					_					_			
1	AD-A112 171	COLD RE EFFECT DEC 81	GIONS RES OF SOIL T	EARCH A Emperat	ND ENG	INEERIN D PH ON DAR, D	G LAB NITRIF C LEGGE	HANOVER	NH KINETI	F/6 ICS IN	8/13 ETC(U	) 1977 -	ľ
1	UNCLASSIFIED	CRREL-S	R-81-33							. NL			ł
	co∓   ∎Q, A ···2··2	_											
		j		END BATE FILWED D4-B21 DTIC									
					_	_							
													ŕ



# Special Report 81-33



December 1981

ADAILLIT

# 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





Prepared for OFFICE OF THE CHIEF OF ENGINEERS By



UNITED STATES ARMY CORPS OF ENGINEERS COLD REGIONS RESEARCH AND ENGINEERING LABORATORY HANOVER, NEW HAMPSHIRE, U.S.A.



£



Unclassified SECURITY CLASSIFICATION OF THIS PAGE (When Date	Entered)	
REPORT DOCUMENTATION	PAGE	READ INSTRUCTIONS
1. REPORT NUMBER	2. GOVT ACCESSION NO.	BEFORE COMPLETING FORM
Createl Depart (1, 22		
Special Report 81-33		
EFECT OF COLL TEMPEDATURE AND ALL	ON	S. TYPE OF REPORT & PERIOD COVERED
NITRIFICATION KINETICS IN SOLLS BE	CETVING A	
LOW LEVEL OF AMMONIUM ENRICHMENT	OLIVINO A	6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR()		8. CONTRACT OR GRANT NUMBER(a)
L.V. Parker, I.K. Iskandar and D.C	. Leggett	
9. PERFORMING ORGANIZATION NAME AND ADDRESS		10. PROGRAM ELEMENT. PROJECT, TASK
U.S. Army Cold Regions Research an	d	
Hanover New Hampshire 03755		CW15 31033
hanover, New hampshile 03/33		
CONTROLLING OFFICE NAME AND ADDRESS	İ	December 1001
Uttice of the Chief of Engineers		13. NUMBER OF PAGES
wasnington, D.C. 20314		33
14. MONITORING AGENCY NAME & ADDRESS(II differen	t from Controlling Office)	15. SECURITY CLASS. (of this report)
		Unclassified
		15a, DECLASSIFICATION/DOWNGRADING SCHEDULE
17. DISTRIBUTION STATEMENT (of the ebstract entered	in Block 20, il different fro	n Report)
18. SUPPLEMENTARY NOTES		
19. KEY WORDS (Continue on reverse aids if necessary an	d identify by block number)	
Ammonium Nitrites		
Microorganisms pH factor		
Land Treatment Removal		
NITTATES Waste water		
20. ABSTRACT (Continue on reverse side if necessary and	identify by black number)	
Two soil samples from an on-going wastewater were spiked with low let temperature on nitrification kinet nitrite-plus-nitrate, and the numbe were monitored periodically during nitrite-plus-nitrate production at period was temperature-dependent, est temperature. The maximum rate	field study of la vels of ammonium ics. The concent rof autotrophic a the study. The all temperatures with the longest of nitrification	and application of municipal to determine the effect of trations of ammonium and mmonium and nitrite oxidizers re was a lag period prior to s, and the length of this lag period occurring at the low- n increased with temperature
D FORM 1473 EDITION OF I HOW AS IS OPEOU	FTF	
I JAN 73 THE CONTINUE OF THOM OF THOM OF THOM OF THOM OF THE OBSOL		Unalgest C: 1

Unclassified SECURITY CLASSIFICATION OF THIS PAGE (When Date Entered) N.

Unclass if ied

SECURITY CLASSIFICATION OF THIS PAGE(When Date Entered)

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.

i i

PREFACE

This report was prepared by L.V. Parker, Microbiologist, Dr. l.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.

Financial support for the study was provided from Civil Works project CWIS 31633, Optimization of Automated Procedures for Design and Management of Land Treatment Systems, and CWIS 31297, Nitrogen Transformations in Land Treatment.

The authors acknowledge the technical assistance of Dr. E.L. Schmidt of the University of Minnesota, St. Paul; Dr. D.R. Keeney, of the University of Wisconsin, Madison; Col. John Atkinson, U.S. Army Reserve; B. Blake, former Physical Science Technician, CRREL; P. Coderre, former Biological Lab Technician, CRREL; and Dr. A.P. Edwards, Visiting Scientist, CRREL. Col. Atkinson provided statistical support for this project as part of his annual active duty. Dr. Keeney also provided soil samples of different pH.

