

DIVISION S-3—SOIL MICROBIOLOGY AND BIOCHEMISTRY

The Effect of Carbon Mineralization on Denitrification Kinetics in Mineral and Organic Soils¹

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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 NH₄-N.

The rate of organic C mineralization followed first-order kinetics in all soils with the mineralization rate coefficient (k_c) 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_d) value was essentially constant for the mineral soils [0.00147 ± 25% day⁻¹ (μg C/ml)⁻¹], while k_d 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 (C_{max}), and WSC and C_{max}, respectively. Water-soluble C represented 0.4 to 0.9% of TOC, while C_{max} 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.

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THE ROLE of organic carbon (C) as an electron donor in the denitrification process has been widely recognized. Several researchers (Wijler 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 relationship was 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 Fe³⁺, Mn⁴⁺, 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:

$$(dC/dt) = -k_c C \quad [1]$$

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_c 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:

$$C = C_{max} \exp(-k_c t) \quad [2]$$

The rate of denitrification coupled with organic C miner-

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Table 1—Selected characteristics of the soils used in the study.

Soil series	pH	Total		C/N ratio	Initial NO ₃ ⁻	NH ₄ ⁺ produced in 7-day anaerobic incubation	Water-soil ratio
		N	C				
		%		μg N/ml			
Mineral soils							
Webster #1	7.0	0.36	3.02	8.4	14.5	51.5	2.0
Webster #2	7.10	0.40	3.74	9.4	5.0	52.5	2.0
Sharpsburg	5.65	0.23	1.65	7.2	9.0	44.9	2.0
Immokalee	5.85	0.06	0.77	12.8	13.5	13.6	2.0
Oliver	6.45	0.09	0.75	8.3	5.0	19.3	2.0
Crowley	6.15	0.08	0.72	9.0	2.0	14.4	2.0
Yolo	5.60	0.06	0.62	10.3	21.0	7.3	2.0
Cecil	6.10	0.06	0.53	8.8	6.0	10.2	2.0
Reservoir sediment	7.5	0.61	6.67	10.9	0.8	45.0	2.0
Organic soils							
Pahokee	6.25	3.71	46.90	12.6	65.8	42.0	3.1
Terra Ceia	6.30	3.37	44.02	13.1	98.4	21.4	14.1
Lauderhill	6.40	3.83	43.54	11.4	31.3	28.7	5.0
Brighton	5.65	2.94	38.49	13.1	0.7	19.9	4.4
Oklawaha	5.35	3.13	36.66	11.7	17.1	56.5	5.7
Montverde	6.30	3.70	36.56	9.9	24.3	39.9	5.5
Montverde† (V)	+5.95	3.86	36.42	9.4	9.8	51.8	13.7

† Virgin soils.

alization in an anaerobic soil was described as a combined first-order process (Rao et al., 1981; Rolston et al., 1980):

$$(dN/dt) = -k_n CN, \quad [3]$$

where N is NO₃⁻ concentration (μg N/ml), and k_n is the denitrification rate coefficient [day⁻¹ (μg C/ml)⁻¹]. Substitution of Eq. [2] into Eq. [3] yields:

$$(dN/dt) = -k_n N [C_{max} \exp(-k_c t)]. \quad [4]$$

The solution of Eq. [4] is:

$$(N/N_0) = \exp\{-(C_{max} k_n/k_c) [1 - \exp(-k_c t)], \quad [5]$$

where N₀ is the initial NO₃⁻ concentration (μg N/ml).

