The Effect of Carbon Mineralization on Denitrification Kinetics in Mineral and Organic Soils


ABSTRACT

Rates of denitrification and organic carbon (C) mineralization were measured simultaneously in soil suspensions maintained at 30° C under anoxic conditions. Nine mineral and seven organic soils were used in the study. Disappearance of NO3- and production of CO2 were measured at various times during the 12-day incubation. Labeled NO3- was used to differentiate denitrification from immobilization and reduction to NH3-N.

The rate of organic C mineralization followed first-order kinetics in all soils with the mineralization rate coefficient (k) values varying from 0.075 to 0.405 day^-1. The denitrification rates in anaerobic soils were shown to be proportional to the concentration of the two substrates: NO3- and available C. The denitrification rate coefficient (k) value was essentially constant for the mineral soils [0.00147 ± 25% day^-1 (µg C/ml)^{-1}], while k values for the organic soils were somewhat more variable [0.00155 ± 65% day^-1 (µg C/ml)^{-1}].

Significant correlations were observed between NO3- consumption and CO2 production. The molar ratio of NO3- consumption to CO2 production ranged from 0.6 to 1.8. Significant relationships were also observed between water-soluble C (WSC) and total organic C (TOC), maximum available C (Cmax), and WSC and Cmax, respectively. Water-soluble C represented 0.4 to 0.9% of TOC, while Cmax represented about 0.6 to 1.4% of TOC. Results also showed that denitrification rates were influenced by the rate at which available C is mineralized and made available to the organisms.

Additional Index Words: nitrate reduction, water-soluble carbon, first-order kinetics, anaerobic soil, flooded soil, Michaelis-Menton kinetics.


The role of organic carbon (C) as an electron donor in the denitrification process has been widely recognized. Several researchers (Wiljer and Delwiche, 1954; Bremner and Shaw, 1958; Nommik, 1956; Bowman and Focht, 1974; Reddy et al., 1978) have shown increased denitrification rates following the addition of energy source (organic C) to a soil. Significant relationships were reported between NO3- losses via denitrification and "available" C, as evaluated either by glucose-equivalent C (Stanford et al., 1975a), by water-soluble C (Burford and Bremner, 1975; Reddy et al., 1980), or by mineralizable C (Burford and Bremner, 1975).

A considerable portion of available C would be used in normal oxidative respiration by denitrifiers and other microorganisms (using O2 as an electron acceptor) until the system becomes anoxic. When O2 is depleted from the system, the denitrifiers use NO3- as an electron acceptor during their oxidative respiration. Once the easily decomposable C is used by the denitrifiers, the rate of NO3- loss would depend on the rate of soil organic C conversion to mineralizable organic C and to soluble organic C (Focht and Verstraete, 1977), while C loss in the same system may not depend on the availability of NO3-, because some organisms use Fe2+, Mn2+, and SO42- as their electron acceptors during C oxidation.

Denitrification rates, expressed either as zero-order (Wiljer and Delwiche, 1954; Nommik, 1956; Patrick, 1960; Reddy et al., 1978) or first-order (Stanford et al., 1975a; Reddy et al., 1980) rate coefficients, and which do not account for the soluble soil organic C availability will be specific to the soil and a given set of environmental conditions, and not of general value in modeling the denitrification process. Also, most researchers (Stanford et al., 1975a; Bowman and Focht, 1974; Burford and Bremner, 1975; Kohl et al., 1976) measured denitrification rates under static conditions where NO3- was present in the overlying water and the underlying soil. Since denitrification occurs only in the anaerobic soil layer, diffusion of NO3- from the floodwater to the anaerobic soil layer also controls the rate of denitrification (Reddy et al., 1978). No data are available to date on the kinetics of available C mineralization coupled with the kinetics of denitrification measured under diffusion nonlimiting conditions.

The objectives of the present investigation were (i) to determine simultaneously the rates of denitrification and C mineralization in several mineral and organic soils; (ii) to develop a relationship between the kinetics of denitrification and organic C mineralization; and (iii) to obtain a denitrification rate coefficient as a function only of available C (i.e., independent of soil type).