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

The contents of this report are not to be used for advertising or promotional purposes. Citation of brand names does not constitute an official endorsement or approval of the use of such commercial products.

est :

## CONTENTS

		Page
Abstract	•	i
Preface	•	iii
Introduction	•	1
Materials and methods	•	4
Effect of temperature on nitrification kinetics	•	4
Effect of pH on nitrification kinetics		5
Results and discussion		6
Effect of temperature on nitrification kinetics	•	6
Effect of pH on nitrification kinetics	•	13
Summary and conclusions	•	16
Literature cited	•	17
Appendix A: Data collected for the temperature experiment		21
Appendix B: Statistical analysis of data collected	•	25

## FIGURES

Figure	
1. The effect of temperature on $NH_4^+$ utilization and $(NO_2^-+NO_3^-)$ production in Windsor soil $\cdots$	9
2. The effect of temperature on NH <sup>+</sup> <sub>4</sub> utilization and $(NO_{\overline{2}}+NO_{\overline{3}})$ production in Charlton soil	9
3. Ammonium oxidizer population at 5°, 15° and 23°C $\ldots$ 1	0
4. Nitrate oxidizer population at $5^{\circ}$ , $15^{\circ}$ and $23^{\circ}$ C 1	.2
5. Production of (NO2+NO3)-N and loss of NH4-N in Plano silt loam at different soil pHs	.4

## TABLES

Table					
1.	Changes in pH	in the	Plano	soils with time	13
2.	Equations for	1n (NO	$\frac{1}{2} + NO_{3}$	concentration with time	15

### 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 µg 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. <u>Nitroso-</u> <u>monas</u>, <u>Nitrosococcus</u>, <u>Nitrosospira</u>, <u>Nitrosolobus</u>, and <u>Nitrosovibrio</u> are the terrestrial  $NH_4^+$  oxidizers. However, only <u>Nitrosomonas</u>, <u>Nitrosospira</u>, and <u>Nitrosolobus</u> are widely distributed in soils (Belser 1979). The other two genera have been only occasionally isolated from the soils. <u>Nitrobacter</u> 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  $0_2$ , 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  $0_2$  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  $2^{\circ}$  to  $35^{\circ}$ C (Frederick 1956). The optimum is reported to vary between  $27^{\circ}$  and  $30^{\circ}$ C (Chandra 1962). Focht and Chang (1975), in a review of the literature, indicated a higher optimum, between  $30^{\circ}$  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). <u>Nitrosomonas</u> is quite sensitive to acid conditions, while <u>Nitrobacter</u> is more susceptible to alkaline conditions. The susceptibility of <u>Nitrobacter</u> to alkaline pHs results in an accumulation of  $NO_2^-$  in alkaline soils (Norrill 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 tor  $\delta H_4^+$ ,  $\delta O_2^-$ , 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 tor 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 (Bohlool 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<sub>4</sub>Cl solution in 125-mL Erlenneyer 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  $(NO_2^- + NO_3^-)$  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. Amnonium-oxidizing microorganisms were enumerated in Walker medium with 0.04% bromthymol blue solution (0.5 g [NH<sub>4</sub>]<sub>2</sub>SO<sub>4</sub>/L). Nitrite oxidizers were enumerated in Watson's Nitrosomonas

medium by replacing the  $(NH_4)_2SO_4$  with  $KNO_2$  (0.00085 g/L).\* Five tubes were used for each dilution. The  $NH_4^+$  oxidizers were incubated 6 weeks at  $28^{\circ}C$ , and the  $NO_2^-$  oxidizers were incubated 8 weeks at  $23^{\circ}C$ . 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-l-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  $8^{\circ}$ C, preincubated at  $23^{\circ}$ C for 1 week, air dried for 24 hr at  $23^{\circ}$ 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

<sup>\*</sup> 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.

 $(N_2^- + NO_3^-)$  using the steam distillation method of Bremner and Keeney (1966). Soil pH was determined in defonized 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  $(NO_2^- + NO_3^-)$  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  $(NO_2^- + NO_3^-)$  concentrations with time. The rate of  $(NO_2^- + NO_3^-)$  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  $(NO_2^- + NO_3^-)$  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 "In  $NH_4^+$  oxidizers" will be used instead.

