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## **Nitrate Reduction in an Organic Soil-Water System**

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## Nitrate Reduction in an Organic Soil-Water System<sup>1</sup>

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### ABSTRACT

Flooded organic soil as a treatment system for nitrate-nitrogen ( $\text{NO}_3\text{-N}$ ) removal from agricultural drainage water was evaluated under controlled conditions. Nitrate-N reduction rates in the flood water (agricultural drainage water) and in the underlying organic soil column were measured as a function of  $\text{NO}_3\text{-N}$  concentration (10, 25, and 50  $\mu\text{g NO}_3\text{-N/ml}$ ), energy source, and temperature (8, 18, and 28°C). Labeled  $\text{NO}_3\text{-N}$  was used to differentiate between denitrification and  $\text{NO}_3\text{-N}$  reduction to ammonium-N ( $\text{NH}_4\text{-N}$ ) or organic N.

Nitrate-N reduction rates in the soil column incubated at 28°C with no excess flood water were best described by first-order kinetics with an average rate constant ( $k$ ) of 0.751  $\text{day}^{-1}$ . Flood water  $\text{NO}_3\text{-N}$  removal rates were also described by first-order kinetics. Nitrate removal rates from low oxygen-demand flood water (expressed as first-order rate constants) with an underlying soil column were 0.038, 0.750  $\text{day}^{-1}$  for an incubation temperature of 8, 18, and 28°C, respectively. In soil columns incubated with high oxygen-demand flood water, most of the added  $\text{NO}_3\text{-N}$  disappeared before it reached the underlying soil column, with  $k$  values of 0.172, 0.292, and 0.790  $\text{day}^{-1}$  for an incubation temperature of 8, 18, and 28°C, respectively. In all treatments, denitrification was the dominant process in the removal of flood water  $\text{NO}_3\text{-N}$ . At low temperature (8°C), a greater portion of  $^{15}\text{NO}_3\text{-N}$  was reduced to  $^{15}\text{NH}_4\text{-N}$  and organic N. Most of the immobilized  $^{15}\text{NO}_3\text{-N}$  appeared as soluble organic N. At 8°C, flood water  $\text{NO}_3\text{-N}$  diffused deeper into the soil column as compared to the flooded soil columns incubated at 28°C.

The results obtained in this study indicate that flooded organic soil can function as an effective sink for reducing  $\text{NO}_3\text{-N}$  levels of agricultural drainage waters.

**Additional Index Words:** flooded soil,  $\text{NO}_3\text{-N}$  diffusion, denitrification, first-order kinetics, waste waters, drainage waters.

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Flooding a soil creates anoxic (oxygen-deficient) conditions. Under these conditions nitrate-nitrogen ( $\text{NO}_3\text{-N}$ ) is used as an electron acceptor during microbial respira-

tion, a process widely known as *denitrification*. Nitrate loss through denitrification has been widely studied by several research workers (Bremner and Shaw, 1958a; Yamane, 1957; Stanford et al., 1975a; Burford and Bremner, 1975) for various soil types and under varying environmental conditions. In agricultural soils planted to crops, denitrification is undesirable because of  $\text{NO}_3\text{-N}$  loss to gaseous end products. On the other hand, the beneficial use of this process was recently recognized in the waste water treatment process, where  $\text{NO}_3\text{-N}$  removal is the primary objective (Dawson and Murphy, 1972; Raveh and Avnimelech, 1973; Stensel et al., 1973). When soils are flooded with  $\text{NO}_3\text{-N}$  rich waste waters, the underlying soil will function as a sink for the  $\text{NO}_3\text{-N}$  present in the flood water. Since little or no denitrification occurs in the flood water not treated with additional carbon (C) source (Engler and Patrick, 1974),  $\text{NO}_3\text{-N}$  removal from the overlying flood water is dependent on the diffusion of  $\text{NO}_3\text{-N}$  into the underlying soil where it is denitrified (Reddy et al., 1978). Kinetics of the denitrification process under these systems are dependent on diffusion phenomena.

