Effect of Temperature on Consecutive Denitrification Reactions in Brookston Clay and Fox Sandy Loam

D. J. MCKENNEY, G. P. JOHNSON, AND W. I. FINDLAY

Department of Chemistry, University of Windsor, Windsor, Ontario N9B 3P4, and Agriculture Canada, Research Station, Harrow, Ontario N9R 1G0, Canada

Received 4 October 1983/Accepted 2 February 1984

The kinetics of several steps in the microbial denitrification process in Brookston clay and Fox sandy loam, two soils common to Southwestern Ontario, were studied in the temperature range of 5 to 25°C. The extent of chemical denitrification was also determined in otherwise identical but sterilized soils at temperatures up to 80°C. A gas flow system was used in which soil gases were continuously removed from anaerobic soil columns by argon carrier gas. Net steady-state rates of NO and N₂O production, rates of loss of NO₂⁻ and production and loss of NO₃⁻ were measured over periods of up to 5 days. Arrhenius activation energies for the zero-order process NO₃⁻ → NO₂⁻ were calculated to be 50 ± 9 kJ mol⁻¹ for Brookston clay and 55 ± 13 kJ mol⁻¹ for Fox sandy loam. The overall reaction, NO₂⁻ → NO (chemodenitrification), in both sterile soils was accurately first order with respect to NO₂⁻; the activation energy was 70 ± 2.8 kJ mol⁻¹ in Brookston clay and 79 ± 1.2 kJ mol⁻¹ in the sandy loam, and the preexponential factors were (2.3 ± 1.2) x 10⁸ and (5.7 ± 1.2) x 10⁶ min⁻¹, respectively.

In recent years a number of publications have reported on the effect of temperature on denitrification in soils (1, 2, 5, 6, 9–11, 13, 20, 21). Generally most studies have focused on the effect of temperature on either the overall loss of NO₃⁻ or the accumulation of nitrogenous gases over static soil systems. Several of these emphasized the effects of temperature on the relative proportions of nitrogenous gases produced (1, 2). Earlier studies by Nomnik (16) and by Brenner and Shaw (4) suggested that the optimum temperature for biological denitrification was in the range of 60 to 65°C. The more recent results of Keeney et al. (11) indicate that this optimum temperature is too high for biological denitrification and that chemical reactions may dominate at temperatures above 50°C. They suggest that a combination of biological and chemical processes at these relatively high temperatures results in an apparent optimum rate at 60 to 67°C.

In our flow system (14), most of the evolved gases are quickly purged out of the soil when argon is used as the carrier gas, thus reducing the rates of their subsequent reactions. This permits us to more easily measure rates of some reactive intermediates.

In this paper we report data on the effect of temperature on several steps in the denitrification process for two soils, Brookston clay (BC) and Fox sandy loam (FSL). Arrhenius parameters for the rate of decrease of NO₃⁻ concentration were measured. Kinetic rate constants for chemical removal of NO₂⁻ and formation of NO were also obtained. Kinetic parameters such as these are required before realistic models for nitrogen transformation in soils can be developed.

(Some of this work was presented at the 74th Annual Meeting of the American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Anaheim, Calif., 28 November to 3 December 1982.)

MATERIALS AND METHODS

The soils from which samples were taken for use in the investigation are situated in southwestern Ontario and are typical of those developed on water-sorted parent materials of glacial origin (Table 1). The methods and materials used were generally as described previously (14). Briefly, the apparatus consisted of a gas flow system whereby a constant flow of water-saturated argon stripped gases evolved from anaerobic soil columns. These columns were Pyrex tubes, 2.5 cm (inside diameter) by 40 cm long.