Least significant difference (LSD) calculations on the In  $(NO_2^- + NO_3^-)$  concentrations showed that the length of the lag or dely phase was dependent on the temperature of incubation. After 9 days there was a significant increase in  $(NO_2^- + NO_3^-)$  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 rep/.ed 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 tor nitrification rates of 0.0104 and 0.0073 meq N/100 g soil per day tor 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  $28^{\circ}$ C, they compare well with the values obtained in the present study at  $23^{\circ}$ 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<sub>4</sub>+ concentration in the Windsor soil.

Analysis of variance was performed to test the effect of temperature and soil on  $\ln (NO_2^- + NO_3^-)$  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  $(NO_2^- + NO_3^-)$  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  $(NO_2^- + NO_3^-)$  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:

> at 5°C In  $(NO_2^- + NO_3^-) = (0.02132)x - 3.1516$   $r^2 = 0.83$ at 15°C In  $(NO_2^- + NO_3^-) = (0.02694)x - 3.0145$   $r^2 = 0.74$ at 23°C In  $(NO_2^- + NO_3^-) = (0.03932)x - 2.9482$  $r^2 = 0.94$

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<sub>4</sub><sup>+</sup> concentration, it was of interest to look at the change in concentration of  $NH_{u}^{+}$  with time at the three temperatures. Analysis of variance to test the effect of temperature, time and soil type (Table B2) on the ln  $NH_{L}^{+}$  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  $Nil_{\mu}^{+}$  concentration was the same for both soils. At the lowest temperature (5°C) there was a net increase in  $NH_{L}^{+}$  while at the intermediate temperature (15°C) there was no significant change in the  $NH_{u}^{+}$ concentration. At 23°C the loss in  $NH_{\mu}^{+}$  was nearly equal to the  $(NO_{2}^{-} +$  $NO_3^{-}$ ) 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  $NH_{\mu}^{+}$ 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  $NH_{\mu}^{+}$ . Regression analysis of the ln  $NH_{\mu}^{+}$  values by day at each temperature gave significant statistical evidence for a linear relationship between the ln  $NH_{\mu}^{+}$  concentration and time (in days) at 5° and 23°C. Statistical evidence was stronger for a linear relationship between the ln  $NH_4^+$  concentration and time than for the concentration and time. This indicates that  $NH_{\mu}^{+}$  was being used by a growing population, either nitrifiers or microorganisms which assimilated  $NH_{\mu}^{+}$ .

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  $15^{\circ}$ 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



Figure 1. The effect of temperature on  $NH_4^+$  utilization and  $(NO_2^- + NO_3^-)$  production in Windsor soil.



Figure 2. The effect of temperature on  $NH_4^+$  utilization and  $(NO_2^- + NO_3^-)$  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  $NH_{\mu}^{+}$  oxidizers in the Windsor and Charlton soils at 5°, 15°, and 23°C can be found in Table A3. The mean initial number of  $NH_{h}^{+}$ oxidizers was  $7.5 \times 10^5$ /g soil for the Windsor soil and  $6.3 \times 10^5$ /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  $5^{\circ}$ ,  $15^{\circ}$  and  $23^{\circ}$ 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 In  $NH_4^+$  oxidizers did not change significantly with time at 5° and 15°C. However, by day 6 at 23°C the number of In  $NH_4^+$  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  $(NO_2^- + NO_3^-)$  production and growth of the  $NII_4^+$  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  $(NO_2^- + NO_3^-)$  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  $NII_4^+$ concentration. Therefore, growth would not be expected at this relatively low  $NII_4^+$  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  $1.7 \times 10^7$ /g soil and  $2.4 \times 10^7$ /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<sub>2</sub><sup>-</sup> oxidizers to be  $10^6$  to  $10^7$ /g soil.

Analysis of variance of the  $\ln NO_2^-$  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  $\ln NO_2^-$  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  $NO_2^{-1}$ 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  $(NO_2^- + NO_3^-)$  production. During this lag period NO<sub>2</sub><sup>-</sup> 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  $(NO_2^{-} + NO_3^{-})$  production. The decrease in the bacterial number





was largest and of the greatest duration at 5°C where the lag in (NO $_2$ <sup>-</sup> + NO $_3$ <sup>-</sup>) production was most pronounced.

