+ oxidizers in soils declined more drastically with depth than did the number of NO_2^- oxidizers. This can be explained by the soils' ability to retain NH_4^+ at the surface.

The rate of nitrification depends on several environmental conditions including water content, dissolved O_2 , temperature, and pH. The effect of water content on nitrifier activity involves at least two factors: the effect of pore space in which enough water is retained to support life and activity, and the rate limiting factor of O_2 diffusion (Seifert 1962). The optimum water content for nitrification is a soil water tension of 0.15 to 5.0 kPa (Miller and Johnson 1964, Sabey 1969). In the sandy and silty loam soils used for wastewater treatment, moisture tensions are invariably within this range.

Skinner and Walker (1961) and Laudelout et al. (1976) present evidence to suggest that O_2 can also become limiting in the presence of relatively high concentrations of NH_4^+ . However, the NH_4^+ concentration is normally low in land-treated soils.

Nitrification occurs at a wide range of temperatures, from 2° to $35^\circ C$ (Frederick 1956). The optimum is reported to vary between 27° and $30^\circ C$ (Chandra 1962). Focht and Chang (1975), in a review of the literature, indicated a higher optimum, between 30° and $36^\circ C$. These differences in optimum temperature might exist because of differences in nitrifier adaptation to the temperature regimes of the areas from which they originate (Mahendrappa et al. 1966, Anderson et al. 1971).

The optimum pH for NH_4^+ and NO_2^- oxidizers is neutral to slightly alkaline (Focht and Chang 1975). Nitrosomonas is quite sensitive to acid conditions, while Nitrobacter is more susceptible to alkaline conditions. The susceptibility of Nitrobacter to alkaline pHs results in an accumulation of NO_2^- in alkaline soils (Morrill and Dawson 1967, Dancer et al. 1973).

Most studies in the past were conducted using a relatively high NH_4^+ concentration. Since Michaelis-Menten kinetics are expected (Leggett and Iskandar 1980, 1981) low concentrations are needed to reveal the interactions with pH and temperature and to avoid oxygen limitations. Therefore, one objective of this study was to investigate the effect of soil temperature and pH on nitrification kinetics in soils receiving a level of NH_4^+ enrichment which would ensure N limitation and simulate most closely the slow addition of wastewater N.

Several methods are available for studying nitrification in soils. Column techniques usually involve a soil-sand mixture to allow proper drainage. The nitrification rate is then determined by measuring the concentrations of NH_4^+ , NO_2^- , and NO_3^- in the effluent with time. Static incubation methods involve treating soil samples with a specific amount of oxidizable N, and then analyzing the soil sample for NH_4^+ , NO_2^- , and NO_3^- . This method has two advantages over column techniques: it reflects more closely the true ecological conditions, and the soil may be sampled for microbial counts without disturbing the system. For these reasons the static incubation method was selected for this study.

Enumeration of nitrifiers has been hindered by the lack of a quick and accurate method. Several methods exist; the most commonly used one is the most probable number (MPN) technique (Alexander and Clark 1965). This method has two disadvantages: it requires a long incubation time (3 weeks to 4 months), and there is a large statistical uncertainty inherent with the method. The degree of uncertainty depends on the number of tubes used per dilution and the dilution factor used. The number of nitrifiers may be underestimated if the growth conditions and media do not allow all of the nitrifiers present to grow, or if the cells are not separated from the soil particles so that each cell is individually dispersed.

Two other techniques for enumerating nitrifiers, which have been developed recently and may yield better precision and require less time to execute, are the microtechnique MPN (Curtis et al. 1975, Rowe et al. 1977) and the fluorescent antibody (FA) technique (Bohloul and Schmidt 1973, Rennie and Schmidt 1977, Belser and Schmidt 1978a). The microtechnique MPN needs further testing. The major drawback of the FA technique is the existence of multiple serotypes requiring many FAs for each genus (Belser 1979). The problem is compounded by the difficulty in isolating nitrifiers and preparing antibodies to them (Belser 1979). Since both of these techniques are still being developed, the tube MPN technique was used for this experiment.

MATERIALS AND METHODS

Effect of temperature on nitrification kinetics

Windsor sandy loam and Charlton silty loam soils were collected from an experimental slow infiltration land treatment facility at CRREL. For information on the test facilities and the soil characteristics the reader should consult Iskandar et al. (1976) and Iskandar et al. (1979). Fresh soil samples were collected from the top 7.5-cm layer, air dried overnight, and sieved through a 2-mm mesh sieve.

Forty-gram subsamples were incubated with 5 mL of a 120-ppm NH_4Cl solution in 125-mL Erlenmeyer flasks, which were fitted with one-hole rubber stoppers to allow air exchange while keeping moisture loss to a minimum. The soil moisture content was maintained at approximately two-thirds of field capacity to prevent N losses due to denitrification. Denitrification ceases when soils become drier than field capacity (Bremner and Shaw 1958, Mahendrappa and Smith 1967, Pilot and Patrick 1972, Abd-el-Malek et al. 1975).

The flasks were incubated at 5°, 15° and 23°C to mimic field conditions at the CRREL slow infiltration test sites. Iskandar et al. (1979) reported a maximum soil surface temperature of 21°C for the test site during the period from September 1976 to April 1978. Samples were sacrificed on days 0, 3, 6, 9, 15, 21, and 30 for analyses.

Inorganic forms of N were determined by the steam distillation method of Bremner and Keeney (1966). The relative rate of nitrification was determined from the rate of ($\text{NO}_2^- + \text{NO}_3^-$) production. Soil pH was determined on soil slurries made with deionized water on days 0 and 30. Moisture content was determined by oven drying at 105°C for 24 hr.