A portion of the flood water  $\text{NO}_3\text{-N}$  may also be removed through immobilization to organic N and reduction to ammonium-N ( $\text{NH}_4\text{-N}$ ). Immobilization of  $\text{NO}_3\text{-N}$  is dependent on the nature of the organic residues added to the system. Residues with wider C/N ratios (generally >23) stimulate immobilization, while the residues with narrow C/N ratios (generally <23) favor mineralization of N (Alexander, 1977). Reduction of  $\text{NO}_3\text{-N}$  to  $\text{NH}_4\text{-N}$  occurs under intense reduced conditions (Buresh and Patrick, 1978). However,  $\text{NO}_3\text{-N}$  removal through these processes is generally insignificant compared to the denitrification process (Chen et al., 1972; Reddy et al., 1978).

The rate of  $\text{NO}_3\text{-N}$  removal from flood water is influenced by depth of flood water, pH, temperature, energy source, and flood water  $\text{NO}_3\text{-N}$  concentration. The influence of some of these factors on denitrification in soil systems has been studied by several research workers (Bremner and Shaw, 1958b; Nommik, 1956; Stanford et al., 1975b). Very limited data are available on the influence of these factors on flood water  $\text{NO}_3\text{-N}$  removal when pollution abatement is the primary objective.

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The objectives of the present study were to quantitatively evaluate the flood water  $\text{NO}_3\text{-N}$  removal capacity of an organic soil, as influenced by concentration of  $\text{NO}_3\text{-N}$  in the flood water, energy source, and temperature, where water treatment for  $\text{NO}_3\text{-N}$  removal is the primary concern.

## MATERIALS AND METHODS

To evaluate the rate of  $\text{NO}_3\text{-N}$  reduction in a soil-water system, a series of experiments were conducted on an organic soil. The soil used in this study was obtained from the plow layer of an organic soil (Lithic Medisaprists, euic, hyperthermic) from the Agricultural Research and Education Center's experimental farm in Zellwood, Fla. The soil had a total N content of 2.5%, total C content of 45.1, and pH of 7.8. Upon flooding, the soil reduced immediately, with the Eh value decreasing to  $-250$  mv within 5 days. Agricultural drainage water used in this study was obtained from drainage canals surrounding organic soils planted to vegetable crops located in Zellwood (near Lake Apopka), Orange County, Fla.

### EXPERIMENT I

This study was designed to evaluate the  $\text{NO}_3\text{-N}$  reduction capacity of the organic soil. Fifteen g (oven-dry basis) of field-moist soil were weighed into test tubes (OD = 2.5 cm, length = 15.0 cm) containing enough deionized distilled water (with 27, 42, 60, 94, and 200  $\mu\text{g}$   $\text{NO}_3\text{-N}/\text{cm}^3$ ) to saturate the soil. The test tubes were stoppered and incubated in the dark at  $28^\circ\text{C}$  for a period of 0, 6, 12, 24, 48, 72, and 120 hours. At the end of each incubation period, duplicate soil samples were extracted with 2N KCl and analyzed for  $\text{NO}_3\text{-N}$ .

In another study, 15 g (oven-dry basis) field-moist soil were transferred to test tubes containing enough labeled  $\text{NO}_3\text{-N}$  (100  $\mu\text{g}$   $^{15}\text{N}/\text{ml}$  enriched with 95% atom  $^{15}\text{N}$  excess) solution to saturate the soil. Tubes were stoppered and incubated in the dark at  $28^\circ\text{C}$  for a period of 0, 24, 48, 72, 96, and 192 hours. At the end of each incubation period, triplicate tubes were removed and the soil was extracted with 2N KCl and filtered through Whatman no. 44 filter paper. The filtered samples were analyzed for  $\text{NO}_3\text{-N}$ ,  $\text{NH}_4\text{-N}$ , and soluble organic N. The remaining soil was dried at  $40^\circ\text{C}$  and analyzed for total N. All fractions were analyzed for labeled N content.