Regression was used to analyze the relationship between the  $\ln (NO_2^- + NO_3^-)$  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  $(NO_2^- + NO_3^-)$  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  $(NO_2^- + NO_3^-)$  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  $(NO_2^- + NO_3^-)$  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  $(NO_2^- + NO_3^-)$  equals the loss of  $NH_4^+$  during the first week for all three soil pHs. Therefore nitrification of the added  $NII_4^+$  was complete within the first 7 days.

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

Incubation time (days)

Initial				
рH	0	7	10	14
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  $(NO_2^- + NO_3^-) - N$ and loss of  $NH_4^+ - N$  in Plano silt loam at different soil pHs.

After the first 7 days there was some loss of  $(NO_2^- + NO_3^-)$  from the soil at pH 7.0. At pH 5.5 there was an increase in the  $(NO_2^- + NO_3^-)$  levels, and at pH 4.5 there may have been a very slight increase in  $(NO_2^- + NO_3^-)$  concentration. Least significant difference calculations revealed that the increase in the ln  $(NO_2^- + NO_3^-)$  concentration from day 7 to day 14 was significant at pH 5.5, and that the decrease in ln  $(NO_2^- + NO_3^-)$  concentration from day 7 to day 14 at pH 7.0 was also significant. However, there was no significant difference between the ln  $(NO_2^- + NO_3^-)$  values on days 7 and 14 at pH 4.5. The loss of  $(NO_2^- + NO_3^-)$  tron 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  $(NO_2^- + NO_3^-)$  after the first week at pH 5.5 indicates that in this soil organic N was mineralized and subsequently nitrified. The continued production of  $(NO_2^- + NO_3^-)$  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  $NH_4^+$  and  $(NO_2^- + NO_3^-)$  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  $(NO_2^- + NO_3^-)$  production.

Linear regression analyses were performed on the  $\ln (NO_2^- + NO_3^-)$  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  $\ln (NO_2^- + NO_3^-)$  concentration with time.

рН	Equation	r <sup>2</sup>	Significance level
4.5	$\ln (NO_2^{-} + NO_3^{-}) = (0.0175)x - 0.2871$	0 <b>.9</b> 805	NS
5.5	$\ln (NO_2^- + NO_3^-) = (0.0232)x - 0.3199$	0.7744	NS
7.0	$\ln (NO_2^{-} + NO_3^{-}) = (0.0215)x - 0.3389$	0.8921	NS

x = Time (days)

NS = Not significant

Maximum rates of  $NH_4^+$  loss and  $(NO_2^- + NO_3^-)$  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  $(NO_2^- + NO_3^-)$  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  $(NO_2^- + NO_3^-)$  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  $(NO_2^- + NO_3^-)$  production at various temperatures. There was a lag period prior to  $(NO_2^- + NO_3^-)$  producto ( $NO_3^-$ ) 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  $NII_4^+$  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<sub>2</sub><sup>-</sup> oxidizers was most pronounced at 5°C where the lag in NO<sub>2</sub><sup>-</sup> production was also longest. After this reduction in number, the NO<sub>2</sub><sup>-</sup> oxidizers grew as nitrification proceeded and there was a statistically significant correlation between nitrification and growth of the NO<sub>2</sub><sup>-</sup> 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  $(NO_2^- + NO_3^-)$  production. The maximum rate of  $(NO_2^- + NO_3^-)$  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  $(NO_2^- + NO_3^-)$  (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.

#### LITERATURE CITED

- Abd-el-Malek, Y., I. Hasny and N.F. Eman (1975) Studies on some environmental factors affecting denitrification in soil. <u>Zentralbl.</u> <u>Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 2</u>, vol. 130, p. 644-653 (Cited in Focht and Verstraete 1977.)
- Alexander, M. and F.E. Clark (1965) Nitrifying bacteria. In <u>Methods of</u> soil analysis. Part 2. Chemical and <u>microbiological properties</u> (C.A. Black, Ed.). Madison, Wisconsin: American Society of Agronomy Inc., p. 1477-1483.
- Anderson, O.E., F.C. Boswell and R.M. Harrison (1971) Variations in low temperature adaptability of nitrifiers in acid soils. <u>Soil Science</u> Society of America Proceedings, vol. 35, p. 68-71.
- Ardakani, M.S., J.T. Rehbock and A.D. McLaren (1974) Oxidation of ammonium to nitrate in a soil column. Soil Science Society of America Proceedings, vol. 37, p. 53-56.
- Beek, J. and M.J. Frissel (1973) <u>Simulation of nitrogen behavior in</u> <u>soils</u>. Wageningen, Netherlands: Centre for Agricultural Publishing and Documentation.
- Belser, L.W. (1979) Population ecology of nitrifying bacteria. <u>Annual Re</u>view of Microbiology, vol. 33, p. 309-333.