The method selected for enumerating nitrifiers in this study is a modified MPN method as outlined by Belser and Schmidt (1978b and pers. comm.). Briefly, 10 g of soil was mixed with 90 mL of basic Walker medium and 5 drops of Tween 80. The mixture was then shaken 1 hr. Serial 1:10 dilutions in 1-mM potassium phosphate buffer (pH 7.4 - 7.6) were performed on the suspended soil solution. Ammonium-oxidizing microorganisms were enumerated in Walker medium with 0.04% bromthymol blue solution (0.5 g $[\text{NH}_4]_2\text{SO}_4/\text{L}$). Nitrite oxidizers were enumerated in Watson's Nitrosomonas

medium by replacing the $(\text{NH}_4)_2\text{SO}_4$ with KNO_2 (0.00085 g/L).^{*} Five tubes were used for each dilution. The NH_4^+ oxidizers were incubated 6 weeks at 28°C, and the NO_2^- oxidizers were incubated 8 weeks at 23°C. After incubation, growth was determined in the MPN tubes for the NH_4^+ oxidizers by testing for production of NO_2^- by a modification of the spot test of Strickland and Parsons (1972). The concentration of the reagents was changed slightly so that Greiss reagent A was 0.5% sulfanilamide in 2.4 M HCl, and Greiss reagent B was 0.3% N-1-naphthylethylenediamine dihydrochloride in 0.12 M HCl. Growth was determined for the NO_2^- oxidizers by testing for the disappearance of NO_2^- in the MPN tubes using the same spot test.

Effect of pH on nitrification kinetics

To study the effect of pH on the nitrification rate at low NH_4^+ concentrations, it is essential that other soil physical and chemical characteristics remain constant. Plano silt loam, classified as a Typic Argiudoll, from the University Experimental Farm at Arlington, Wisconsin, was selected for this study. The soil had an initial pH of 4.8, organic matter content of 3.5%, and a clay content of 22% (Dancer et al. 1973). In 1972 the plots were limed with different amounts of dolomitic limestone. This treatment resulted in soils of different pH. Three soils, pH 4.5, 5.5, and 7.0, were selected for this study. Over the years these soils may have developed populations of nitrifiers adapted to each of the different pHs.

Field moist samples were collected and stored for approximately 6 months at 8°C, preincubated at 23°C for 1 week, air dried for 24 hr at 23°C, and then sieved through a 2-mm sieve.

Samples were incubated with NH_4^+ (as NH_4Cl) in Erlenmeyer flasks at 23°C as described previously. Subsamples were randomly selected and sacrificed on days 0, 3, 7, 10, and 14. The soil was analyzed for NH_4^+ and

^{*} Later correspondence revealed that this concentration should have been 0.085 g/L. The concentration used in this medium was detectable using the spot test for NO_2^- . However, this low concentration of NO_2^- may have been subject to chemical breakdown leading to falsely positive growth of NO_2^- oxidizers in a random manner. For this reason the tubes were read after 8 weeks incubation instead of the recommended 4 months incubation.

($\text{NO}_2^- + \text{NO}_3^-$) using the steam distillation method of Bremner and Keeney (1966). Soil pH was determined in deionized water slurries on days 0, 7, 10, and 14. Moisture content was determined by oven drying of subsamples at 105°C for 24 hr.

RESULTS AND DISCUSSION

Effect of temperature on nitrification kinetics

Figures 1 and 2 show the changes in NH_4^+ and ($\text{NO}_2^- + \text{NO}_3^-$) concentrations in relation to temperature and time of incubation for Windsor and Charlton soils respectively. At all temperatures there was a lag phase, after which there was an increase in the ($\text{NO}_2^- + \text{NO}_3^-$) concentrations with time. The rate of ($\text{NO}_2^- + \text{NO}_3^-$) production was slowest at 5°C and greatest at 23°C . The concentration of NH_4^+ decreased at 23°C , remained steady at 15°C , and increased at 5°C in both soils.

Statistical analyses were performed on the actual values of the ($\text{NO}_2^- + \text{NO}_3^-$) and NH_4^+ concentrations, and the numbers of NO_2^- and NH_4^+ oxidizers, and on the natural logarithms of those values. Natural logarithms were used because of the exponential nature of microbial growth. Hereafter, instead of saying, for example, "the natural logarithm of the number of NH_4^+ oxidizers," the shorthand "ln NH_4^+ oxidizers" will be used instead.

Least significant difference (LSD) calculations on the ln ($\text{NO}_2^- + \text{NO}_3^-$) concentrations showed that the length of the lag or delay phase was dependent on the temperature of incubation. After 9 days there was a significant increase in ($\text{NO}_2^- + \text{NO}_3^-$) concentration at 23°C . While at 15° and 5°C , the lag phase was 15 and 21 days respectively. The dependence of the length of the lag phase on soil temperature has been previously reported by Sabey et al. (1969).

In both soils, the rate of nitrification increased with increasing temperature. The maximum rates of nitrification for Windsor soil at 5° , 15° , and 23°C were 0.0049, 0.0056 and 0.0089 meq N/100 g soil per day, respectively, or 1.6, 1.8 and 2.8 kg N/ha per day. The corresponding maximum nitrification rates for the Charlton soil were 0.0009, 0.0034, 0.0069 meq N/100 g soil per day, or 0.3, 1.1, and 2.2 kg N/ha per day. In a separate experiment, Schmidt and co-workers (pers. comm.) obtained values for nitrification rates of 0.0104 and 0.0073 meq N/100 g soil per day for similar

Windsor and Charlton soils, respectively, or 3.3 and 2.3 kg N/ha per day. When one considers that those nitrification rates were obtained at 28°C, they compare well with the values obtained in the present study at 23°C. The higher nitrification rates in the Windsor soil as compared with the Charlton soil may have been due to the higher pH or the higher initial NH_4^+ concentration in the Windsor soil.

Analysis of variance was performed to test the effect of temperature and soil on $\ln(\text{NO}_2^- + \text{NO}_3^-)$ production (Table B1). It showed that soil type and the interaction between time and temperature were highly significant, and that time and temperature were both highly significant within that interaction. The $(\text{NO}_2^- + \text{NO}_3^-)$ values for the Charlton soil (Fig. 1 and 2) were significantly lower than those for the Windsor soil. Since none of the interactions of other variables (time, temperature) with soil were significant, the soils do not have to be considered independently. The effect of temperature was similar for both soils. Regression analysis showed that there was significant statistical evidence for a linear relationship at all three temperatures and that the slope of the line, representing the increase in the production of $(\text{NO}_2^- + \text{NO}_3^-)$ with time, increased with temperature. The linear equations for both soils and the coefficients of determination (r^2) for these equations at each temperature are:

$$\begin{aligned} \text{at } 5^\circ\text{C} \quad \ln(\text{NO}_2^- + \text{NO}_3^-) &= (0.02132)x - 3.1516 \\ r^2 &= 0.83 \end{aligned}$$