### EXPERIMENT II

The capacity of the organic soil to function as a sink for flood water  $\text{NO}_3\text{-N}$  was evaluated under simulated soil column conditions. Five hundred g (oven-dry basis) of field-moist soil were transferred into a wide-mouth bottle and were subsequently flooded. This resulted in a saturated soil column of 10 cm. Soil columns were preincubated with 10 cm flood water for a period of 10 days to insure the removal of  $\text{NO}_3\text{-N}$  through denitrification. At the end of preincubation, overlying flood water was removed and then replaced with (i) drainage water with low oxygen demand; and (ii) drainage water with high oxygen demand (waste water was treated with 2 mg plant material/ml of water, to create high oxygen demand) to obtain flood water depths of 8 cm. The plant material used in this study was water hyacinth [*Eichhornia crassipes* (Mart. solms.)], which was dried at  $60^\circ\text{C}$  and ground to pass through a 2-mm sieve. The ground plant material was added to the drainage water and equilibrated for a period of 5 days before use. Characteristics of the agricultural drainage water used in the study are presented in Table 1. For both types of waste water,  $\text{NO}_3\text{-N}$  removal rates were evaluated at initial concentrations of 10, 25, and 50  $\mu\text{g}$  N/ml. Triplicate soil columns for each treatment were incubated in the dark at 8, 18, and  $28^\circ\text{C}$ . To determine active sites of  $\text{NO}_3\text{-N}$  reduction, the two types of waste water with varying levels of  $\text{NO}_3\text{-N}$  were also incubated without an underlying soil column at the same incubation temperatures. A 5-ml sample of flood water was obtained 0, 1, 2, 4, 6, 9, 13, 17, and 21 days after incubation by lowering a pipette 4 cm from the surface of the flood water, and analyzed for  $\text{NO}_3\text{-N}$  content. Dissolved oxygen and pH were also measured before each sampling period.

To differentiate between  $\text{NO}_3\text{-N}$  immobilization and denitrification, labeled  $^{15}\text{NO}_3\text{-N}$  (95 atom %  $^{15}\text{N}$  excess) was added to the two

Table 1—Characteristics of the organic soil drainage water used in the study.

Parameter	High oxygen-demand waste water†	Low oxygen-demand waste water
pH	7.4	8.0
EC, $\mu\text{mhos}/\text{cm}$	1,000	578
	mg/liter	
BOD	183	52
$\text{NO}_3\text{-N}$	0.2	0.5
$\text{NH}_4\text{-N}$	1.5	1.5
Organic N	17.0	5.7
Ortho-P	0.8	0.5
Total P	2.5	1.0
Dissolved $\text{O}_2$	0.5	7.3

† All parameters were measured after drainage water was equilibrated 5 days with 2 mg plant material/ml of drainage water.

types of drainage water at each level of initial  $\text{NO}_3\text{-N}$  concentration, and incubated under similar conditions described above. At the end of the 21-day incubation period, flood water was analyzed for  $\text{NO}_3\text{-N}$ ,  $\text{NH}_4\text{-N}$ , and organic N. The soil column (4-cm-depth interval) was extracted with 2N KCl and the extract was analyzed for  $\text{NO}_3\text{-N}$ ,  $\text{NH}_4\text{-N}$ , and soluble organic N. Remaining soil was analyzed for total N. All fractions were analyzed for labeled N content.