- Belser, L.W. and E.L. Schmidt (1978a) Serological diversity within a terrestrial ammonia-oxidizing population. <u>Applied and Environmental</u> Microbiology, vol. 36, p. 589-593.
- Belser, L.W. and E.L. Schmidt (1978b) Diversity in the ammonia-oxidizing nitrifier population of a soil. <u>Applied and Environmental Micro-</u> <u>biology</u>, vol. 36, p. 584-588.
- Bohlool, B.B. and E.L. Schmidt (1973) A fluorescent antibody technique for determination of growth rates of bacteria in soil. <u>Bull, Ecol.</u> <u>Res.</u> Commun., vol. 17, p. 336-338.
- Bremner, J.M. and K. Shaw (1978) Denitrification in soil, II. Factors affecting denitrification. <u>Journal of Agricultural Science</u>, vol. 51, p. 40. (Cited in Focht and Verstraete 1977.)
- Bremner, J.M. and D.R. Keeney (1966) Determination and isotope-ratio analysis of different forms of nitrogen in soils: 3. Exchangable ammonium, nitrate, and nitrite by extraction-distillation methods. <u>Soil</u> Science Society of America Proceedings, vol. 30, p. 577-582.
- Chandra, P. (1962) Note on the effect of shifting temperatures on nitrification in a loam soil. <u>Canadian Journal of Soil Science</u>, vol. 42, p. 314-315. (Cited in Chopp 1979.)
- Chopp, K.M. (1979) <u>Microbial populations and activity in soil irrigated</u> with municipal wastewater effluent. University of Minnesota: Master's Thesis.
- Curtis, E.J.C., K. Durrant, and M.M.I. Harman (1975) Nitrification in rivers in the Trent Basin. Water Research, vol. 9, p. 255-268.
- Dancer, W.S., L.A. Peterson, and G. Chesters (1973) Ammonification and nitrification of N as influenced by soil pH and previous N treatments. <u>Soil Science Society of America Proceedings</u>, vol. 37, p. 67-69.
- Day, P.R., H.E. Doner, and A.D. McLaren (1978) Relationships among microbial populations and rates of nitrification and denitrification in a Hanford soil. In <u>Nitrogen in the Environment. Vol. 2. Oil-</u> <u>Plant-Nitrogen Relationships</u> (D.R. Nielson and J.C. MacDonald, Eds.). New York: Academic Press, p. 305-363.
- Delwiche, C.C. and B.A. Bryan (1976) Denitrification. <u>Annual Review of</u> Microbiology, vol. 30, p. 241-262.
- Focht, D.D. and A.C. Chang (1975) Nitrification and denitrification processes related to wastewater treatment. Advances in Applied Microbiology, vol. 19, p. 153-186.
- Focht, D.D. and W. Verstraete (1977) Biochemical ecology of nitrification and denitrification. In <u>Advances in Microbial Ecology</u>, vol. 1 (M. Alexander, Ed.). New York: Plenum Press, p. 135-214.