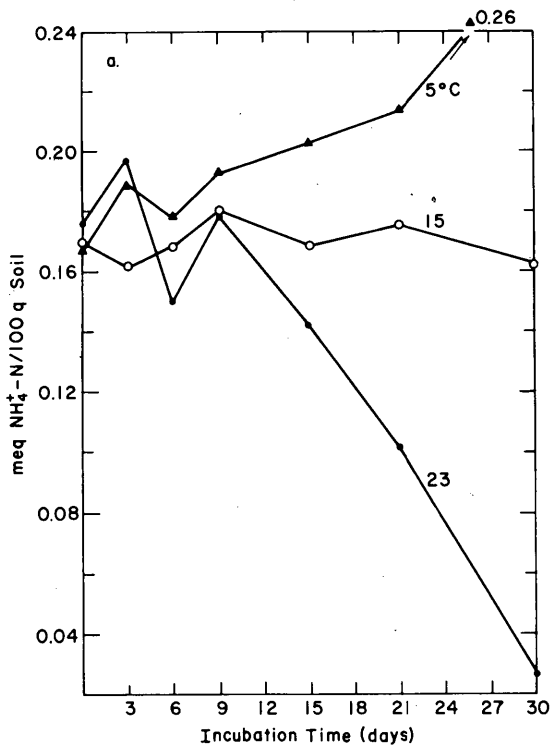
$$\begin{aligned} \text{at } 15^\circ\text{C} \quad \ln(\text{NO}_2^- + \text{NO}_3^-) &= (0.02694)x - 3.0145 \\ r^2 &= 0.74 \end{aligned}$$

$$\begin{aligned} \text{at } 23^\circ\text{C} \quad \ln(\text{NO}_2^- + \text{NO}_3^-) &= (0.03932)x - 2.9482 \\ r^2 &= 0.94 \end{aligned}$$

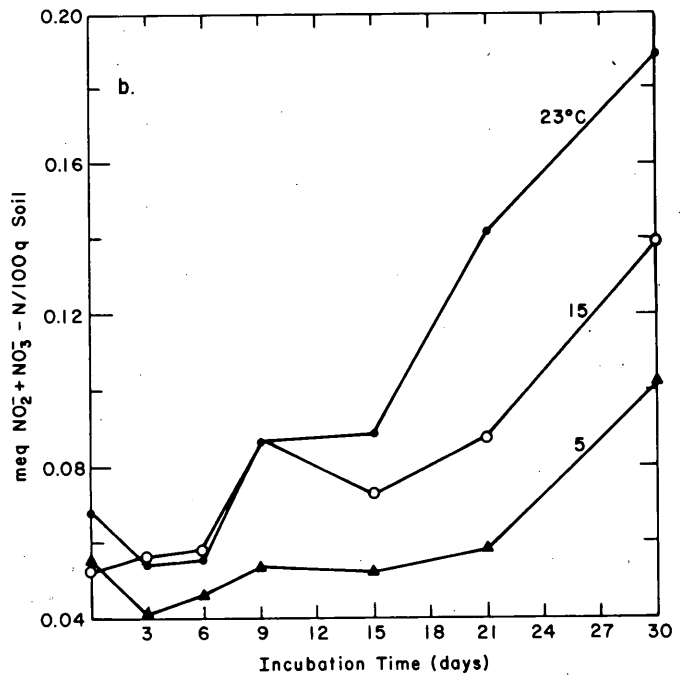
where x = time (days). Although the relationship between concentration and time could also be expressed as linear, statistically it was better represented by the logarithm of the concentration with time. A logarithmic increase in concentration is indicative of a corresponding logarithmic increase in nitrifiers.

Since in nitrification, production of NO_2^- and NO_3^- is accompanied by a corresponding decrease in NH_4^+ concentration, it was of interest to look at the change in concentration of NH_4^+ with time at the three temperatures. Analysis of variance to test the effect of temperature, time and soil type (Table B2) on the $\ln \text{NH}_4^+$ concentration indicated that the interaction of time with temperature was significant. Since the interactions of soil with time and soil with temperature were not significant, the effect of temperature on NH_4^+ concentration was the same for both soils. At the lowest temperature (5°C) there was a net increase in NH_4^+ while at the intermediate temperature (15°C) there was no significant change in the NH_4^+ concentration. At 23°C the loss in NH_4^+ was nearly equal to the ($\text{NO}_2^- + \text{NO}_3^-$) produced, although there was a slight net decrease in total mineral N at 23°C . Belser (1979) reported that low temperature affects nitrification more adversely than mineralization. At 15°C mineralization equaled NH_4^+ losses, which were due to nitrification and immobilization (microbial assimilation). At 5°C mineralization was greater than nitrification and immobilization, which resulted in an accumulation of NH_4^+ . Regression analysis of the $\ln \text{NH}_4^+$ values by day at each temperature gave significant statistical evidence for a linear relationship between the $\ln \text{NH}_4^+$ concentration and time (in days) at 5° and 23°C . Statistical evidence was stronger for a linear relationship between the $\ln \text{NH}_4^+$ concentration and time than for the concentration and time. This indicates that NH_4^+ was being used by a growing population, either nitrifiers or microorganisms which assimilated NH_4^+ .

The pHs of the soils were determined at the beginning and conclusion of the experiment. The pH of the Windsor soil was 5.55 on day 0. By day 30 the pH of the Windsor soil at 5°C was 5.80. This increase was probably due to a depletion of the organic matter (acids). The pH of this soil at 15°C on day 30 was nearly the same as on day 0, 5.53. At this temperature the rate of nitrification was slightly greater and neutralized any increase in pH that resulted from depletion of the organic matter. At 23°C the rate of nitrification was much greater and the pH had dropped by day 30 to 5.31. The Charlton soil showed very similar trends. The initial pH of this soil was 5.50. By day 30 at 5°C the pH of the soil had risen to