The gases were analyzed for NO and N₂O. Nitric oxide was analyzed with an NO/NO₂/N₂O analyzer (Columbia Scientific Industries, model 1600). The N₂O was determined by gas chromatography (Perkin-Elmer model 3920B, with a 63Ni electron capture detector). Five soil columns were used in parallel, each individually thermostatted to selectively span a temperature range of approximately 5 to 25°C. The soil in each column was taken from the same field-moist sample which had been passed through a 5-mm sieve and thoroughly mixed but otherwise untreated. Three series of runs were carried out on each soil under as close to identical conditions as possible. In the first series, each of the five columns was monitored for NO and N₂O production as a function of time from the onset of anaerobiosis, defined as the time when argon flow was started. In the second series, analyses of NO₃⁻ and NO₂⁻ were obtained as a function of time. Occasional water and pH measurements were also made. In these runs NO was also measured as a means of assessing the reproducibility of the first series. At selected time intervals approximately 15-g soil samples were withdrawn, using a stainless-steel spatula from each column without interruption of the argon carrier gas flow, thus ensuring that the soil remained anaerobic. NO₃⁻ and NO₂⁻ were extracted immediately, using 100 cm³ of distilled water, stirring vigorously for about 55 min, and vacuum filtering through a no. 3 Whatman filter followed by vacuum filtration through a 0.22-µm membrane filter (type GS, Millipore...
TABLE 1. Selected chemical and physical properties of the soils used

<table>
<thead>
<tr>
<th>Property</th>
<th>% Dry wt</th>
<th>BC*</th>
<th>FSLb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clay</td>
<td></td>
<td>40</td>
<td>12</td>
</tr>
<tr>
<td>Silt</td>
<td></td>
<td>30</td>
<td>9</td>
</tr>
<tr>
<td>Sand</td>
<td></td>
<td>26</td>
<td>78</td>
</tr>
<tr>
<td>Organic matter</td>
<td></td>
<td>4.1</td>
<td>1.0</td>
</tr>
<tr>
<td>Organic carbon</td>
<td></td>
<td>2.4</td>
<td>0.58</td>
</tr>
<tr>
<td>Field capacity (1/3 bar)</td>
<td></td>
<td>21.0</td>
<td>13.5</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td>6.9</td>
<td>6.8</td>
</tr>
</tbody>
</table>

* BC designation: orthic humic gleysoil (U.S. equivalent, haplaquoll).

b FSL designation: gray-brown luvisol (U.S. equivalent, typic hapludalfic).

Corps) to remove soil bacteria. The filtrates were then stored in the dark at 4°C for later analysis. Storage of filtrates under those conditions had no effect on the subsequent analyses (14). In the third series of runs, sterile but otherwise identical soil was studied to measure the chemical rate of NO3- disappearance. Sterilization of sieved soil was obtained by autoclaving at 123°C and 18-lb/in² pressure for 35 min. After sterilization, enough freshly prepared NaNO2 solution, \(9 \times 10^{-3} \text{ mol of N dm}^{-3}\), was sprayed quickly and as uniformly as possible on the soil to bring the concentration up to \(1.6 \times 10^{-7} \text{ mol of N g of soil}^{-1}\). The soil was thoroughly mixed and divided into subsamples, which were then packed into the columns and subsequently made anaerobic with argon. Nitric oxide was then monitored for periods of up to 31 h with occasional checks for N2O production. Nitrate loss was also obtained by analysis for NO3- in aqueous extracts of soil taken at the beginning and end of the run. In one case, using sterile FSL at 80°C, NO3- was also determined at a number of time intervals to compare the kinetics of NO3- loss to NO production.

Methods of analysis, estimates of random errors, and other details are generally as described previously (14); however, in the current investigation the method developed by Cox (7) was chiefly used for NO3- analysis. Analyses of NO3- concentrations in the 27-ppm (27-μg g⁻¹) range were crosschecked, using the specific ion electrode (Microprocessor analyzer, model 901; Orion Research Inc.) with satisfactory results. At low concentrations (<1 ppm) Cox's method was preferable and convenient in that it was very similar to his method that we used for NO3- determination. Mildly acidic I⁻ solution reduces NO2- to NO, which is then measured with the NO/NO2/NO3 analyzer. Iron(II) with strongly acidic (1:1 H2SO4) molybdate ion is the reducing agent for NO3- (7). Calibrations for the NO3- were made with NaNO2 solutions prepared daily from 100 ppm of stock preserved with boric acid (Orion instruction manual; nitrate ion electrode, model 92-07).