REACTION RATE EQUATIONS

In this study, the rate of available C mineralization during denitrification was assumed to follow first-order kinetics:

\[
\frac{dC}{dt} = -kC
\]  [1]

where C = (C_{max} - CO2), i.e., the available C remaining in the soil, C_{max} is the maximum available C. CO2 is the C utilized during denitrification, and k is C mineralization coefficient (day^{-1}); C concentrations are expressed as µg C/ml of soil water. The value of C_{max} was determined from the 2-week aerobic incubation, as described in a later section. Integration of Eq. [1] yields:

\[
C = C_{max} \exp(-kt).
\]  [2]

The rate of denitrification coupled with organic C miner-
alization in an anaerobic soil was described as a combined first-order process (Rao et al., 1981; Rolston et al., 1980):

\[
(dN/dt) = -k_cCN,
\]

where \( N \) is NO\(_3^-\) concentration (\( \mu g \) N/ml), and \( k_c \) is the denitrification rate coefficient [day\(^{-1}\) (\( \mu g \) C/ml)\(^{-1}\)]. Substitution of Eq. [2] into Eq. [3] yields:

\[
(dN/dt) = -k_cN [C_{max} \exp (-k_c t)].
\]

The solution of Eq. [4] is:

\[
(N/N_0) = \exp \left\{- \frac{C_{max}}{K_c} \frac{N}{(K_c + N)} \right\}.
\]

where \( N_0 \) is the initial NO\(_3^-\) concentration (\( \mu g \) N/ml).

In order to have applicability of the data over a wider concentration range, attempts were made to obtain \( k_c \) and \( K_c \) values in the Michaelis-Menten equation to describe the denitrification rate coefficient for two substrates (C and N in the present case), the modified Michaelis-Menten equation (Bray and White, 1966; Bowman and Focht, 1974) can be stated as:

\[
\frac{dN}{dt} = -v_{max} \frac{C}{(K_c + C)'(K_n' + N)}\]

where \( K_c \) and \( K_n \) are saturation constants, respectively, for N and C, \( v_{max} \) is the maximum denitrification rate (\( \mu g \) N ml\(^{-1}\) day\(^{-1}\)) under substrate nonlimiting conditions (i.e., N \( \gg \) \( K_c \) and C \( \gg \) \( K_n \)), and C and N are as defined earlier. Separation of variables in Eq. [6] gives:

\[
\int_{N_0}^{N} \frac{[K_c + N]}{N_0} dN = - \int_{0}^{t} \frac{v_{max} C}{(K_c + C)} dt.
\]


\[
(N - N_0) + K_c \ln \left( \frac{N}{N_0} \right) = (v_{max}/k_c) \ln \left\{ (K_c + C_{max}) \exp \left(-k_c t\right)/\left(K_c + C_{max}\right) \right\}.
\]

### MATERIALS AND METHODS

#### Soils

Nine mineral soils collected from various locations in the continental U.S.A. and seven organic soils collected from various locations in Florida were used in this study. Selected properties of these soils are shown in Table 1. Air-dried mineral soils were ground to pass through a 20-mesh sieve, adjusted to 0.3 atm soil water tension, and equilibrated for a period of 24 hours before use. Organic soils contained different amounts of soil water at the time of collection and were used without drying. Organic soils were stored for a maximum period of 4 weeks at 4°C in tightly sealed containers before their use in the experiments.

#### Incubation Procedure

Mineral soil (150 g on an oven-dry basis) was transferred into each of two 500-ml Erlenmeyer flasks. Enough distilled water was added to each flask so that the soil could be maintained as a well-stirred suspension. The water-to-soil ratio for mineral soils was 2, while for organic soils it ranged from 3 to 14 (Table 1), depending upon initial soil water content. Each flask was fitted with a rubber stopper consisting of a sampling port and inlet and outlet tubing for continuous flow of N\(_2\) gas (30 ml/min) for creating O\(_2\)-free conditions. Soils were then incubated at 30°C under continuous stirring. After 3 hours of continuous flow of N\(_2\) through the stirred soil suspensions, a 50-ml aliquot of soil suspension was removed and used in additional experiments, as described later. A known amount of NO\(_2^-\) was then added to the soil suspension to obtain a soil solution concentration of 200 \( \mu g \) N/ml.