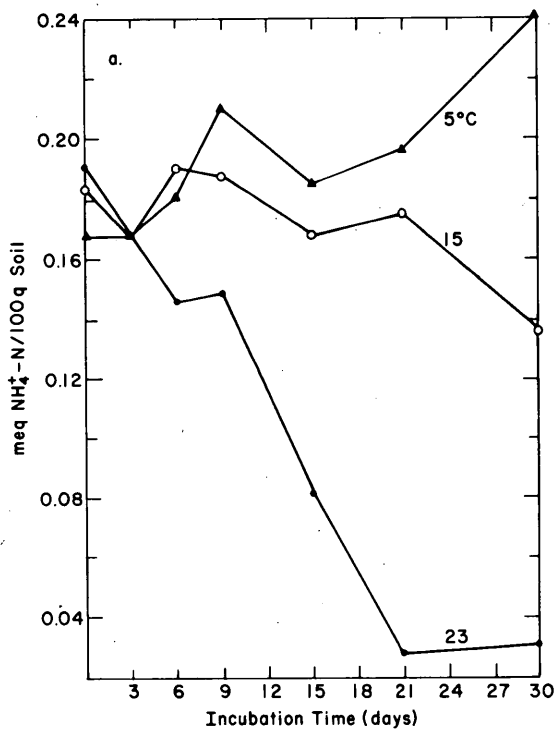


a. Utilization of NH_4^+

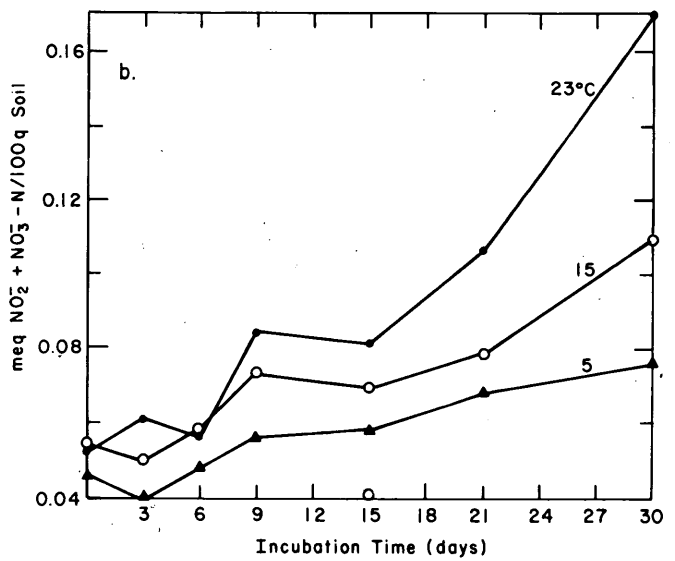


b. Production of $(\text{NO}_2^- + \text{NO}_3^-)$

Figure 1. The effect of temperature on NH_4^+ utilization and $(\text{NO}_2^- + \text{NO}_3^-)$ production in Windsor soil.



a. Utilization of NH_4^+



b. Production of $(\text{NO}_2^- + \text{NO}_3^-)$

Figure 2. The effect of temperature on NH_4^+ utilization and $(\text{NO}_2^- + \text{NO}_3^-)$ production in Charlton soil.

5.80. At 15°C the pH had dropped slightly to 5.36 by day 30, and at 23°C the pH had dropped to 5.23 by day 30.

Many modelers assume that the rate of nitrification is determined by the number of nitrifiers (Ardakani et al. 1973, Beek and Frissel 1973, Day et al. 1978, Leggett and Iskandar 1980, 1981) and that growth and nitrification are coupled, provided growth is not limited by other factors. In this study oxygen and moisture content should not have been limiting. The MPN data for the NH_4^+ oxidizers in the Windsor and Charlton soils at 5°, 15°, and 23°C can be found in Table A3. The mean initial number of NH_4^+ oxidizers was $7.5 \times 10^5/\text{g}$ soil for the Windsor soil and $6.3 \times 10^5/\text{g}$ for the Charlton soil. These numbers agree well with MPN determinations made by Schmidt and co-workers on similar samples where they found 10^5 to 10^6 organisms/g soil (pers. comm.). Analysis of variance was performed on the \ln number of NH_4^+ oxidizers (Table B3). There was no significant difference in the number of NH_4^+ oxidizers in the two soils. The interactions of soil with temperature and soil with time were also not significant. Therefore the data for the two soils were pooled and plotted for each temperature (Fig. 3). There may not have been a large enough difference between the nitrification rates of the two soils to result in a statistically significant difference in the number of nitrifiers, or the MPN technique may not

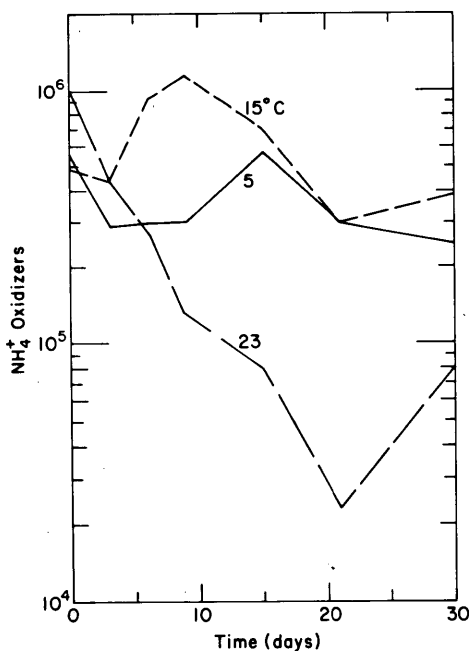


Figure 3. Ammonium oxidizer population at 5°, 15° and 23°C.

be sensitive enough to reflect these differences. Analysis of variance indicated that temperature was highly significant within the interaction of time with temperature, which also was significant. Examination of the difference between the ln number of NH_4^+ oxidizers at the different temperatures (by LSD calculations) demonstrated that there was no significant difference between those values at 5° and 15°C, while the values at 23°C, surprisingly, were significantly lower than those at 5° and 15°C.

Least significant difference determinations performed for each day at each temperature showed that the ln NH_4^+ oxidizers did not change significantly with time at 5° and 15°C. However, by day 6 at 23°C the number of ln NH_4^+ oxidizers was significantly lower than it was at day 0. Linear regression and analysis of variance of the regression demonstrated that the relationship between ln NH_4^+ oxidizers and time was not linear at 5° and 15°C. At 23°C the probability of obtaining the estimated slope, given that the true slope was zero, was significant. Therefore, a linear relationship did exist at 23°C. The slope for this line was negative, indicating that death of the NH_4^+ oxidizers was logarithmic. The substrate was rapidly depleted at 23°C, but not at 5° and 15°C (Fig. 1 and 2), which may explain why the NH_4^+ oxidizers died at 23°C. The low level of substrate at 5° and 15°C was apparently enough to allow maintenance of the population but not growth.