- Frederick, L.R. (1956) The formation of nitrate from ammonium nitrogen in soils: I. Effect of temperature. Soil Science Society of America Proceedings, vol. 20, p. 496-500.
- Iskandar, I.K., S. Quarry, R. Bates, and J. Ingersoll (1979) Changes in soil characteristics and climatology during five years of wastewater application to CRREL test cells. U.S. Army Cold Regions Research and Engineering Laboratory Special Report 79-23.
- Iskandar, I.K., R.S. Sletten, D.C. Leggett, and T.F. Jenkins (1976) Wastewater renovation by a prototype slow infiltration land treatment system. CRREL Report 76-19.
- Laudelout, H., R. Lambert, and M.L. Pham (1976) Influence du pH et de la pression partielle d'oxygene sur la nitrification. <u>Ann. Microbiolo.</u> (Institute Pasteur), vol. 127(A), p. 367-382.
- Leggett, D.C. and I.K. Iskandar (1980) Improved enzyme kinetic model for nitrification in soils amended with ammonium. 1. Review of literature. CRREL Report 80-1.
- Leggett, D.C. and I.K. Iskandar (1981) Evaluation of a nitrification model. Chapter 12 in <u>Modeling wastewater renovation: land treatment</u> (I.K. Iskandar, Ed.). New York: Wiley-Interscience.
- Mahendrappa, M.K. and R.L. Smith (1967) Some effects of moisture on denitrification in acid and alkaline soils. <u>Soil Science Society of America Proceedings</u>, vol. 31, p. 212. (Cited in Focht and Verstraete 1977.)
- Mahendrappa, M.K., R.L. Smith, and A.T. Christiansen (1966) Nitrifying organisms affected by climatic region in western United States. <u>Soil</u> Science Society of America Proceedings, vol. 30, p. 60-62.
- Miller, R.D. and D.D. Johnson (1964) The effect of soil moisture tension on carbon dioxide evolution, nitrification, and nitrogen mineralization. <u>Soil Science Society of America Proceedings</u>, vol. 28, p. 644-647.
- Morrill, L.G. and J.E. Dawson (1967) Patterns observed for the oxidation of ammonium to nitrite by soil organisms. <u>Soil Science Society of</u> <u>America Proceedings</u>, vol. 31, p. 757-760.
- Pilot, L. and W.H. Patrick, Jr. (1972) Nitrate reduction in soils: Effect of soil moisture tension. <u>Soil Science</u>, vol. 118, p. 78. (Cited in Focht and Verstraete 1977.)
- Rennie, R.J. and E.L. Schmidt (1977) Immunofluorescence studies of <u>Nitrobacter</u> populations in soils. <u>Canadian Journal of Microbiology</u>, vol. 23, no. 8, p. 1011-1017.
- Rowe, R., R. Todd, and J. Waide (1977) Microtechnique for most-probable number analysis. <u>Applied and Environmental Microbiology</u>, vol. 33, p. 675-680.

- Sabey, B.K. (1969) Influence of soil moisture tension on nitrate accumulation in soils. Soil Science Society of America Proceedings, vol. 33, p. 263-266.
- Sabey, B.R., L.R. Frederick and W.V. Bartholomew (1969) The formation of nitrate from ammonium nitrogen in soils. IV. Use of the delay and maximum rate phases for making quantitative predictions. <u>Soil Science</u> Society of America Proceedings, vol. 33, p. 276-278.
- Schloesing, J.J.T. and A. Muntz (1877) Sur la nitrification par les ferments organisés. <u>Comptes Rendus</u>, vol. 84, p. 301-303. Paris: Academy of Sciences.
- Seifert, J. (1962) Influence of soil structure and moisture content on number of bacteria and degree of nitrification. <u>Folia Microbiologia</u>, vol. 7, p. 234-238.
- Skinner, R.A. and N. Walker (1961) Growth of <u>Nitrosomonas europea</u> in batch and continuous culture. <u>Archiv fuer Mikrobiologie</u>, vol. 38, p. 339-349.
- Steel, R.G.D. and E.H. Torrie (1960) <u>Principles and procedures of</u> <u>statistics with special reference to the biological sciences</u>. New York: McGraw-Hill Book Co.
- Strickland, J.D. and T.R. Parsons (1972) <u>A practical handbook of seawater</u> analysis, Bulletin 167. Ottawa: Fisheries Research Board of Canada.

					Inc	ubation ti	me		
Soil	l and	Before				(days)			
incul	bation	NH <sub>4</sub> C1	0	3	6	9	15	21	30
Let	<u>ap</u> .	additio	n						
Charl	lton								
5°C	A	0.0743	0.1688	0.1661	0.1794	0.2151	U.1572	0,1987	0.2382
<i>.</i>	В	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
	В		0.1781	0.1663	0.1913	0.1864	0.1811	0 <b>.</b> 1759	0.1396
23°C	A		0.1763	0.1636	0.1422	0.1467	0.0597	0.0255	0.0302
	В		0.2044	0.1712	0.1502	0.1511	0.0917	0.0311	0.0328
Winds	sor								
5°C	A	0.0616	0.1696	0.1920	0.1772	0.1925	0.2003	0.2151	0.2695
	В	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
	В		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
	В		0.1786	0.1918	0.1842	0.1802	0.1414	0.0916	0.0265