Carbon dioxide evolved during anaerobic respiration (NO\(_3^-\) reduction or denitrification) was trapped by bubbling the effluent gas through a 0.5N KOH solution. During the first 5 days of incubation, 20-ml aliquots of soil suspensions were removed more frequently, and thereafter samples were taken at longer intervals. None of the soils was incubated longer than 12 days. Each soil suspension aliquot was treated with 50 ml of 0.01M CaCl\(_2\) and then filtered through Whatman no. 42 filter paper after 5 min of shaking. The filtered solutions were analyzed for NH\(_4^+\) and NO\(_3^-\). At the same sampling periods, 0.5N KOH in the CO\(_2\) traps were replaced with fresh solution and CO\(_2\) trapped in the KOH solution was analyzed.

In a companion study, duplicate 30-ml portions of untreated soil suspension were transferred into 170-ml glass bottles fitted with a serum cap. The soil suspension was amended with \( ^{15} \)NO\(_3^-\) (enriched with 10.05 atom % \(^{15}\)N for mineral soils and 99.10 atom % \(^{15}\)N for organic soils) to obtain a concentration of 200 \( \mu g \) N/ml in the soil solution. The bottles were purged with N\(_2\) gas to create anoxic conditions and incubated at 30°C with continuous shaking for 14 days. At the end of incubation, soils were treated with 50 ml of 2M KCl solution and filtered through Whatman no.
42 filter paper after 1 hour of shaking. Labeled N was analyzed in organic N, NH₄⁺, and NO₃⁻ fractions.

**Water Soluble Carbon (WSC)**

A portion of untreated soil suspension (20 ml) was treated with 25 ml of deionized water (1:5 soil-to-solution ratio) and filtered through a prewashed 0.2-μm filter paper after 30 min of shaking. The filtered solutions were analyzed for soluble C.

**Maximum Available Carbon (C_max)**

Ten grams of mineral soil and 3 g of organic soil (oven-dry basis) treated with enough water to obtain a soil water tension of 0.3 atm were transferred into a wide-mouth flask along with a test tube containing 0.57V KOH. The flask was sealed with a rubber stopper and silicone glue. A flask with no soil was also included in the study. All flasks were incubated in the dark at 30°C for 14 days. At the end of incubation, CO₂ trapped in the KOH was analyzed.

**Analytical Methods**

The CO₂ absorbed in KOH solutions was titrated with standard acid (Stotzky, 1965). Total carbon in the soil samples was determined by dry combustion. Soluble carbon in the solution was measured using a modified K₂Cr₂O₇ oxidation method (Mebius, 1960; Burford and Brenner, 1975). Nitrate and NH₄⁺ were determined using steam distillation (Brenner, 1965). An isotope ratio mass spectrometer was used to determine ¹⁵N in the samples.

**Estimation of Model Parameters**

From the measured values of C_max and the amounts of CO₂ produced during anoxic incubation, the values of kₜ were estimated by minimizing the sum of squares (SSQ):

\[
SSQ = \sum_{i=1}^{n} (\text{calc}_i - \text{meas}_i)^2, \tag{9}
\]

where the subscripts calc and meas denote, respectively, the calculated and measured values of C, the superscript i denotes a given measurement, and n is the number of measurements. The value of C_max in Eq. [6] was estimated as C_max minus CO₂ produced during denitrification.

As an index of the goodness-of-fit of calculations to the measurements, the values of standard error (SE) were computed as follows:

\[
SE = \left( \frac{SSQ}{n} \right)^{1/2}.
\]

Note that SE represents the average deviation between measured and calculated values at a given time. Using the NO₃⁻ data and the values of C_max, kₜ, and N₀, the values of kₜ in Eq. [5] were determined for each soil in a manner similar to those for C data. Equation [8] was solved using numerical techniques. Given the values of N₀, C_max, and kₜ, the values of vₜₕ, kₜ, and kₜ in Eq. [8] were estimated for the NO₃⁻ loss data using nonlinear, least-squares (NLLS) optimization procedure (Rao et al., 1979).