### EXPERIMENT III

This study was designed to determine the distribution of  $\text{NO}_3\text{-N}$  that diffused from the overlying flood water into the soil column. An open-end 5-cm (OD) PVC pipe was fitted with a rubber stopper and field-moist organic soil was transferred into duplicate PVC containers to obtain a 10-cm-long soil column. Soil was flooded and preincubated for a period of 10 days to insure denitrification of the initial  $\text{NO}_3\text{-N}$ . At the end of preincubation, flood water was replaced with drainage water containing 100  $\mu\text{g}$   $\text{NO}_3\text{-N}/\text{ml}$  to a depth of 10 cm and incubated for 96 hours at 8, 18, and  $28^\circ\text{C}$ . In a companion study, after preincubation (as described above) duplicate soil columns were sterilized by autoclaving for a period of 40 min. After autoclaving, flood water was displaced with the sterilized drainage water (100  $\mu\text{g}$   $\text{NO}_3\text{-N}/\text{ml}$ ) to obtain a 10-cm floodwater depth, and incubated in the dark for a period of 78 hours at 8 and  $28^\circ\text{C}$ . At the end of the incubation period, soil columns were sectioned into 5-mm fractions using similar procedure described by Reddy and Patrick (1976). Each sectioned soil sample was extracted with 2N KCl and analyzed for  $\text{NO}_3\text{-N}$ .

### ANALYTICAL METHODS

Nitrate-N and  $\text{NH}_4\text{-N}$  were analyzed using steam distillation (Bremner, 1965a). Soluble organic N in the extracts and total N in the soil samples were analyzed by Kjeldahl digestion and distillation (Bremner, 1965b). Labeled N ( $^{15}\text{N}$ ) content of the samples were analyzed using a 21-614 isotope ratio (DuPont model) mass spectrometer. In experiment II, where 5-ml water samples were obtained,  $\text{NO}_3\text{-N}$  was analyzed using the Brucine method (APHA, 1971).

The data obtained on  $\text{NO}_3\text{-N}$  disappearance was best described by a first-order kinetic equation

$$d(\text{NO}_3\text{-N})/dt = -k(\text{NO}_3\text{-N}). \quad [1]$$

Integrating Eq. [1], we obtain

$$(\text{NO}_3\text{-N})_L = (\text{NO}_3\text{-N})_0 [1 - \exp(-kt)] \quad [2]$$

where

$(\text{NO}_3\text{-N})_L$  = nitrate-N disappeared from soil column or flood water  $\mu\text{g}/\text{ml}$

$(\text{NO}_3\text{-N})_0$  = initial  $\text{NO}_3\text{-N}$  concentration,  $\mu\text{g}/\text{ml}$

$k$  = first-order rate constant,  $\text{day}^{-1}$

$t$  = time, days.

Using least square fit of the data to Eq. [2],  $k$  values were estimated for several treatments. The  $k$  value reflects the  $\text{NO}_3\text{-N}$  removal as a re-

sult of denitrification,  $\text{NO}_3\text{-N}$  reduction to  $\text{NH}_4\text{-N}$ ,  $\text{NO}_3\text{-N}$  immobilization, and  $\text{NO}_3\text{-N}$  diffusion.

## RESULTS AND DISCUSSION

### Nitrate N Reduction Capacity of the Organic Soil

Data pertaining to the  $\text{NO}_3\text{-N}$  reduction capacity of an organic soil incubated without excess flood water are presented in Fig. 1. Results indicate a rapid decrease in soil-water  $\text{NO}_3\text{-N}$  concentration, with about 80% of the initial  $\text{NO}_3\text{-N}$  lost within 48 hours after incubation. Nitrate-N reduction rate increased with increasing soil-water  $\text{NO}_3\text{-N}$  concentration. Nitrate reduction rate followed first-order kinetics in the initial  $\text{NO}_3\text{-N}$  concentration range of 27 to 200  $\mu\text{g N/cm}^3$ , with an average rate constant of  $0.751 \pm 0.180 \text{ day}^{-1}$ . Diffusion of  $\text{NO}_3\text{-N}$  within the soil-water to the enzymatic sites was probably controlling the rate and order of  $\text{NO}_3\text{-N}$  reduction reaction. The bulk density of the organic soil column was  $0.39 \text{ g/cm}^3$ , indicating a larger volume of water-filled pores than mineral soils which have bulk densities  $>1$ . In a recent study, Reddy et al. (1978) described  $\text{NO}_3\text{-N}$  reduction by zero-order kinetics for mineral soils when C is nonlimiting.