To obtain best estimates of first-order rate constants of or Arrhenius parameters involving soil N transformation, a weighted least-squares analysis of the experimental data was used. The statistical procedures, estimates of weighting factors, and confidence limits were obtained following Cvetanović et al. (8) and Mortimer (15).

RESULTS AND DISCUSSION

The results and conclusions are discussed for convenience in terms of the simplified scheme suggested by Payne (17):

\[
\text{NO}_3^- \xrightarrow{\text{f_{NO}} \cdot [\text{NO}]/m} \text{NO}_2 \xrightarrow{\text{f_{NO2}} \cdot [\text{N}_2\text{O}]/m} \text{N}_2
\]

Reactions 1 to 4 are known to occur through biological enzymatic processes, whereas step 5 is added to account for chemical reduction of NO3-. The kinetic differential equations relating to the N species in each step in the overall mechanism are given by McKenney et al. (14).

The rates of formation of NO and of N2O are balanced by the rate of removal by carrier, \(f_{\text{NO}}\) and \(f_{\text{N}_2\text{O}}\), respectively, and by further reaction within the soil column. These net rates are calculated as \(f_{\text{NO}} = f_c \cdot [\text{NO}]/m\) and \(f_{\text{N}_2\text{O}} = f_c \cdot [\text{N}_2\text{O}]/m\), where \(f_c\) is the measured total flow rate of effluent gas from the column, \(m\) is the mass of soil in the column, and [NO] and [N2O] are concentrations (parts per million, by volume) in the effluent gas stream. At Ar flow rates above 100 cm³ min⁻¹, the proportion of NO or N2O removed as compared with the quantity remaining which undergoes further reaction is independent of the flow rate (14). Thus, a steady state is obtained where the N transformation rates remain approximately constant for periods usually considerably longer than that required for NO or N2O analyses or for the time required to withdraw soil samples and filter the subsequent aqueous extracts for NO3- and N2O determinations. These reaction rates, however, change slowly over periods of several hours, reflecting changes in substrate, carbon, enzyme, etc., within the soil (13, 14).

Nitrate loss. (i) BC. Loss of soil NO3- as a function of time at five different soil temperatures is shown in Fig. 1 plotted as [NO3-] versus time. The linearity of the plots suggests that NO3- removal is reasonably described by zero-order kinetics (\(r^2 = 0.961\) to 0.988), but it is emphasized that the extent of reaction in each case is insufficient to accurately distinguish between zero and first-order removal. First-order plots, \(ln [\text{NO}_3^-]\) versus time (not shown), are also linear with similar correlation coefficients, ranging from \(r^2 = 0.971\) to 0.991. Our choice of zero-order removal is based on the following considerations. Phillips et al. (18) and Reddy et al. (19) showed that for many soils zero-order kinetics applies unless the supply of NO3- to the soil is limited by diffusion from overlying flood water or the reaction is limited by the supply of available carbon. Also, Michaelis-Menten kinetics may apply to this system, in which case zero-order removal

![FIG. 1. Soil nitrate concentrations ([NO3-]) in BC (pH 6.9) as a function of time at temperatures of 4.9 to 25.0°C.](http://aem.asm.org/)

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would be observed provided that the Michaelis constant, \( K_m \), for nitrate removal is much less than \( [\text{NO}_3^-] \). Betlach and Tiedje (3) obtained a \( K_m \) of \(<15 \mu\text{M} \) for cell suspensions of three different denitrifiers and \( \text{NO}_3^- \) concentrations in this study were several orders of magnitude greater than the \( K_m \).

Rate constants, \( k_1 \), obtained from the slopes of the curves shown in Fig. 1 are plotted in Fig. 2 according to the Arrhenius equation, \( \ln k_1 = \ln A - E/RT \), where \( A \) is the preexponential factor, \( E \) is the activation energy, \( R \) is the gas constant, and \( T \) is the temperature (\( K \)). Good linearity is shown and the results can be expressed as \( k_1 = 0.18 \exp(-50 \text{kJ mol}^{-1}/RT) \text{mol g}^{-1} \text{min}^{-1} \) for BC.