**RESULTS**

**Organic Carbon Mineralization**

Carbon dioxide production owing to mineralization of available soil organic C during denitrification could be described well for all soils by Eq. [2] with the estimated kₜ value being different for each soil (Table 2). The average kₜ value, denoted by kₜ', for mineral and organic soils was 0.145 day⁻¹ (C.V. = 63%) and 0.191 day⁻¹ (C.V. = 74%), respectively. No significant relationship was observed between the kₜ values and total organic C (TOC) or maximum available organic C (C_max). The calculated values for CO₂ production in selected soils, as estimated by Eq. [2] using a different kₜ value for each soil (Table 2), are shown in Fig. 1 as solid lines along with the measured data. The soils shown in Fig. 1 were selected to represent the entire range in the goodness-of-fit (range of SE was 4 to 32 μg C/ml) between the data and Eq. [1]. The dashed lines in Fig. 1 were calculated using kₜ'.

**Table 2—Summary of parameters for describing the kinetics of CO₂ production and denitrification in mineral and organic soils**

(see Eq. [16]).

<table>
<thead>
<tr>
<th>Soil series</th>
<th>WSC</th>
<th>C_max</th>
<th>CO₂</th>
<th>N₀</th>
<th>kₜ x 10⁻⁴</th>
<th>hₜ x 10⁻⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μg C/ml</td>
<td>μg N/ml</td>
<td>day⁻¹</td>
<td>day⁻¹ (μg C/ml)⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mineral soils</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Webster #1</td>
<td>174</td>
<td>360</td>
<td>125</td>
<td>214</td>
<td>0.75</td>
<td>1.04</td>
</tr>
<tr>
<td>Webster #2</td>
<td>189</td>
<td>420</td>
<td>150</td>
<td>205</td>
<td>0.81</td>
<td>1.22</td>
</tr>
<tr>
<td>Sharpshurg</td>
<td>144</td>
<td>215</td>
<td>186</td>
<td>209</td>
<td>3.61</td>
<td>2.14</td>
</tr>
<tr>
<td>Immokalee</td>
<td>36</td>
<td>60</td>
<td>52</td>
<td>213</td>
<td>2.58</td>
<td>1.26</td>
</tr>
<tr>
<td>Oliver</td>
<td>75</td>
<td>150</td>
<td>68</td>
<td>205</td>
<td>1.00</td>
<td>1.25</td>
</tr>
<tr>
<td>Crowley</td>
<td>96</td>
<td>150</td>
<td>77</td>
<td>202</td>
<td>1.16</td>
<td>1.86</td>
</tr>
<tr>
<td>Yolo</td>
<td>54</td>
<td>90</td>
<td>46</td>
<td>221</td>
<td>1.29</td>
<td>1.66</td>
</tr>
<tr>
<td>Cecil</td>
<td>66</td>
<td>180</td>
<td>75</td>
<td>206</td>
<td>0.91</td>
<td>1.74</td>
</tr>
<tr>
<td>Reservoir</td>
<td>222</td>
<td>240</td>
<td>98</td>
<td>200</td>
<td>0.93</td>
<td>1.05</td>
</tr>
<tr>
<td>Avg. (%)</td>
<td>57%</td>
<td>57%</td>
<td>48%</td>
<td>3%</td>
<td>68%</td>
<td>25%</td>
</tr>
<tr>
<td>Organic soils</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pahokee</td>
<td>528</td>
<td>1,019</td>
<td>261</td>
<td>266</td>
<td>0.52</td>
<td>0.31</td>
</tr>
<tr>
<td>Terra Ceia</td>
<td>147</td>
<td>170</td>
<td>99</td>
<td>298</td>
<td>1.80</td>
<td>0.96</td>
</tr>
<tr>
<td>Lauderhill</td>
<td>183</td>
<td>276</td>
<td>276</td>
<td>231</td>
<td>3.64</td>
<td>1.72</td>
</tr>
<tr>
<td>Brighton</td>
<td>249</td>
<td>219</td>
<td>173</td>
<td>201</td>
<td>1.63</td>
<td>1.66</td>
</tr>
<tr>
<td>Oklawaha</td>
<td>258</td>
<td>576</td>
<td>177</td>
<td>217</td>
<td>0.55</td>
<td>0.61</td>
</tr>
<tr>
<td>Montverde</td>
<td>183</td>
<td>364</td>
<td>134</td>
<td>224</td>
<td>1.15</td>
<td>2.39</td>
</tr>
<tr>
<td>Montverde (V)</td>
<td>144</td>
<td>228</td>
<td>224</td>
<td>210</td>
<td>4.05</td>
<td>0.41</td>
</tr>
<tr>
<td>Avg. (%)</td>
<td>55%</td>
<td>76%</td>
<td>34%</td>
<td>15%</td>
<td>74%</td>
<td>63%</td>
</tr>
</tbody>
</table>