In order to have applicability of the data over a wider concentration range, attempts were made to obtain K_n and v_{max} values in the Michaelis-Menten equation to describe the denitrification process. For reactions controlled by 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:

$$\frac{dN}{dt} = -v_{max} \left[\frac{C}{(K_c + C)} \cdot \frac{N}{(K_n + N)} \right], \quad [6]$$

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

$$\int_{N_0}^N [(K_n + N)/N] dN = - \int_0^t [(v_{max} C)/(K_c + C)] dt. \quad [7]$$

Substituting Eq. [2] for C in Eq. [7] and integration yields:

$$(N - N_0) + K_n \ln(N/N_0) = (v_{max}/k_c) \ln \{ [K_c + C_{max} \cdot \exp(-k_c t)] / (K_c + C_{max}) \}. \quad [8]$$

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-dry 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) were 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 N₂ gas (30 ml/min) for creating O₂-free conditions. Soils were then incubated at 30°C under continuous stirring. After 3 hours of continuous flow of N₂ 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 NO₃⁻ was then added to the soil suspension to obtain a soil solution concentration of 200 μg N/ml.

Carbon dioxide evolved during anaerobic respiration (NO₃⁻ 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 CaCl₂ and then filtered through Whatman no. 42 filter paper after 5 min of shaking. The filtered solutions were analyzed for NH₄⁺ and NO₃⁻. At the same sampling periods, 0.5N KOH in the CO₂ traps were replaced with fresh solution and CO₂ 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 ¹⁵NO₃⁻ (enriched with 10.05 atom % ¹⁵N for mineral soils and 99.10 atom % ¹⁵N for organic soils) to obtain a concentration of 200 μg N/ml in the soil solution. The bottles were purged with N₂ 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, NH_4^+ , and NO_3^- 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 0.5N 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, CO_2 trapped in the KOH was analyzed.

Analytical Methods

The CO_2 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 $\text{K}_2\text{Cr}_2\text{O}_7$ oxidation method (Mebius, 1960; Burford and Bremner, 1975). Nitrate and NH_4^+ were determined using steam distillation (Bremner, 1965). An isotope ratio mass spectrometer was used to determine ^{15}N in the samples.

Estimation of Model Parameters

From the measured values of C_{max} and the amounts of CO_2 produced during anoxic incubation, the values of k_c were estimated by minimizing the sum of squares (SSQ):

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

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_{meas} in Eq. [6] was estimated as C_{max} minus CO_2 produced during denitrification.

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

$$\text{SE} = (\text{SSQ}/n)^{1/2}. \quad [10]$$

Note that SE represents the average deviation between measured and calculated values at a given time. Using the NO_3^- data and the values of C_{max} , k_c , and N_o , the values of k_n 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 N_o , C_{max} , and k_c , the values of v_{max} , k_n , and k_c in Eq. [8] were estimated for the NO_3^- 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 k_c value being different for each soil (Table 2). The average k_c value, denoted by k'_c 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 k_c values and total organic C (TOC) or maximum available organic C (C_{max}). The calculated values for CO_2 production in selected soils, as estimated by Eq. [2] using a different k_c 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 $\mu\text{g C/ml}$) between the data and Eq. [1]. The dashed lines in Fig. 1 were calculated using k'_c

Table 2—Summary of parameters for describing the kinetics of CO_2 production and denitrification in mineral and organic soils (see Eq. [5]).

Soil series	WSC†	C_{max}	CO_2 †	N_o	$k_c \times 10^1$	$k_n \times 10^1$
		$\mu\text{g C/ml}$		$\mu\text{g N/ml}$	day^{-1}	$\text{day}^{-1} (\mu\text{g C/ml})^{-1}$
			Mineral soils			
Webster #1	174	360	125	214	0.75	1.04
Webster #2	189	420	150	205	0.81	1.22
Sharpsburg	144	215	186	209	3.61	2.14
Immokalee	36	60	52	213	2.58	1.26
Oliver	75	150	68	205	1.00	1.25
Crowley	96	150	77	202	1.16	1.86
Yolo	54	90	46	221	1.29	1.66
Cecil	66	180	75	206	0.91	1.74
Reservoir	222	240	98	200	0.93	1.05
Avg.	117	207	97	208	1.45	1.47
(% C.V.)	57%	57%	48%	3%	68%	25%
			Organic soils			
Pahokee	528	1,019	261	266	0.52	0.31
Terra Ceia	147	170	99	298	1.80	0.96
Lauderhill	183	276	276	231	3.64	1.72
Brighton	249	219	173	201	1.63	1.66
Oklawaha	258	576	177	217	0.55	0.61
Montverde	183	304	134	224	1.15	2.39
Montverde (V)	144	228	224	210	4.05	0.41
Avg.	241	399	192	235	1.91	1.15
(% C.V.)	55%	76%	34%	15%	74%	63%