(ii) FSL. The carbon content in FSL was considerably less than that in BC (Table 1), as was \( \text{NO}_3^- \), \( [\text{NO}_3^-] = 3 \times 10^{-7} \text{mol g}^{-1} (\sim 4 \text{ppm of N}) \). Although more scatter was observed in the \( \text{NO}_3^- \) analyses, the data (not shown) appeared to follow zero-order kinetics over most of the time period, with correlation coefficients \( (r^2) \) ranging between 0.923 and 1.00. First-order plots were also linear: \( r^2 = 0.945 \) to 0.999. More significantly, however, \( \text{NO}_3^- \) concentrations at all temperatures were depleted to no less than 0.5 \times 10^{-7} \text{mol g}^{-1} \text{min}^{-1} \) (about 0.7 ppm of N), at which time the removal of \( \text{NO}_3^- \) stopped. Rate constants determined from slopes of \( [\text{NO}_3^-] \) versus time were used to obtain Arrhenius parameters for the FSL, \( k_1 = 3.4 \exp(-55 \text{kJ mol}^{-1}/RT) \text{mol g}^{-1} \text{min}^{-1} \), which are similar to those obtained with BC soil.

Nitrite production and loss. (i) BC. Figure 3 shows the variation of soil \( \text{NO}_2^- \) concentration as a function of time at five different temperatures. At each temperature \( [\text{NO}_2^-] \) increases to a maximum, with the most rapid increase corresponding to the highest temperature. The maximum concentration obtained also increases slightly with increasing temperature (i.e., \( 1.03 \times 10^{-7} \text{mol g of soil}^{-1} \) at 4.9°C to \( 1.38 \times 10^{-7} \text{mol g of soil}^{-1} \) at 25.0°C).

Approximate values for the initial rate of increase of soil

![FIG. 2. Arrhenius plot of \( k_1 \) in BC (pH 6.9) as determined from the zero-order kinetics of \( [\text{NO}_2^-] \) loss (Fig. 1) and estimates based on initial rates of \( [\text{NO}_2^-] \) increase.](image)


![FIG. 3. Soil nitrite concentration ([\( \text{NO}_2^- \)]) in BC (pH 6.9) as a function of time at temperatures of 4.9 to 25.0°C.](image)

\([\text{NO}_2^-]\) were obtained from the initial slopes of the curves, assuming linearity from time zero to the first point shown on each curve in Fig. 3. These rates, obtained before \( \text{NO}_2^- \) accumulated significantly, were expected to be approximately equal to the rate of loss of \( \text{NO}_3^- \) at each temperature. The rate constants (slopes) are also plotted on Fig. 2 for comparison with the data obtained directly from the soil \( [\text{NO}_3^-] \) removal plots. Excellent agreement is shown at the lower temperature, but as the temperature increases an increasing discrepancy appears. The rate constants estimated from \( \text{NO}_2^- \) production are greater at the higher temperatures than values obtained from the soil \( [\text{NO}_3^-] \) loss curves. Although the reason for this is not obvious, the shapes of the curves in Fig. 3 show that the net rate of change of \( \text{NO}_2^- \) concentration is strongly time and temperature dependent. The time dependency points to changing substrate, carbon, or enzyme levels and precludes extraction from this data of a meaningful rate constant for biological \( \text{NO}_2^- \) removal. It is possible, however, to measure the extent of chemical denitrification (chemodenitrification), \( k_s \), and thereby deduce the relative importance of the chemical versus biological \( \text{NO}_2^- \)-to-NO process in these soils.