†CO₂ produced during 7-day incubation was used to compare with WSC and C_max. In some soils, CO₂ produced after 7 days was due to other processes because NO₃⁻ in these soils reached near zero level after 7 days.

‡WSC = water-soluble C.
values in Eq. [1]; the agreement between data and calculated lines is only fair. Thus, in further work, individual $k$ values were used. In some soils, the rate of CO$_2$ production during the first 3 days was higher than that during the later periods. In these soils, the initial surge of CO$_2$ production was probably due to a rapid consumption of WSC during denitrification. Also, slightly more C was probably made available in some of these soils, as a result of air drying (Powelson, 1980), sieving, and mixing of the anaerobic slurry.

**Rate of Denitrification**

Denitrification rate was evaluated by following NO$_3$ disappearance in a stirred soil suspension maintained under O$_2$-free conditions. For all soils, labeled NO$_3$ data indicated $<$ 3% of the added N was incorporated into organic N and NH$_4$ fractions. Using the NO$_3$ loss data and C mineralization, denitrification rate coefficient ($k_a$) was determined for each soil using Eq. [5]. The estimated $k_a$ values, shown in Table 2, were essentially constant for the nine mineral soils (0.00147 ± 25% day$^{-1}$ (µg C/ml)$^{-1}$), while those for the organic soils were somewhat more variable (0.00155 ± 65% day$^{-1}$ (µg C/ml)$^{-1}$). Good agreement was found between measured NO$_3$ loss and that predicted using $k_a$ and $k_c$ values specific to a given soil (Fig. 2). The soils included in Fig. 2 were selected to represent the entire range of SE values (4 to 13 µg N/ml for mineral soils and 8 to 22 µg N/ml for organic soils). Also shown as dashed lines in Fig. 2 are the curves calculated using an average $k_c$ value (averaged separately for mineral and organic soils) and individual $k_a$ values. The deviations between the measured and calculated NO$_3$ loss are not considered large given the practical utility of a single denitrification rate coefficient for all mineral (or organic) soils.

Although the fits between Eq. [8] and the measured data were good, the range in parameter values was very large. For example, $v_{max}$ ranged from 66 to 260,400 µg N/ml day, $K_v$ from 0 to 18,330 µg N/ml, and $K_n$ from 0 to 8182 µg C/ml. Compared with the Michaelis constants reported in the literature (Focht and Verstraete, 1977), these values may be unacceptable. The reason for the large range in estimated values for these parameters can be attributed to the nonlinear nature of Eq. [8] and the fact that the three parameters ($v_{max}$, $K_v$, and $K_n$) are partially correlated. Thus, essentially the same curve can be calculated using widely different sets of parameter values. This behavior is illustrated in Fig. 3, where the hatched area bounds the curves calculated by Eq. [8], using four sets of $v_{max}$ and $K_v$ values shown while holding $k_a$ constant. It is evident from Fig. 3 that an increase in $v_{max}$ can almost be compensated for by a proportional increase in $K_v$. A similar inter-relationship be-

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![Fig. 1](image1.png) - Cumulative CO$_2$ production during denitrification in selected mineral and organic soils. (Closed circles represent experimental data; dashed lines represent values calculated using average $k_c$ values; and solid lines represent the values calculated using independent $k_c$ values.)