Regression analyses were performed to determine if a correlation existed between $(\text{NO}_2^- + \text{NO}_3^-)$ production and growth of the NH_4^+ oxidizers. The data from each population were treated separately to allow for the significant effect of temperature. Both the actual values and the natural logarithms of the values were tested for correlation. There was no significant correlation between the production of $(\text{NO}_2^- + \text{NO}_3^-)$ and the number of NH_4^+ oxidizers at either 5° or 15°C. There was significant negative correlation at 23°C. Since the original soil for this experiment was taken from an on-going wastewater treatment site, it is likely that the number of nitrifiers was close to the maximum population density for the given NH_4^+ concentration. Therefore, growth would not be expected at this relatively low NH_4^+ concentration.

The number of NO_2^- oxidizers for each day and temperature are given in Table A3. The mean number of NO_2^- oxidizers on day 0 for the Windsor soil

was 1.7×10^7 /g soil and 2.4×10^7 /g soil for the Charlton soil. These numbers agree well with MPN counts made by Schmidt and co-workers (pers. comm.) on similar samples where they estimated the number of NO_2^- oxidizers to be 10^6 to 10^7 /g soil.

Analysis of variance of the $\ln \text{NO}_2^-$ oxidizers indicated that time was the only significant factor (Table B4). Since temperature, soil, and their interactions were not significant, the data for the two soils were pooled and plotted for each temperature (Fig. 4).

Linear regression and analysis of variance of the regression demonstrated that the relationship between the $\ln \text{NO}_2^-$ oxidizers and time was not linear at any temperature or for all temperatures combined. Least significant difference calculations were performed for each day at each temperature. At 5°C the number of NO_2^- oxidizers was significantly lower (at the 0.05 significance level) for days 9 and 15 than the number of NO_2^- oxidizers on day 0. Day 21 was only significantly lower at the 0.10 level, not at the 0.05 level. At 15°C day 9 was significantly lower at the 0.10 level. At 23°C there was no significant change in the number of NO_2^- oxidizers at either the 0.05 or 0.10 levels. The significant decrease in the bacterial number by day 9 at 5° and 15°C may be correlated with the lag period that was observed prior to $(\text{NO}_2^- + \text{NO}_3^-)$ production. During this lag period NO_2^- was not produced and was thus unavailable for nitrite oxidizer growth and maintenance. Although the decrease in the number on day 9 was not significant at 23°C , the observed decrease may also reflect the lag in $(\text{NO}_2^- + \text{NO}_3^-)$ production. The decrease in the bacterial number

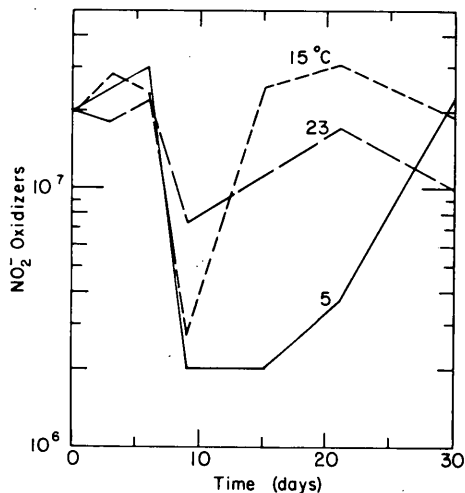


Figure 4. Nitrite oxidizer population at 5° , 15° and 23°C .

was largest and of the greatest duration at 5°C where the lag in ($\text{NO}_2^- + \text{NO}_3^-$) production was most pronounced.

Regression was used to analyze the relationship between the $\ln(\text{NO}_2^- + \text{NO}_3^-)$ values and \ln number NO_2^- oxidizers for days 9 through 30. Day 9 was considered the starting day for growth of the NO_2^- oxidizers. There was no statistically significant correlation at 5°, 15° or 23°C. However, when the mean concentration of ($\text{NO}_2^- + \text{NO}_3^-$) and number of NO_2^- oxidizers for each day at 5°C were used, a highly significant correlation was found. Therefore, there was a real positive correlation between ($\text{NO}_2^- + \text{NO}_3^-$) production and NO_2^- oxidizer growth at 5°C, although there was a lot of noise in the data. Use of the means in similar analyses at 15° and 23°C did not indicate any significant relationships. This was partially due to the decrease in NO_2^- oxidizers on day 30, and no doubt the statistical uncertainty inherent in the MPN technique was also a factor.

In general, while there appears to be no correlation between nitrification and growth of the NH_4^+ oxidizers, which is most likely due to their initially high numbers, there appears to be a correlation between growth of the NO_2^- oxidizers and nitrification, at least at the lower temperatures. This correlation occurs after the NO_2^- oxidizers have died off, their death being due to the lag in NO_2^- production.

Effect of pH on nitrification kinetics

Figure 5 shows the reduction of NH_4^+ concentration and the production of ($\text{NO}_2^- + \text{NO}_3^-$) with time at pH 4.5, 5.5 and 7.0 for the Plano soils, which are from the experimental field plots at the University of Wisconsin. Production of ($\text{NO}_2^- + \text{NO}_3^-$) equals the loss of NH_4^+ during the first week for all three soil pHs. Therefore nitrification of the added NH_4^+ was complete within the first 7 days.

Table 1. Changes in pH in the Plano soils with time.

Initial	<u>Incubation time (days)</u>			
	<u>0</u>	<u>7</u>	<u>10</u>	<u>14</u>
pH				
4.50	4.63	4.55	4.52	4.33
5.52	5.56	5.35	5.37	5.31
7.00	7.00	6.80	6.74	6.79

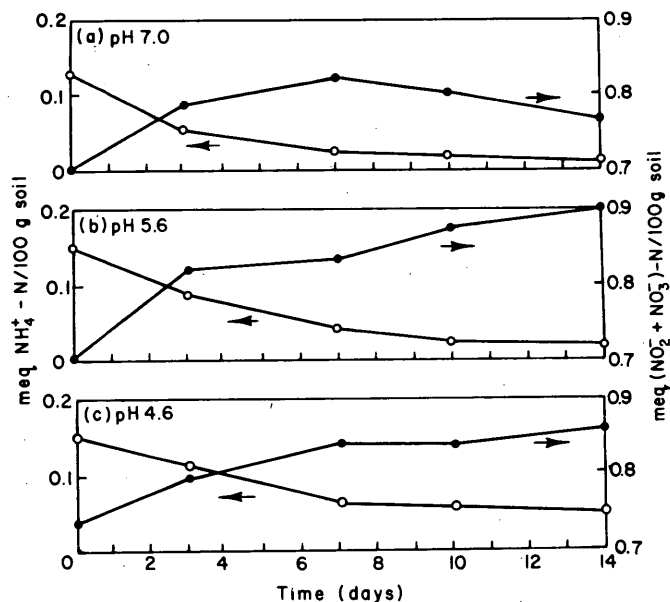


Figure 5. Production of $(\text{NO}_2^- + \text{NO}_3^-)\text{-N}$ and loss of $\text{NH}_4^+\text{-N}$ in Plano silt loam at different soil pHs.