In sterile BC soil \( \text{NO}_2^- \) is reduced, forming NO. A number of analyses were made to determine whether \( \text{N}_2\text{O} \) was also produced in sterile soil. None was detected although the minimum detectable level of \( \text{N}_2\text{O} \) in gas samples analyzed by the gas chromatograph utilized under our operating conditions was 1.0 ppb (vol/vol). Starting with \( \text{NO}_2^- \), for the chemical reaction \( \text{NO}_2^- \rightarrow \text{NO} \), where \( a \) is an unknown stoichiometric coefficient, if first-order behavior is assumed, \( \frac{1}{a} f_{\text{NO}} = \frac{1}{a} \frac{d[\text{NO}]}{dt} = \frac{d[\text{NO}_2^-]}{dt} = k_d[\text{NO}_2^-] \)

Substituting for \([\text{NO}_2^-]\),

\( f_{\text{NO}} = ak_s[\text{NO}_2^-]_0 e^{-k_st} \)

where \([\text{NO}_2^-]_0 \) is the initial concentration at any time and \([\text{NO}_2^-]_0 \) is the initial concentration. Therefore, \( \ln f_{\text{NO}} = \ln(ak_s[\text{NO}_2^-]_0) - k_st \) or \( \ln(\text{fNO}) = \ln(\text{fNO}_0) - k_st \).

Figure 4 shows a plot of \( \ln f_{\text{NO}} \) versus time at 35°C. The excellent linearity clearly indicates first-order behavior over 97% of the \( \text{NO}_2^- \) conversion in the case shown. Rate constants, \( k_s \), were calculated from the slopes of similar plots at temperatures between 25 and 60°C (data not shown). The slight deviation of the points from the line apparent at
longer times in Fig. 4 results from the least-squares weighting procedure (8, 15). Since the actual observations are \( f_{NO} \) and not \( \ln(f_{NO}) \), the weighting more correctly minimizes the sums of squares of the residuals of \( f_{NO} \), not \( \ln(f_{NO}) \), with each \( f_{NO} \) weighted equally. If an unweighted least-squares calculation was made, the points representing smaller \( f_{NO} \) values would have been incorrectly weighted more heavily as a result of the change in variable to logarithm form (8).

Approximate values of rate constants as determined by soil \([NO_2^-]\) loss, \( k'_5 \), were also obtained from soil \([NO_2^-]\) determinations at each temperature, where the concentrations were determined at the beginning and end of each run. All \( k_5 \) and \( k'_5 \) values are shown in Table 2. Reasonably close agreement is obtained in the rate constants determined from either \( f_{NO} \) or \([NO_2^-]\) data. The constants evaluated from Arrhenius plots (not shown) can be expressed as \( k_5 = 2.3 \times 10^9 \exp(-70.0 \text{kJ mol}^{-1}/RT) \text{min}^{-1} \), based on \( f_{NO} \), or \( k'_5 \approx 3.2 \times 10^9 \exp(-65 \text{kJ mol}^{-1}/RT) \text{min}^{-1} \) based on \([NO_2^-]\) for BC.

The \( k'_5 \) values by nitrite determination are based upon only two measurements, initial and final, and consequently are less precise and perhaps less accurate. Whereas the temperature dependence is quite similar to that of \( k_5 \) from NO output, the individual \( k'_5 \) values are uniformly lower over the temperature range studied, indicating that more NO is produced than \( NO_2^- \) lost at a given temperature. Typically, at 51°C for 98% \( NO_2^- \) loss, about 30% of the NO produced is in excess of the \( NO_2^- \) reacted for a ratio \( k'_5/k_5 = 0.082 \). A similar observation at 60°C in a Pano silt loam soil was reported by Keeney et al. (11), who discussed a possible reason in terms of reactions of \( NO_2^- \) with existing nitroso or oximino compounds in the soil. Further work is required to investigate this possibility.

A van Slyke-type reaction involving oxidation of organic N by \( NO_2^- \) to yield \( N_2 \) was recently studied over a temperature range of +20 to -20°C by Christianson and Cho (6). They found that \( N_2 \) was not produced by this reaction at \( NO_2^- \) levels below a threshold concentration of 40 \( \mu \text{g} \) of N g\(^{-1}\), which is well above the levels of 2 to 3 \( \mu \text{g} \) of N g\(^{-1}\) used in this work. Using \( NO_2^- \) concentrations of 100 and 200 \( \mu \text{g} \) of N g\(^{-1}\), they determined activation energies for van Slyke-type \( N_2 \) production of 45.6 and 52.7 kJ mol\(^{-1}\) for unfrozen and frozen soils, respectively.