Data (Table 2) on the fate of added  $^{15}\text{NO}_3\text{-N}$  indicate that about 97% of the added N was lost through denitrification, 2.5% of the added N was reduced to  $^{15}\text{NH}_4\text{-N}$ , and 0.6% of the added N was immobilized into the organic  $^{15}\text{N}$  fraction. These data confirm the significance of the denitrification process in organic soils. The redox potential (Eh) of the soil system decreased from 452 mv at the start of the incubation to -252 at the end of the 8-day incubation period, indicating a high demand for electron acceptors.

### Flood Water $\text{NO}_3\text{-N}$ Removal

Figures 2, 3, and 4 show the  $\text{NO}_3\text{-N}$  disappearance rate from the overlying flood water as influenced by temperature and flood water  $\text{NO}_3\text{-N}$  concentration. Flood water  $\text{NO}_3\text{-N}$  removal rate increased with increasing initial  $\text{NO}_3\text{-N}$  concentration. In the presence of low oxygen-demand flood water,  $\text{NO}_3\text{-N}$  loss was dependent on the diffusion of  $\text{NO}_3\text{-N}$  from the overlying flood water to the underlying soil column, since very little or no  $\text{NO}_3\text{-N}$  reduction occurred in the flood water, as evidenced in the treatments where low oxygen-demand flood water was incubated without an underlying soil column. In the treatments with high oxygen-demand

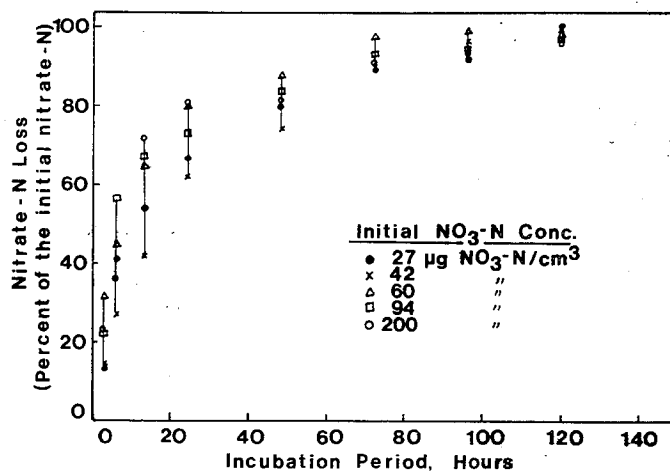


Fig. 1—Rate of  $\text{NO}_3\text{-N}$  disappearance in an organic soil incubated under saturated soil moisture conditions.

flood water, most of the flood water  $\text{NO}_3\text{-N}$  was lost within 5 days after initiation of incubation. With high oxygen-demand flood water, a significant portion of the flood water  $\text{NO}_3\text{-N}$  was reduced in the flood water itself, before it diffused into the underlying soil column, as evidenced in the treatment where high oxygen-demand flood water was incubated without an underlying soil column. This type of trend was seen at all initial flood water  $\text{NO}_3\text{-N}$  concentrations. Flood water  $\text{NO}_3\text{-N}$  removal was increased with increasing temperature in all treatments with maximum and minimum  $\text{NO}_3\text{-N}$  removal observed at 28 and  $8^\circ\text{C}$ , respectively.

Nitrate-N removal rate from the overlying flood water was best described by using a first-order kinetic equation. Using least square fit of the data to Eq. [2], first-order kinetic rate ( $k$ ) constants were calculated. These data fit fairly well to this equation with  $R$  values  $>0.9$  for all treatments. Since the differences in  $k$  values were not appreciably different for various initial flood water  $\text{NO}_3\text{-N}$  concentrations, an average  $k$  value for all three concentrations is presented in Table 3. At 8, 18, and  $28^\circ\text{C}$ ,  $\text{NO}_3\text{-N}$  removal rate was more rapid in high oxygen-demand flood water incubated with or without an underlying organic soil column, as compared to the low oxygen-demand flood water.