After the first 7 days there was some loss of $(\text{NO}_2^- + \text{NO}_3^-)$ from the soil at pH 7.0. At pH 5.5 there was an increase in the $(\text{NO}_2^- + \text{NO}_3^-)$ levels, and at pH 4.5 there may have been a very slight increase in $(\text{NO}_2^- + \text{NO}_3^-)$ concentration. Least significant difference calculations revealed that the increase in the $\ln(\text{NO}_2^- + \text{NO}_3^-)$ concentration from day 7 to day 14 was significant at pH 5.5, and that the decrease in $\ln(\text{NO}_2^- + \text{NO}_3^-)$ concentration from day 7 to day 14 at pH 7.0 was also significant. However, there was no significant difference between the $\ln(\text{NO}_2^- + \text{NO}_3^-)$ values on days 7 and 14 at pH 4.5. The loss of $(\text{NO}_2^- + \text{NO}_3^-)$ from the soil at pH 7.0 was most likely due to immobilization (microbial assimilation). Microbial growth, and therefore assimilation, appears to be most rapid at the neutral pH.

The continued production of $(\text{NO}_2^- + \text{NO}_3^-)$ after the first week at pH 5.5 indicates that in this soil organic N was mineralized and subsequently nitrified. The continued production of $(\text{NO}_2^- + \text{NO}_3^-)$ was not significant at pH 4.5, indicating that mineralization and nitrification were much slower at this pH, typical of an acid soil.

The pHs of the three soils were monitored during the course of the experiment (Table 1). All three soil pHs dropped as nitrification proceeded. The decline in pH was more rapid at pHs 5.5 and 7.0.

Analysis of variance of the $\ln \text{NH}_4^+$ and $(\text{NO}_2^- + \text{NO}_3^-)$ concentrations indicated that the interaction of pH and time was significant, or highly significant, and that the effects of pH and time were significant or highly significant within this interaction (Table B5). Therefore, pH had a significant effect on the rates of NH_4^+ loss and $(\text{NO}_2^- + \text{NO}_3^-)$ production.

Linear regression analyses were performed on the $\ln (\text{NO}_2^- + \text{NO}_3^-)$ concentration data from the first 7 days (Table 2). None of the equations proved to be significantly linear. This lack of linearity may be due to an inadequate number of data points. Nitrification was more spontaneous than expected, and it is clear that more samples should have been taken earlier in the experiment.

Table 2. Equations for $\ln (\text{NO}_2^- + \text{NO}_3^-)$ concentration with time.

<u>pH</u>	<u>Equation</u>	<u>r²</u>	<u>Significance level</u>
4.5	$\ln (\text{NO}_2^- + \text{NO}_3^-) = (0.0175)x - 0.2871$	0.9805	NS
5.5	$\ln (\text{NO}_2^- + \text{NO}_3^-) = (0.0232)x - 0.3199$	0.7744	NS
7.0	$\ln (\text{NO}_2^- + \text{NO}_3^-) = (0.0215)x - 0.3389$	0.8921	NS

x = Time (days)

NS = Not significant

Maximum rates of NH_4^+ loss and $(\text{NO}_2^- + \text{NO}_3^-)$ production were determined from values on days 0 and 3, and these maximum rates were then used for comparisons. The maximum rate of NH_4^+ loss was greatest at pH 7.0 (0.0250 meq N/100 g soil per day) and least at pH 4.5 (0.0133 meq N/100 g soil per day). The maximum rate of NH_4^+ loss at pH 5.5 was 0.0212 meq N/100 g soil per day. The maximum rate of $(\text{NO}_2^- + \text{NO}_3^-)$ production was greatest at pH 5.5 (0.0378 meq N/100 g soil per day or 12.0 kg N/ha per day) and least at pH 4.5 (0.0175 meq N/100 g soil per day or 5.6 kg N/ha per day). The apparent rate at pH 7.0 was less (0.0277 meq N/100 g soil per day or 8.8 kg N/ha per day) than at pH 5.5 due to the loss of $(\text{NO}_2^- + \text{NO}_3^-)$ and NH_4^+ by immobilization. These maximum rates were tentative since more data points were required.

The high rate of immobilization was most likely a result of the handling the samples received prior to the start of the experiment. These samples had been refrigerated for 6 months and were then brought to room

temperature for 1 week prior to being used. The microbial population could then have used the newly added NH_4^+ and newly formed NO_3^- . This growth was most rapid at the neutral pH.

SUMMARY AND CONCLUSIONS

Windsor sandy loam and Charlton silty loam soils from a slow infiltration land treatment test facility were used to study the effect of temperature on nitrification kinetics at low NH_4^+ concentrations. The net rate of nitrification was determined by measuring the rate of $(\text{NO}_2^- + \text{NO}_3^-)$ production at various temperatures. There was a lag period prior to $(\text{NO}_2^- + \text{NO}_3^-)$ production at all temperatures that was longest at the lowest temperature. The maximum rate of nitrification increased with increasing temperature ranging from 1.6 kg N/ha per day at 5°C to 2.8 kg N/ha per day at 23°C in the Windsor soil, and from 0.3 kg N/ha per day at 5°C to 2.2 kg N/ha per day at 23°C in the Charlton soil. Nitrification was more rapid in the Windsor soil at all temperatures. This may have been due to its higher pH, or higher initial NH_4^+ values.

The concentration of NH_4^+ was also monitored. At 23°C nitrification proceeded rapidly, and the added NH_4^+ had been used by day 21 in the Windsor and by day 30 in the Charlton soil. Since at the lower temperatures (5° and 15°C) the rate of nitrification was slower, the relative rate of mineralization became greater at these temperatures. Therefore there was no net loss of NH_4^+ at these temperatures, and at 5°C NH_4^+ accumulated.