† CO_2 produced during 7-day incubation was used to compare with WSC and C_{max} . In some soils, CO_2 produced after 7 days was due to other processes because NO_3^- 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_c 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 (Powlson, 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_n) was determined for each soil using Eq. [5]. The estimated k_n values, shown in Table 2, were essentially constant for the nine mineral soils [$0.00147 \pm 25\% \text{ day}^{-1} (\mu\text{g C/ml})^{-1}$], while those for the organic soils were somewhat more variable [$0.00155 \pm 65\% \text{ day}^{-1} (\mu\text{g C/ml})^{-1}$]. Good agreement was found between measured NO_3^- loss and that predicted using k_c and k_n 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 $\mu\text{g N/ml}$ for mineral soils and 8 to 22 $\mu\text{g N/ml}$ for organic soils). Also shown as dashed lines in Fig. 2 are the curves calculated using an average k_n value (averaged separately for mineral and organic soils) and individual k_c 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 $\mu\text{g N/ml day}$, K_n from 0 to 18,330 $\mu\text{g N/ml}$, and K_c from 0 to 8182 $\mu\text{g C/ml}$. 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_n , and K_c) 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_n values shown while holding k_c constant. It is evident from Fig. 3 that an increase in v_{max} can almost be compensated for by a proportional increase in K_n . A similar inter-relationship be-

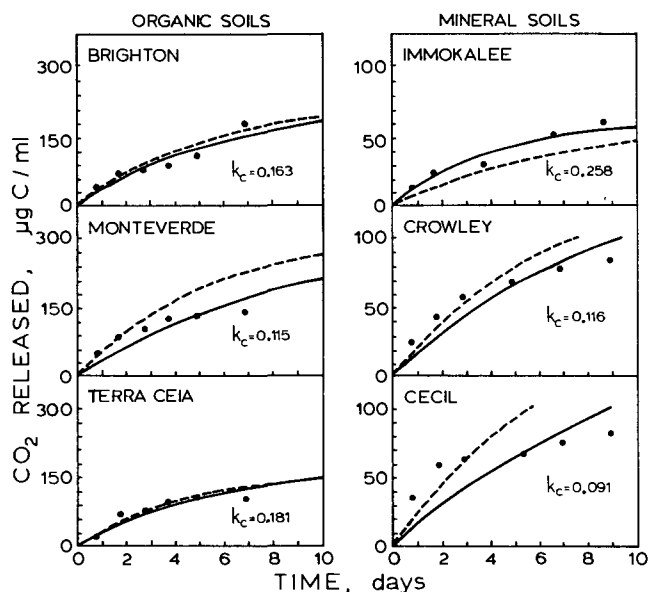


Fig. 1—Cumulative CO_2 production during denitrification in selected mineral and organic soils. (Closed circles represent experimental data; dashed lines represent values calculated using average k_c values; and solid lines represent the values calculated using independent k_c values.)