![Fig. 2](image2.png) - NO$_3$ loss in selected mineral and organic soils. (Closed circles represent experimental data; dashed lines represent values calculated using average $k_c$ values; and solid lines represent the values calculated using independent $k_c$ values.)

![Fig. 3](image3.png) - Relationship between NO$_3$ loss and CO$_2$ production in mineral and organic soils. Middle line represents the average linear regression for all soils. Two outside lines represent the regression in 2 extreme soils.
Therefore, when the measured data being fitted is between \( v_{\text{max}} \) and \( K_c \) was observed (data not shown). The bars in Fig. 3 represent the total range in measured data for \( \text{NO}_3^- \) loss in two mineral soils (Crowley and Oliver) with similar \( C_{\text{max}}, N_0, \) and \( k_c \) values. Therefore, when the measured data being fitted is rather variable (±10 \( \mu \text{g} \) N/ml for the data in this study), it is difficult to select with any reliability among the four sets of parameter values shown in Fig. 3.

**Denitrification vs. Carbon Mineralization**

The relationship between \( \text{NO}_3^- \) loss and \( \text{CO}_2 \) production during anaerobic incubation of mineral and organic soils is shown in Fig. 4. Because \( \text{NO}_3^- \) was added to the soil suspensions containing no \( \text{O}_2 \), it may be assumed that the measured \( \text{CO}_2 \) was released primarily during the respiration of denitrifying bacteria. A linear regression equation with zero intercept (i.e., \( y = mx \)) was fitted to the \( \text{CO}_2 \) vs. \( \text{NO}_3^- \) for each soil. Three separate linear regression lines are presented in Fig. 4 for the organic and mineral soils. The solid line represents the average slope for all mineral (or organic) soils, while the dashed lines are for soils with the largest and least slopes. These equations show that for each mole of \( \text{CO}_2 \) released, on an average the amounts of \( \text{NO}_3^- \) denitrified were 1.27 mol (0.72 to 1.80) for mineral soils and 0.78 mol (0.58 to 1.48) for organic soils (Fig. 4).

The water soluble C represents about 0.4 to 0.9% of TOC, whereas \( C_{\text{max}} \) represents about 0.6 to 1.4% of TOC (Table 3), probably because much of TOC in most of the soils is resistant to decomposition. Maximum available C (\( C_{\text{max}} \)) measured in this study was based on a 14-day aerobic incubation. Even though WSC and \( C_{\text{max}} \) concentrations were adequate to denitify 200 \( \mu \text{g} \) \( \text{NO}_3^- \)– \( \text{N} \) ml, only 70 to 75% of WSC and 40 to 43% of \( C_{\text{max}} \) was used in 7 days while the remaining C was apparently available at a relatively slower rate.

**DISCUSSION**

The results presented here have demonstrated that in anaerobic soils, the denitrification rates are proportional to the concentrations of the two substrates: \( \text{NO}_3^- \) and available C. The denitrification rate coefficient \( (k_s) \) was essentially independent of soil type among the nine mineral soils studied. The \( k_s \) value for the organic soils was somewhat more variable compared to the mineral soils (C.V. = ± 65% vs. ± 25%). The causes of this variability remain unclear at this time since the nature of organic C in these soils is apparently similar (Volk and Zelazny, 1974). It should be noted that while the same soil/solution ratios were maintained for all mineral soils, different ratios had to be used for organic soils owing to the differences in their initial soil water contents.