In earlier studies Reddy et al. (1978) observed that flood water  $\text{NO}_3\text{-N}$  loss was dependent on the diffusion of  $\text{NO}_3\text{-N}$  into the underlying soil, resulting in apparent

Table 2—Distribution of applied  $^{15}\text{NO}_3\text{-N}$  and Eh in an organic soil incubated at  $28^\circ\text{C}$ .

Labeled N-fraction	Incubation period, days					
	0	1	2	3	4	8
	% added $^{15}\text{NO}_3\text{-N}$					
$\text{NO}_3\text{-N}$	100	10.8	1.4	0.0	0.0	0.0
$\text{NH}_4\text{-N}$	—	1.7	0.9	1.4	2.2	2.4
Soluble organic N	—	0.9	0.3	1.0	0.3	0.3
Organic N†	—	0.2	0.2	0.2	0.9	0.3
Total	100	13.5	2.8	2.6	3.4	3.0
N-loss	—	86.5	97.2	97.4	96.6	97.0
Eh (mv)	452	173	-77	-179	-208	-252

† Does not include soluble organic N.

Table 3—First-order rate coefficients (expressed as an average of all initial  $\text{NO}_3\text{-N}$  concentrations) for  $\text{NO}_3\text{-N}$  disappearance from the flood water.

Treatment	Temperature, $^\circ\text{C}$		
	8	18	28
	First order rate constant, $\text{day}^{-1}$ *		
1) Low oxygen-demand flood water with underlying soil column	$0.038 \pm 0.004$	$0.079 \pm 0.013$	$0.118 \pm 0.019$
2) High oxygen-demand flood water with underlying soil column	$0.172 \pm 0.059$	$0.292 \pm 0.125$	$0.750 \pm 0.153$
3) High oxygen-demand flood water without soil column	$0.125 \pm 0.105$	$0.289 \pm 0.144$	$0.604 \pm 0.094$

\* All  $R$  values are  $>0.90$  (range 0.90 to 0.99) and were significant at 0.05 level of probability.

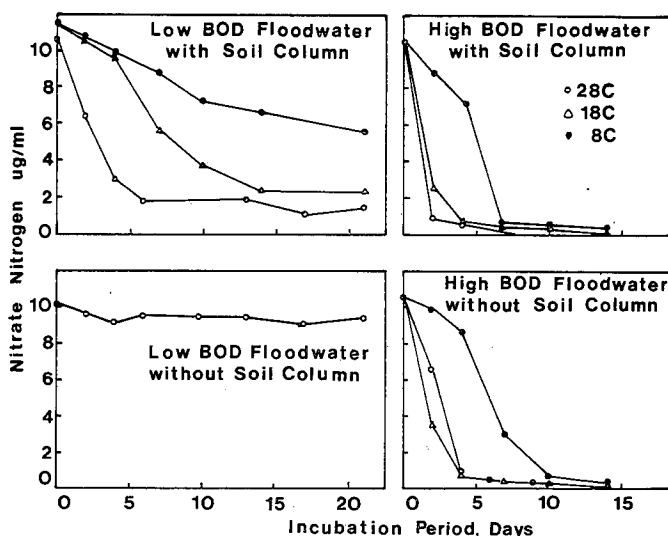


Fig. 2—Rate of flood water  $\text{NO}_3\text{-N}$  disappearance as influenced by temperature at an initial  $\text{NO}_3\text{-N}$  concentration of  $10 \mu\text{g N/ml}$ .

first-order kinetics for denitrification process. These studies were conducted under complete oxygen-free conditions with no additional energy source added to the flood water. The results obtained in the present study also show flood water  $\text{NO}_3\text{-N}$  removal to follow first-order reaction. In the treatment with high oxygen-demand flood water without an underlying soil,  $\text{NO}_3\text{-N}$  loss from flood water also followed first-order kinetics, even though diffusion was not a factor. It should be remembered that these studies were not conducted under oxygen-free conditions, because flood water surface was exposed to the air. Oxygen diffusion into the flood water can also interfere with the  $\text{NO}_3\text{-N}$  reduction process, because facultative aerobes prefer  $\text{O}_2$  over  $\text{NO}_3\text{-N}$  as an electron acceptor (Alexander, 1977).