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

# APPENDIX A: DATA COLLECTED FOR THE TEMPERATURE EXPERIMENT

Soil and Before				Incubation time (days)						
incul tem	np.	on NH <sub>4</sub> Cl addition	0 n	3	6	9	15	21	30	
Charl	lton									
5°C	A B	0.0381 0.0393	0.0497 0.0422	0.0385 0.0398	0.0463 0.0498	0.0617 0.0512	0.0565 0.0600	0.0625 0.0729	0.0754 0.0761	
15°C	A B		0.0542 0.0462*	0.0508 0.0497	0.0561 0.0607	0.0668 0.0802	0.0497 0.0322	0.0851 0.0738	0.0965 0.1244	
23°C	A B		0.0525 0.0508	0.0523 0.0697	0.0566 0.0581	0.0687 0.1003	0.0660 0.0970	0.0981 0.1156	0.1455 0.1930	
Winds	sor									
5°C	A B	0.0329 0.0393	0.0607 0.0495	0.0390 0.0429	0.0537 0.0379	0.0488 0.0568	0.0476 0.0560	0.0483 0.0677	0.1031 0.1018	
15°C	A B		0.0622 0.0428	0.0459* 0.0566	0.0578 0.0588	0.0799 0.0932	0.0661 0.0794	0.0810 0.0945	0.1324 0.1449	
23°C	A B		0.0746 0.0614	0.0498 0.0591	0.0510 0.0610	0.0787 0.0948	0.0825 0.0947	0.1202 0.1639	0.1815 0.1960	

Table A2. The  $(NO_2^{-} + NO_3^{-}) - N \text{ meq}/100 \text{ g soil for short term nitrification}$  experiment testing the effect of temperature.

Ļ

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

22

.^

			MPN NH +	MPN NO
	Soil	Temp.	oxidizers/g soil	oxidizers/g soil
Day		(°C)	U	
0	Charlton	5	4.6x10 <sup>5</sup>	2.8x10 <sup>7</sup>
		15	7.1x10 <sup>5</sup>	$2.9 \times 10^7$
		23	7.1x10 <sup>5</sup>	$1.6 x 10^{7}$
	Windsor	5	$6.6 \times 10^{5}$	$2.6 \times 10^{7}$
		15	2 <b>.9x</b> 10 <sup>5</sup>	$2.2 \times 10^{7}$
		23	$1.3 \times 10^{6}$	$4.2 \times 10^{6}$
3	Charlton	5	$2.8 \times 10^{5}$	$2.9 \times 10^{7}$
		15	$7.1 \times 10^{5}$	$1.4 x 10^{7}$
		23	$4.6 \times 10^{5}$	$2.8 \times 10^{6}$
	Windsor	5	$2.9 \times 10^{5}$	$1.9 \times 10^{7}$
		15	$1.6 \times 10^5$	$4.3 \times 10^{7}$
		23	$4.3 \times 10^{5}$	$3.4 \times 10^{7}$
6	Charlton	5	1.8x10 <sup>5</sup>	$1.4x10^{7}$
		15	$7.1 \times 10^{5}$	$2.4 \times 10^{7}$
		23	$4.6 \times 10^{5}$	$6.2 \times 10^{6}$
	Windsor	5	$4.2 \times 10^{5}$	$4.7 \times 10^{7}$
		15	1.1x10 <sup>6</sup>	
		23	$8.5 \times 10^{4}$	$3.8 \times 10^{7}$
8	Charlton	5	$3.2 \times 10^5$	$1.2 \times 10^{6}$
		15	$1.2 \times 10^{6}$	$2.2 \times 10^{6}$
		23	$2.2 \times 10^{5}$	$1.2 \times 10^{7}$
	Windsor	5	$2.9 \times 10^{5}$	$2.7 \times 10^{6}$
		15	1.1x10 <sup>6</sup>	$2.9 \times 10^{6}$
		23	$4.0 \times 10^{4}$	$2.7 \times 10^{6}$
15	Charlton	5	4.6x10 <sup>5</sup>	$2.4 \times 10^{6}$
		15	$3.1 \times 10^{5}$	$2.9 \times 10^{7}$
		23	$1.0 \times 10^{5}$	$6.4 \times 10^{5}$
	Windsor	5	$6.6 \times 10^5$	$1.3 \times 10^{6}$
		15	1.1x10 <sup>6</sup>	$2.1 \times 10^{7}$
		23	$6.0 \times 10^{4}$	$2.2 \times 10^{7}$
21	Charlton	5	$3.2 \times 10^5$	$6.5 \times 10^{6}$
		15	$1.7 \times 10^{5}$	$4.6 \times 10^{7}$
		23	$1.7 \times 10^{4}$	$3.1 \times 10^{7}$
	Windsor	5	$2.9 \times 10^{5}$	$2.8 \times 10^{5}$
		15	$4.2 \times 10^{5}$	$1.7 \times 10^{7}$
		23	$2.9 \times 10^{4}$	$4.2 \times 10^{6}$
30	Charlton	5	2.2x10 <sup>5</sup>	$1.4 \times 10^{7}$
		15	$3.6 \times 10^5$	$2.9 \times 10^{7}$
		23	1.0x10 <sup>5</sup>	$1.0 \times 10^{7}$
	Windsor	5	$2.7 \times 10^{5}$	$2.9 \times 10^{7}$
		15	$4.2 \times 10^5$	9.6x10 <sup>6</sup>
		23	$5.9 \times 10^{4}$	9.5x10 <sup>6</sup>