(ii) FSL. The overall pattern of production and loss of \( NO_2^- \) in FSL (Fig. 5) was similar in many respects to that observed in BC (Fig. 3), but the following differences were apparent. Apparent maximum values of \([NO_2^-]\) were reached in much shorter time periods in the sandy loam. For example, at 25°C \([NO_2^-]_{\text{max}} \) appears at \( ~900 \text{ min} \) in BC and at \( ~300 \text{ min} \) in FSL. The decrease of \([NO_2^-]\) after the maxima was much steeper. In BC \([NO_2^-]_{\text{max}} \) decreases with decreasing temperature, whereas the opposite was observed in the sandy loam, although in both cases the differences in \([NO_2^-]_{\text{max}} \) from one temperature to another were not large.

Approximate values of \( k_5 \), which we designate as \( k'_5 \), for FSL calculated from the initial slopes of the \([NO_2^-] \)-versus-time plots yield Arrhenius parameters which compare quite closely with those obtained directly from \( NO_2^- \) loss: \( k'_5 \approx 0.077 \exp(-47 \text{kJ mol}^{-1}/RT) \text{mol g}^{-1} \text{min}^{-1} \) for FSL.

Values for the constants \( k_5 \) for chemical removal of \( NO_2^- \) were obtained in the same manner as was done for BC.Nitric oxide production from sterile soil was measured over

![FIG. 4. Net rate of NO production as a function of time, \( \ln(f_{NO})_t = \ln(f_{NO})_0 - k_5 t \), in sterile BC at pH 6.9 and 35.0°C. Similar results were obtained at 25, 43, 51, and 60°C.](http://aem.asm.org/)

<table>
<thead>
<tr>
<th>Table 2. Rate constants for chemical disappearance of ( NO_2^- ) and formation of NO in sterile soil</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Soil</strong></td>
</tr>
<tr>
<td>BC (pH 6.9)</td>
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<tr>
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<tr>
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<tr>
<td>FSL (pH 6.8)</td>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>

* In this case, \( k'_5 \) was obtained from a weighted least-squares first-order plot, using six approximately equally spaced data points.
the temperature range 25 to 80°C, and excellent first-order plots, comparable to Fig. 4, were obtained (data not shown). Nitrite concentrations again were measured before and after each run. In the case of the 80°C investigation, loss was monitored as a function of time and excellent agreement with assumed first-order kinetics were observed. The rate constants are tabulated in Table 2. The corresponding Arrhenius equations for these data in FSL can be expressed as: 

\[ k_5 = 5.7 \times 10^8 \exp(-78.5 \text{kJ mol}^{-1}/RT) \text{ min}^{-1} \]

based on \( f_{\text{NO}} \), or \( k_5' = 7.8 \times 10^{10} \exp(-87 \text{kJ mol}^{-1}/RT) \text{ min}^{-1} \) based on \( \text{NO}_2^- \) disappearance. Apparently in both the FSL and BC soils, more NO is formed than can be accounted for by \( \text{NO}_2^- \) loss over the temperature range studied. From Table 2, at 80°C, \( k_5'/k_5 = 0.80 \) and about 30% excess NO is formed for 98% conversion.

**Nitric oxide production and loss.** (i) BC. Net rates of NO production, \( f_{\text{NO}} \), show (Fig. 6) a characteristic increase and subsequent decrease with increasing time in parallel with the changing \( \text{NO}_2^- \) concentration shown in Fig. 3. The rate of change in \( f_{\text{NO}} \) is most pronounced at high temperature, whereas at 5°C \( f_{\text{NO}} \) changed very little over a period of up to 8,000 min (Fig. 6).

At the two lowest temperatures, the relative constancy in the measured net NO production rates indicates very slow changes in substrate carbon or enzyme levels, whereas at the higher temperatures the changes are more rapid. Thus, determination of an accurate activation energy is precluded.