From the results presented in Fig. 4, it should not be concluded either that the Michaelis-Menten model, Eq. [8], is incorrect or that a unique set \( v_{\text{max}}, K_s, \) and \( K_c \) values do not exist for a given experimental data set. This study, however, suggests that reliable Michaelis constants cannot be derived from the denitrification rates measured at low concentrations of N and C and apparently limiting as might have been the case in this study. When both substrates are limiting, i.e., \( C < K_c \) and \( N < K_s \), Eq. [8] reduces to:

\[
\frac{dN}{dt} = \left( -\frac{v_{\text{max}}}{K_c} \right) \frac{K_s}{K_c + N} \left( \frac{C}{K_c} + 1 \right) \left( \frac{N}{K_s} + 1 \right)
\]

Comparison of Eq. [11] and Eq. [5] shows that \( k_s \),

**Table 3—Regression equations for relating various indices for "available" soil organic C in mineral and organic soils.**

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Dependent variable</th>
<th>Regression equation</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOC</td>
<td>( C_{\text{max}} )</td>
<td>( C_{\text{max}} = 0.0138 ) (TOC)</td>
<td>0.71*</td>
</tr>
<tr>
<td>TOC</td>
<td>WSC</td>
<td>( WSC = 0.0038 ) (TOC)</td>
<td>0.86**</td>
</tr>
<tr>
<td>WSC</td>
<td>( C_{\text{max}} )</td>
<td>( C_{\text{max}} = 1.699 ) (WSC)</td>
<td>0.92**</td>
</tr>
<tr>
<td>WSC</td>
<td>( \text{CO}_2 )</td>
<td>( \text{CO}_2 = 0.748 ) (WSC)</td>
<td>0.87**</td>
</tr>
<tr>
<td>( C_{\text{max}} )</td>
<td>( \text{CO}_2 )</td>
<td>( \text{CO}<em>2 = 0.427 ) ( C</em>{\text{max}} )</td>
<td>0.89**</td>
</tr>
<tr>
<td>TOC</td>
<td>( C_{\text{max}} )</td>
<td>( C_{\text{max}} = 0.0056 ) (TOC)</td>
<td>0.80**</td>
</tr>
<tr>
<td>TOC</td>
<td>WSC</td>
<td>( WSC = 0.0038 ) (TOC)</td>
<td>0.99**</td>
</tr>
<tr>
<td>WSC</td>
<td>( C_{\text{max}} )</td>
<td>( C_{\text{max}} = 1.563 ) (WSC)</td>
<td>0.94**</td>
</tr>
<tr>
<td>WSC</td>
<td>( \text{CO}_2 )</td>
<td>( \text{CO}_2 = 0.701 ) (WSC)</td>
<td>0.90**</td>
</tr>
<tr>
<td>( C_{\text{max}} )</td>
<td>( \text{CO}_2 )</td>
<td>( \text{CO}<em>2 = 0.407 ) ( C</em>{\text{max}} )</td>
<td>0.80**</td>
</tr>
</tbody>
</table>

**Notes:**
- \( * * * \) Significant at the 0.05 and 0.01 levels of probability, respectively.
- \( \dagger \) \text{CO}_2 produced during 7-day denitrification process.
- \( \ddagger \) TOC = total organic C; WSC = water-soluble C.
described equally well both by Eq. [5] and Eq. [8], suggesting that the present experiments may have been conducted under substrate-limiting conditions. This conclusion may not be valid for NO$_3^-$ since sufficient NO$_3^-$ was added to bring the initial solution concentrations to > 200 µg N/ml. A series of denitrification experiments with varying C and N concentrations are presently being initiated in our laboratory to determine whether a single set of Michaelis–Menten parameters can be derived for the soils included in this study.