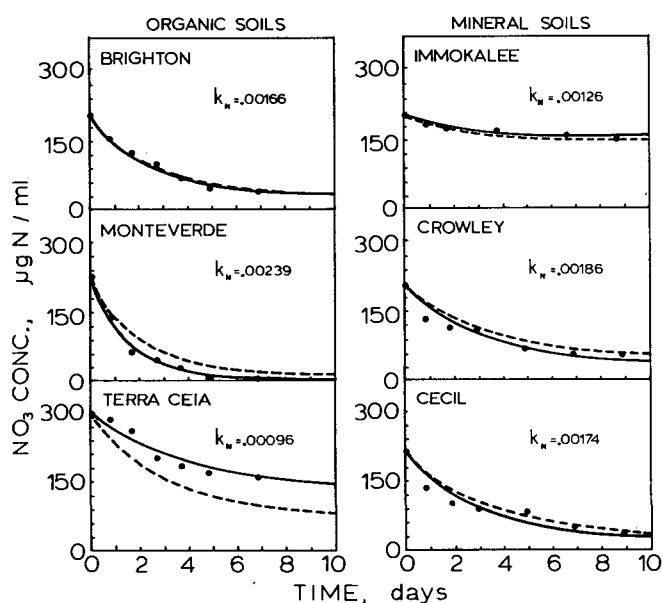


Fig. 2— NO_3^- loss in selected mineral and organic soils. (Closed circles represent experimental data; dashed lines represent values calculated using average k_n values; and solid lines represent the values calculated using independent k_n values.)

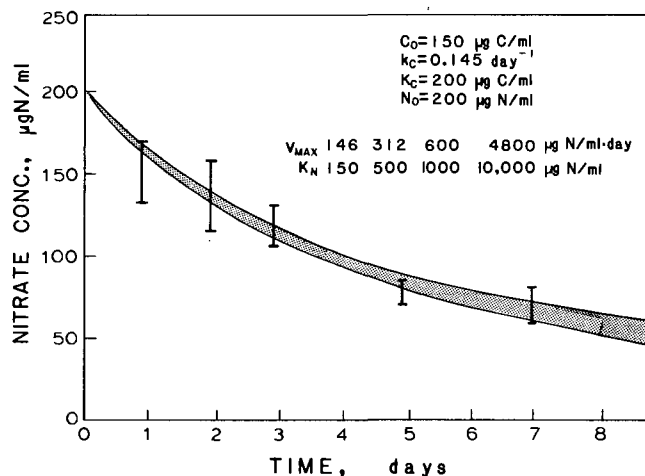


Fig. 3—Relationship between NO_3^- loss and CO_2 production in mineral and organic soils. Middle line represents the average linear regression for all soils. Two outside lines represent the regression in 2 extreme soils.

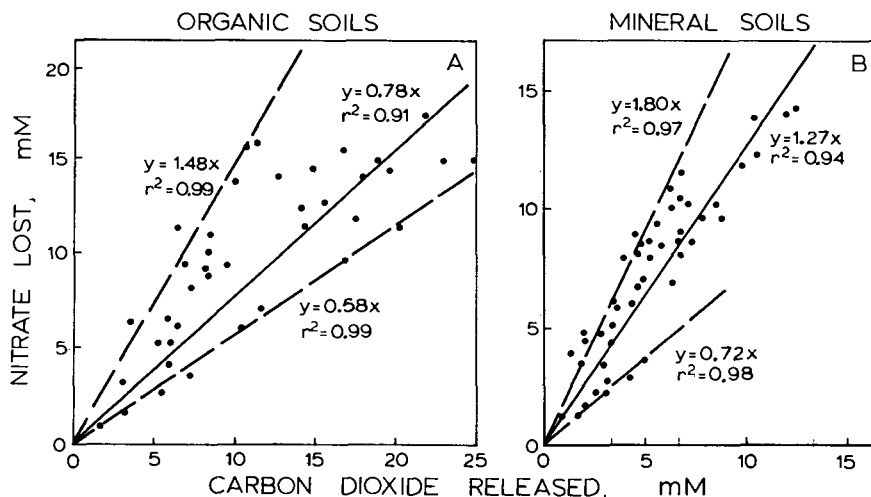


Fig. 4—Comparison of measured and calculated NO_3^- loss in 2 mineral soils. The bars represent the total range in measured data for Crowley and Oliver soils. The hatched area bounds curves calculated using Eq. [8] with 4 sets of widely different parameter values.