The number of NH_4^+ and NO_2^- oxidizers was monitored throughout the experiment. The number of NH_4^+ oxidizers remained relatively constant at 5° and 15°C, while at 23°C it decreased significantly. This pattern appeared to be most closely related to the available NH_4^+ . At 5° and 15°C, NH_4^+ remained available while at 23°C it was depleted. The lack of correlation between nitrification and growth of NH_4^+ oxidizers may be because the number of NH_4^+ oxidizers in these soils had reached the maximum population density allowed by this level of NH_4^+ enrichment. This may have been the result of the periodic wastewater treatment these soils received prior to this experiment.

The number of NO_2^- oxidizers decreased significantly after 9 days at 5° and 15°C because of the lag in production of NO_2^- . At 23°C this decrease was not significant, but the lag period was not as long either.

The decrease in NO_2^- oxidizers was most pronounced at 5°C where the lag in NO_2^- production was also longest. After this reduction in number, the NO_2^- oxidizers grew as nitrification proceeded and there was a statistically significant correlation between nitrification and growth of the NO_2^- oxidizers at 5°C .

The Plano soil was selected for the experiment testing the effect of pH on nitrification kinetics because this would keep soil factors constant while changing only the pH. The experiment was performed at 23°C so that temperature would not be a limiting factor. Lowering the pH from 5.5 (the approximate pH of the Windsor and Charlton soils) to 4.5 resulted in a lowered rate of NH_4^+ loss and ($\text{NO}_2^- + \text{NO}_3^-$) production. The maximum rate of ($\text{NO}_2^- + \text{NO}_3^-$) production went from 12.0 kg N/ha per day at pH 5.5, to 5.6 kg N/ha per day at pH 4.5. Raising the pH from 5.5 to 7.0 resulted in a greater rate of NH_4^+ loss. However, raising the pH from 5.5 to 7.0 did not increase the rate of accumulation of ($\text{NO}_2^- + \text{NO}_3^-$) (8.8 kg N/ha per day). Instead, there was a loss of mineral N from the system at pH 7.0. This loss was most likely accounted for by losses to immobilization, a result of the rapid microbial growth following cold storage.

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APPENDIX A: DATA COLLECTED FOR THE TEMPERATURE EXPERIMENT

Table A1. The NH_4^+ -N meq/100 g soil for short term nitrification experiment testing the effect of temperature.

Soil and incubation temp.	Before NH_4Cl addition	Incubation time (days)						
		0	3	6	9	15	21	30
Charlton								
5°C A	0.0743	0.1688	0.1661	0.1794	0.2151	0.1572	0.1987	0.2382
B	0.0604	0.1661	0.1672	0.1807	0.2044	0.2135	0.1926	0.2432
15°C A		0.1887	0.1692	0.1886	0.1886	0.1557	0.1734	0.1332
B		0.1781	0.1663	0.1913	0.1864	0.1811	0.1759	0.1396
23°C A		0.1763	0.1636	0.1422	0.1467	0.0697	0.0255	0.0302
B		0.2044	0.1712	0.1502	0.1511	0.0917	0.0311	0.0328
Windsor								
5°C A	0.0616	0.1696	0.1920	0.1772	0.1925	0.2003	0.2151	0.2695
B	0.0608	0.1663	0.1854	0.1786	0.1937	0.2052	0.2120	0.2664
15°C A		0.1647	0.1674	0.1651	0.1836	0.1694	0.1797	0.1646
B		0.1746	0.1558	0.1725	0.1762	0.1690	0.1720	0.1616
23°C A		0.1737	0.2022	0.1161	0.1774	0.1435	0.1129	0.0271
B		0.1786	0.1918	0.1842	0.1802	0.1414	0.0916	0.0265

Table A2. The $(\text{NO}_2^- + \text{NO}_3^-)$ -N meq/100 g soil for short term nitrification experiment testing the effect of temperature.

Soil and incubation temp.	Before NH_4Cl addition	Incubation time (days)						
		0	3	6	9	15	21	30
Charlton								
5°C A	0.0381	0.0497	0.0385	0.0463	0.0617	0.0565	0.0625	0.0754
B	0.0393	0.0422	0.0398	0.0498	0.0512	0.0600	0.0729	0.0761
15°C A		0.0542	0.0508	0.0561	0.0668	0.0497	0.0851	0.0965
B		0.0462*	0.0497	0.0607	0.0802	0.0322	0.0738	0.1244
23°C A		0.0525	0.0523	0.0566	0.0687	0.0660	0.0981	0.1455
B		0.0508	0.0697	0.0581	0.1003	0.0970	0.1156	0.1930
Windsor								
5°C A	0.0329	0.0607	0.0390	0.0537	0.0488	0.0476	0.0483	0.1031
B	0.0393	0.0495	0.0429	0.0379	0.0568	0.0560	0.0677	0.1018
15°C A		0.0622	0.0459*	0.0578	0.0799	0.0661	0.0810	0.1324
B		0.0428	0.0566	0.0588	0.0932	0.0794	0.0945	0.1449
23°C A		0.0746	0.0498	0.0510	0.0787	0.0825	0.1202	0.1815
B		0.0614	0.0591	0.0610	0.0948	0.0947	0.1639	0.1960

* Missing value calculated by Yates method (Steel and Torrie [1960], p. 139).

Table A3. Nitrifier MPN values for short term nitrification experiment testing the effect of temperature.