(ii) FSL. Curves showing net production rates of NO versus time at five different temperatures are shown in Fig. 7. At all temperatures a very rapid decrease in \( f_{\text{NO}} \) occurs after initial increases. It appears from the shape of the curves that potentially maximal \( f_{\text{NO}} \) values were not yet reached before the rapidly decreasing phase. In all cases, the time when \( f_{\text{NO}} \) began to decrease was later than that for the corresponding phase of rapidly decreasing \( \text{NO}_2^- \) concentrations (Fig. 5), indicating that NO production from residual \( \text{NO}_2^- \) continued during this period. In contrast, in BC, where \( \text{NO}_3^- \) disappearance persists (Fig. 1), net NO production begins to decrease (Fig. 6) while \( [\text{NO}_2^-] \) continues to increase (Fig. 3).

Table 3 shows approximate time to maxima for \( [\text{NO}_2^-] \), \( f_{\text{NO}} \), and \( f_{\text{NO}} \). Columns under series 2 show that \( f_{\text{NO}} \) begins to decrease from its maximum value about 300 min later than

**Nitrous oxide production and loss.** (i) BC. The activation energy for net \( \text{N}_2\text{O} \) production was reported in an earlier publication (13). The pattern of \( f_{\text{N}_2\text{O}} \) behavior as a function of time was very similar to that observed for \( f_{\text{NO}} \) (shown in Fig. 6), although the decreases in net rates after maxima were
TABLE 3. Approximate time to maxima for [NO$3^-$], $f_{NO}$, and $f_{NO}$ for FSL$^a$

<table>
<thead>
<tr>
<th>Temp (°C)</th>
<th>Series 1</th>
<th>Series 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$t_{NO}$ (min)</td>
<td>$t_{NO}$ (min)</td>
</tr>
<tr>
<td>5.8</td>
<td>2,000</td>
<td>2,300</td>
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<tr>
<td>10.0</td>
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<td>15.6</td>
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<tr>
<td>20.2</td>
<td>600</td>
<td>670</td>
</tr>
<tr>
<td>25.3</td>
<td>420</td>
<td>500</td>
</tr>
</tbody>
</table>

$^a$ Series 2 was carried out 26 days after series 1.

much more gradual. For example, at $20^\circ$C, $f_{NO}$ decreases by only about 7% over a period ~40 h. This long-term constancy in rates, reflecting relatively constant substrate, carbon, etc., therefore permitted estimation of an activation energy for $f_{NO}$. The activation energy reported was 79.9 kJ mol$^{-1}$ (13). By using the weighted least-squares procedure the value becomes 84 kJ mol$^{-1}$, and the Arrhenius expression is $f_{NO}^{max} = 4.58 \times 10^4 \exp(-84 \text{kJ mol}^{-1}/RT)$ mol of N g$^{-1}$ min$^{-1}$ for BC.

(ii) FSL. Figure 8 shows net N$_2$O production data as functions of time at the various temperatures. Similarity to the $f_{NO}$ curves is evident (Fig. 7). Decreases occur rapidly, but are significantly delayed for various periods after NO decreases. Compare columns under series 1 in Table 3. It is clear that N$_2$O production continues provided residual NO remains in the soil.

With the sandy loam sample collected later in the summer (data not shown), decreases from observed maxima occurred at later times and at values presumably closer to potential maxima.

It is interesting to point out that the relative accumulated amounts of N$_2$O to NO produced are very significantly dependent on temperature. Figure 9 shows the ratio of total output of N$_2$O to NO versus temperature for FSL. Apparently the ratio increases directly with temperature. The increase is likely explained by a difference in activation energies for the processes governing production and loss of the two substances.

All Arrhenius parameters are reported in Table 4 along with calculated Arrhenius expressions for anaerobic soil systems at $20^\circ$C. Consideration of the values for $k_1$ based on NO$_3^-$ loss shows that very similar activation energies apply for both soils studied in this work. The average value for the two, $E_1 = 53$ kJ mol$^{-1}$, corresponds to $Q_{10} = 2.1$ at 15 to $25^\circ$C.