tween v_{\max} and K_c was observed (data not shown). The bars in Fig. 3 represent the total range in measured data for NO_3^- loss in two mineral soils (Crowley and Oliver) with similar C_{\max} , N_o , and k_c values. Therefore, when the measured data being fitted is rather variable ($\pm 10 \mu\text{g N/ml}$ 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 NO_3^- loss and CO_2 production during anoxic incubation of mineral and organic soils is shown in Fig. 4. Because NO_3^- was added to the soil suspensions containing no O_2 , it may be assumed that the measured 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 CO_2 vs. 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 CO_2 released, on an average the amounts of 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_{\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_{\max}) measured in this study was based on a 14-day aerobic incubation. Even though WSC and C_{\max} concentrations were adequate to denitrify $200 \mu\text{g NO}_3^- \text{N/ml}$, only 70 to 75% of WSC and 40 to 43% of C_{\max} was readily 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: NO_3^- and available C. The denitrification rate coefficient (k_n) was essentially independent of soil type among the nine mineral soils studied. The k_n value for the organic soils was somewhat more variable compared to the mineral soils (C.V. = $\pm 65\%$ vs. $\pm 25\%$). 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_{\max} , K_n , and K_c values does 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., $C \ll K_c$ and $N \ll K_n$, Eq. [8] reduces to:

$$dN/dt = (-v_{\max}/K_n K_c) CN. \quad [11]$$

Comparison of Eq. [11] and Eq. [5] shows that k_n

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

Independent variable	Dependent variable	Regression equation	R^2
Mineral soils†			
TOC	C_{\max}	$C_{\max} = 0.0138 (\text{TOC})$	0.71*
TOC	WSC	$\text{WSC} = 0.0087 (\text{TOC})$	0.86**
WSC	C_{\max}	$C_{\max} = 1.699 (\text{WSC})$	0.92**
WSC	CO_2^\ddagger	$\text{CO}_2 = 0.748 (\text{WSC})$	0.87**
C_{\max}	CO_2^\ddagger	$\text{CO}_2 = 0.427 (C_{\max})$	0.89**
Organic soils†			
TOC	C_{\max}	$C_{\max} = 0.0056 (\text{TOC})$	0.86**
TOC	WSC	$\text{WSC} = 0.0036 (\text{TOC})$	0.92**
WSC	C_{\max}	$C_{\max} = 1.563 (\text{WSC})$	0.94**
WSC	CO_2^\ddagger	$\text{CO}_2 = 0.701 (\text{WSC})$	0.90**
C_{\max}	CO_2^\ddagger	$\text{CO}_2 = 0.407 (C_{\max})$	0.80*

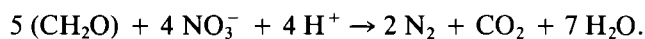
*, ** Significant at the 0.05 and 0.01 levels of probability, respectively.

† CO_2 produced during 7-day denitrification process.

‡ TOC = total organic C; WSC = water-soluble C.

$= (v_{\max}/K_n K_r)$. The measured NO_3^- loss data could be 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 $> 200 \mu\text{g N/ml}$. 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_2O , or N_2 , 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_2O 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:



Accordingly, for each mole of CO_2 produced, about 0.8 mole of NO_3^- are 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 $> 200 \text{ mV}$ (Patrick et al., 1976). Eh value of Lauderhill muck, treated with $200 \mu\text{g N/ml}$ 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 $200 \mu\text{g NO}_3^- \text{ N/ml}$ 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 $200 \mu\text{g NO}_3^- \text{ N/ml}$, 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 denitrification rates 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 $Q_{10} \approx 1.5$ 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. Results of several workers (Hutchinson and Mosier, 1979; Ryden and Lund, 1980a, b; Rolston 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_n 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.

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

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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.