Day	Soil	Temp. (°C)	MPN NH ₄ ⁺ oxidizers/g soil	MPN NO ₂ ⁻ oxidizers/g soil
0	Charlton	5	4.6x10 ⁵	2.8x10 ⁷
		15	7.1x10 ⁵	2.9x10 ⁷
		23	7.1x10 ⁵	1.6x10 ⁷
--	Windsor	5	6.6x10 ⁵	2.6x10 ⁷
		15	2.9x10 ⁵	2.2x10 ⁷
		23	1.3x10 ⁶	4.2x10 ⁶
3	Charlton	5	2.8x10 ⁵	2.9x10 ⁷
		15	7.1x10 ⁵	1.4x10 ⁷
		23	4.6x10 ⁵	2.8x10 ⁶
--	Windsor	5	2.9x10 ⁵	1.9x10 ⁷
		15	1.6x10 ⁵	4.3x10 ⁷
		23	4.3x10 ⁵	3.4x10 ⁷
6	Charlton	5	1.8x10 ⁵	1.4x10 ⁷
		15	7.1x10 ⁵	2.4x10 ⁷
		23	4.6x10 ⁵	6.2x10 ⁶
--	Windsor	5	4.2x10 ⁵	4.7x10 ⁷
		15	1.1x10 ⁶	---
		23	8.5x10 ⁴	3.8x10 ⁷
8	Charlton	5	3.2x10 ⁵	1.2x10 ⁶
		15	1.2x10 ⁶	2.2x10 ⁶
		23	2.2x10 ⁵	1.2x10 ⁷
--	Windsor	5	2.9x10 ⁵	2.7x10 ⁶
		15	1.1x10 ⁶	2.9x10 ⁶
		23	4.0x10 ⁴	2.7x10 ⁶
15	Charlton	5	4.6x10 ⁵	2.4x10 ⁶
		15	3.1x10 ⁵	2.9x10 ⁷
		23	1.0x10 ⁵	6.4x10 ⁵
	Windsor	5	6.6x10 ⁵	1.3x10 ⁶
		15	1.1x10 ⁶	2.1x10 ⁷
		23	6.0x10 ⁴	2.2x10 ⁷
21	Charlton	5	3.2x10 ⁵	6.5x10 ⁶
		15	1.7x10 ⁵	4.6x10 ⁷
		23	1.7x10 ⁴	3.1x10 ⁷
--	Windsor	5	2.9x10 ⁵	2.8x10 ⁵
		15	4.2x10 ⁵	1.7x10 ⁷
		23	2.9x10 ⁴	4.2x10 ⁶
30	Charlton	5	2.2x10 ⁵	1.4x10 ⁷
		15	3.6x10 ⁵	2.9x10 ⁷
		23	1.0x10 ⁵	1.0x10 ⁷
--	Windsor	5	2.7x10 ⁵	2.9x10 ⁷
		15	4.2x10 ⁵	9.6x10 ⁶
		23	5.9x10 ⁴	9.5x10 ⁶

APPENDIX B: STATISTICAL ANALYSIS OF DATA COLLECTED

Table B1. Analysis of variance of $\ln(\text{NO}_2^- + \text{NO}_3^-)$ concentration for the short-term nitrification experiment testing the effect of temperature.

Term	DF	Mean square	F	Num./den.	Level of significance
T	2	1.25815	18.60	T/TD	HS 0.001
S	1	0.175031	8.26	S/WCE	HS 0.010
D	6	1.31364	19.42	D/TD	HS 0.001
TS	2	3.75524×10^{-2}	1.77	TS/WCE	NS
TD	12	6.76592×10^{-2}	3.19	TD/WCE	HS 0.005
SD	6	2.98343×10^{-2}	1.38	SD/WCE	NS
TSD	12	3.15924×10^{-2}	1.42	TSD/WCE	NS
WCE	42	2.11963×10^{-2}			

T = Temperature ($^{\circ}\text{C}$) WCE = Within-cell error
 S = Soil type NS = Not significant
 D = Time (days) HS = Highly significant

Table B2. Analysis of variance of $\ln \text{NH}_4^+$ concentration values for the short-term nitrification experiment testing the effect of temperature.

Term	DF	Mean square	F	Num./den.	Level of significance
T	2	3.07924	3.94	T/TD	NS
S	1	0.237304	2.81	S/TSD	NS
D	6	0.544662	<1	D/TD	NS
TS	2	0.168097	1.99	TS/TSD	NS
TD	12	0.780581	9.26	TD/TSD	HS 0.001
SD	6	9.89457×10^{-2}	1.17	SD/TSD	NS
TSD	12	8.43071×10^{-2}	12.74	TSD/WCE	HS 0.001
WCE	42	6.6198×10^{-3}			

T = Temperature ($^{\circ}\text{C}$) WCE = Within-cell error
 S = Soil type NS = Not significant
 D = Time (days) HS = Highly significant

Table B3. Analysis of variance of ln number NH_4^+ oxidizers for the short-term nitrification experiment testing the effect of temperature.

Term	DF	Mean square	F	Num./den.	Level of significance
T	2	6.18455	16.99	T/TD	HS 0.001
S	1	0.04734	<1	S/TSD	NS
D	6	1.58879	1.36	D/TD	NS
TS	2	0.471907	1.30	TS/TSD	NS
TD	12	1.16444	3.20	TD/TSD	S 0.050
SD	6	0.24623	<1	SD/TSD	NS
TSD	12	0.364002			

T = Temperature ($^{\circ}\text{C}$)

S = Soil type

D = Time (days)

NS = Not significant

HS = Highly significant

Table B4. Analysis of variance of ln number NO_2^- oxidizers for the short-term experiment testing the effect of temperature.

Term	DF	Mean square	F	Num./den.	Level of significance
T	2	0.374663	2.93	T/TSD	NS
S	1	5.08408	<1	S/TSD	NS
D	6	11.8639	3.48	D/TSD	S 0.050
TS	2	2.27132	<1	TS/TSD	NS
TD	12	1.19563	1.46	TD/TSD	NS
SD	6	0.416234	1.97	SD/TSD	NS
TSD	12	1.35107			

T = Temperature ($^{\circ}\text{C}$)

S = Soil type

D = Time (days)

NS = Not significant

S = Significant

Table B5. Analysis of variance of mineral N levels for the short-term nitrification experiment testing the effect of pH.

Term	DF	Mean square	F	Num./den.	Level of significance
ln NH ₄ ⁺ concentration					
P	2	2.5017	14.59	P/PD	HS 0.001
D	4	4.73449	27.61	D/PD	HS 0.001
PD	8	0.171497	2.70	PD/WCE	S 0.025
WCE	30	0.06356			
ln (NO ₂ ⁻ + NO ₃ ⁻) concentration					
P	2	1.91657x10 ⁻²	5.27	P/PD	S. 0.050
D	4	4.014914x10 ⁻²	11.11	D/PD	HS 0.001
PD	8	3.63872x10 ⁻³	4.26	PD/WCE	HS 0.005
WCE	30	8.53266x10 ⁻⁴			

P = pH
 D = time (days)
 WCE = Within-cell error
 S = Significant
 HS = Highly significant