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

23/24

### APPENDIX B: STATISTICAL ANALYSIS OF DATA COLLECTED

Term	DF	Mean square	F	Num./den.	Level of significance
Т	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	3.75524x10 <sup>-2</sup>	1.77	TS/WCE	NS
TĐ	12	6.76592x10 <sup>-2</sup>	3.19	TD/WCE	HS 0.005
SD	6	2.98343x10 <sup>-2</sup>	1.38	SD/WCE	NS
TSD	12	3.15924x10 <sup>-2</sup>	1.42	TSD/WCE	NS
WCE	42	2.11963x10 <sup>-2</sup>			
T = S =	Temperature Soil type	(°C) WCE NS	= Within = Not si	-cell error gnificant	

Table B1. Analysis of variance of  $\ln (NO_2^++NO_3^-)$  concentration for the short-term nitrification experiment testing the effect of temperature.

Т	=	Tempe	erature	( <sup>0</sup> C)	WCE	=	Within-	-cell error
S	=	Soil	tvpe		NS	=	Not sig	gnificant
D	=	Time	(days)		нs	=	Highly	significant

Table B2. Analysis of variance of ln  $NH_4^+$  concentration values for the short-term nitrification experiment testing the effect of temperature.

Term	DF	Mean square	F	Num./den.	Level of significance
т	2	3.07924	3.94	T/TD	NS
S	1	0.237304	2.81	S/TSD	NS
D	6	0.544662	<1	D/TU	NS
TS	2	0.168097	1.99	TS/TSD	NS
TU	12	0.780581	9.26	TD/TSD	HS 0.001
SD	6	9.89457/x10	2 1.17	SD/TSD	NS
TSD	12	8.43071x10 <sup>-2</sup>	12.74	TSD/WCE	HS 0.001
WCE	42	6.6198x10 <sup>-3</sup>			
T = T $S = S$	emperation	ure ( <sup>O</sup> C) WC e N	CE = With NS = Not	nin-cell erro significant	or

_						0 · · · · = - · · · · · ·
D	Ŧ	Time	(days)	HS =	Highly	significant

Term	DF	<u>Mean square</u>	F	Num./den.	Level of	significance
Т	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	<u></u>			

NS = Not significant

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.

ļ

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
Т	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			

 $T = Temperature (^{O}C)$ 

 $T = Temperature (^{O}C)$ 

S = Soil type

D = Time (days)

NS = Not significant

S = Soil type

S = Significant

D = Time (days)

Term	DF	Mean square	F	Num./den.	Level of significance
		ln NH <sub>4</sub> +	concentr	ation	
Р	2	2.5017	14.59	P/PD	HS 0.001
D	4	4.73449	27.61	D/PD	HS C.U.L
PD	8	0.171497	2.70	PD/WCE	S. U. OLS
WCE	30	0.06356			
		ln (NO <sub>2</sub> -	- + NO3-)	concentrati	on
Р	2	$1.91657 \times 10^{-2}$	5.27	P/PD	<b>5.</b> 0.050
D	4	4.014914x10 <sup>-2</sup>	11.11	D/PD	HS 0.001
PD	8	$3.63872 \times 10^{-3}$	4.26	PD/WCE	HS 0.005
WCE	30	8.53266x10 <sup>-4</sup>			

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

P = pH

D = time (days)

WCE = Within-cell error

S = Significant

HS = Highly significant

 $27^{-1} = |\mathfrak{V}_{18}|$  gover ment printing of ECE ( ) by 2.500 ( ) or 2.5