Chemical denitrification in both soils appears to have relatively large and approximately similar activation energies, 79 kJ mol$^{-1}$ for the sandy loam and 70 kJ mol$^{-1}$ for the clay. Using the Arrhenius expressions for $k_5$ and the value of the stoichiometric coefficient, $a$, for reaction 5 obtained from the intercept of Fig. 4, the rate of NO formed chemically when NO$_3^-$ was at its maximum concentration (Fig. 3 and 5) is estimated to be ~1% of the total rate, $f_{NO}$, formed in sandy loam (see Fig. 7) and ~46% of the total rate in BC (Fig. 6). The latter estimate is in good agreement with our earlier estimate (14).

Without having considerably more detailed information concerning specific mechanisms, attaching any theoretical significance to the preexponential factors listed in Table 4 undoubtedly would not be warranted for the biological processes and may be premature for the chemical loss of NO$_3^-$ as well. It is interesting, however, to point to the similarities between $A$ values for NO$_3^-$ loss and for chemical NO$_2^-$ loss in the two soils. The preexponential factors for chemical removal of NO$_2^-$ are quite similar in the two soils and lower by a factor of about $10^7$ to $10^6$ than values typical of unimolecular processes occurring in aqueous solution. Therefore, substantial negative entropies of activation, ~96 to ~115 JK$^{-1}$ mol$^{-1}$ may be involved. The data presented here are not sufficient to identify a specific mechanism for NO$_2^-$ removal. Many possibilities have been suggested by a number of authors (11). Further study is required, but any mechanisms postulated for this process must be consistent with the Arrhenius parameters obtained here.

![FIG. 8. Net rates of N$_2$O production in FSL (pH 6.8) as a function of time at temperatures of 5.8 to 25.3°C.](http://aem.asm.org/)

![FIG. 9. Ratio of total accumulation of N$_2$O and NO (N$_2$O/NO) as a function of temperature in FSL (pH 6.8) for over 3,000 min of experimental time; ratios were determined from data generated by graphical integration of net NO and N$_2$O production rates versus time plots shown in Fig. 7 and 8.](http://aem.asm.org/)
Comparison of the Arrhenius expression $Ae^{-E/RT}$ calculated for each process at 20°C given in Table 4 leads to the following conclusions. Nitrate loss is approximtely twice as fast in FSL as it is in BC. Chemical loss of NO$_3^-$ is a factor of 16 times slower in the sandy loam, and therefore, from a comparison of the data presented in Fig. 3 and 5, loss of NO$_3^-$ by biological processes in the sandy loam is much faster, in this case six times the rate in BC.

Surprisingly maximum net rates of production of nitric oxide and nitrous oxide are both greater in the sandy loam by factors of 1.8 and 1.7, respectively. In an earlier relatively long-term field study (12), N$_2$O evolution from BC was generally observed to be about one or two magnitudes greater than rates measured over FSL. This would suggest that, in the field, aeration was more restricted in the BC, which is fine-textured, poorly drained soil, than in the coarse-textured FSL.

The temperature dependence of $f_{NO}$ and $f_{N2O}$ may be quite similar in both soils, although rigorous comparison of the two would require further study with FSL containing more substrate, either NO$_3^-$ or carbon or both. Presumably, more substrate would allow the reaction to proceed for a greater length of time in order that potential maximum rates would be obtained at all temperatures.

By using our gas flow method we have been able to determine the temperature dependence of several steps in the denitrification process. Precise control of carbon and bacterial levels in these soils would also permit evaluation of Arrhenius parameters for biological NO$_3^-$ loss and net NO production rate. The latter intermediate is very reactive and therefore has proven difficult to measure in the past. The Arrhenius parameters we have obtained should eventually prove useful for developing realistic models for simulating nitrogen transformations and transport in soils.

ACKNOWLEDGMENTS

This work was supported in part by a grant from Environment Canada, Atmospheric Environment Service.

We are grateful to Lucio Gelmini, who carried out some analyses in the FSL runs.

LITERATURE CITED