The end products of NO$_3^-$ reduction are NH$_4^+$, N$_2$, or N$_2$O, while the end product for C decomposition is CO$_2$. Labeled NO$_3^-$ data indicated very little reduction of NO$_3^-$ to NH$_4^+$; thus, in our study, N$_2$ and N$_2$O are probably the dominant end products of this process. Using glucose as the available C equivalent, the following stoichiometric equation for NO$_3^-$ reduction (denitrification) can be written as:

$$5(\text{CH}_3\text{O}) + 4 \text{NO}_3^- + 4 \text{H}^+ \rightarrow 2 \text{N}_2 + \text{CO}_2 + 7 \text{H}_2\text{O}.$$ 

Accordingly, for each mole of CO$_2$ produced, about 0.8 mole of NO$_3^-$ is consumed. The above equation is based on the assumption that NO$_3^-$ is the only electron acceptor during microbial respiration. The above stoichiometric relationship was not observed for organic and mineral soils. The ratio of mmol of NO$_3^-$ consumption to mmol of CO$_2$ production ranged from 0.6 to 1.8 (Fig. 4). It is probable that in addition to NO$_3^-$, other electron acceptors (e.g., microbial reduction of Mn$^{4+}$) were also involved in CO$_2$ production (Turner and Patrick, 1968). However, the contribution of other electron acceptors is likely to be small because the Eh of anaerobic soils treated with NO$_3^-$ would be buffered at values > 200 mV (Patrick et al., 1976). Eh value of Lauderhill muck, treated with 200 µg N/ml and maintained under anaerobic conditions for 2 weeks, varied between 150 and 200 mV (unpublished results, K. R. Reddy). It is possible that some CO$_2$ was also produced during fermentation process which can lead to the observed deviation from the expected stoichiometric relationship between NO$_3^-$ loss and CO$_2$ production (Fig. 4). However, the NO$_3^-$ concentrations are probably sufficiently high to inhibit the activity of fermenting bacteria.

In our experiments, addition of 200 µg NO$_3^-$ N/ml to an anaerobic soil led to a greater demand for available C and resulted in its rapid depletion during the 12-day incubation period. Although the amounts of WSC (0.4 to 0.9% of TOC) and C$_{max}$ (0.6 to 1.4% of TOC) were sufficiently high to deplete 200 µg NO$_3^-$ N/ml, rate of available C consumption during denitrification was different among the soils studied. The half-life for the available C (50% of the C used during denitrification) consumption was in the range of 1.7 to 13.3 days. This indicates that the denitrification rate can be controlled by the rate at which available C is mineralized and made available to the denitrifiers. In soils and sediments, the available C is continually regenerated by solubilization of resistant C to more readily available forms and by the addition of organic wastes and plant residues. In upland soils, additional amounts of available C are produced during wetting and drying cycles (Reddy and Patrick, 1975). In aquatic ecosystems, such as flooded fields, wetlands, and lake bottoms, NO$_3^-$ concentrations are usually low and the available C concentrations in the sediment are generally higher than that needed for denitrification. Therefore, under field conditions, available C is seldom expected to be a limiting factor to denitrify small amounts of NO$_3^-$.

In addition to the concentrations of the two substrates (available C and NO$_3^-$), denitrification rates under field conditions are influenced by temperature and the extent of soil anaerobiosis. Stanford et al. (1975b) observed that denitrification rates approximately doubled with a 10°C increase in temperature. Based on this and other reports (Dawson and Murphy, 1972; Bailey, 1976), a temperature correction factor of Q$_{10} = 1.5$ to 2.1 appears to be satisfactory for denitrification. The extent of soil anaerobiosis is specified by the fraction of the soil volume that is anaerobic (Stanford, 1979). Results of several workers (Hutchinson and Moore, 1979; Ryder and Lund, 1980a, b; Robinson et al., 1980) suggest that the extent of soil anaerobiosis, especially in the top 30-cm depth, is determined by (i) soil texture and structure; (ii) the frequency distribution of rainfall/irrigation events; and (iii) the rates of O$_2$ supply by diffusion and consumption by soil microorganisms. It is possible that certain regions of the soil might be anaerobic while most of the soil volume is aerobic (i.e., presence of anaerobic microsites). The $k_c$ value reported here, after appropriate adjustments for the factors discussed above, may be used in simulation models (e.g., Rao et al., 1981) for N dynamics.

LITERATURE CITED

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The captions for Fig. 3 and Fig. 4 (p. 65–66) should be interchanged; i.e., the illustration shown as Fig. 3 should be Fig. 4, and that shown as Fig. 4 should be Fig. 3.