For low oxygen-demand flood water,  $Q_{10}$  (ratio of the velocity constant at two temperatures differing by  $10^\circ\text{C}$  calculated by dividing the  $k$  value obtained at the high temperature, by the  $k$  value obtained at the low temperature) values of 1.5 and 2.1 were found for

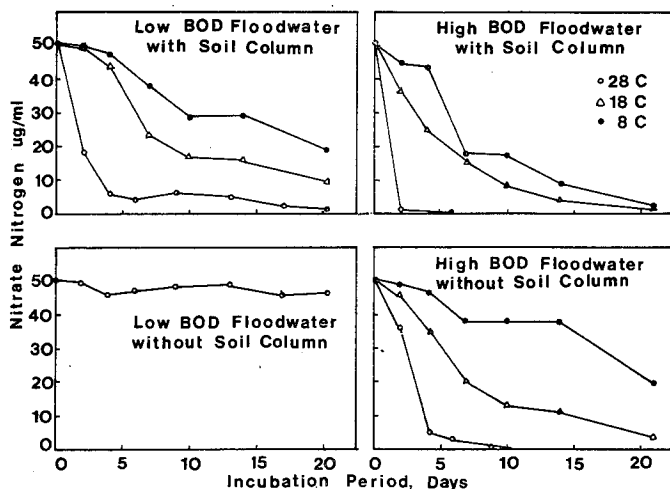


Fig. 4—Rate of flood water  $\text{NO}_3\text{-N}$  disappearance as influenced by temperature at an initial  $\text{NO}_3\text{-N}$  concentration of  $50 \mu\text{g N/ml}$ .

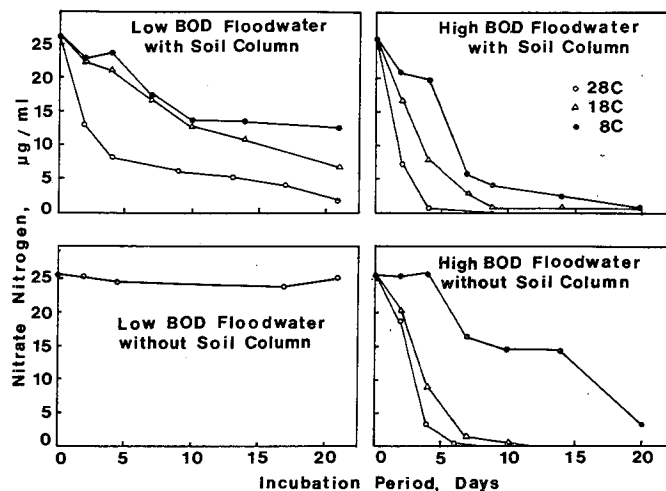


Fig. 3—Rate of flood water  $\text{NO}_3\text{-N}$  disappearance as influenced by temperature at an initial  $\text{NO}_3\text{-N}$  concentration of  $25 \mu\text{g N/ml}$ .

temperature ranges of 18 to  $28^\circ\text{C}$  and 8 to  $18^\circ\text{C}$ , respectively. Similarly,  $Q_{10}$  values for the  $\text{NO}_3\text{-N}$  removal from the high oxygen-demand flood water with an underlying soil column were 2.7 and 1.7 for temperatures 18 to  $28^\circ\text{C}$  and 8 to  $18^\circ\text{C}$ , respectively. Whereas,  $Q_{10}$  values for the  $\text{NO}_3\text{-N}$  removal rates in the treatment with high oxygen-demand flood water without an underlying soil column were 2.0 and 2.3 for temperatures 18 to  $28^\circ\text{C}$  and 8 to  $18^\circ\text{C}$ , respectively. These data indicate that  $Q_{10}$  values are not influenced by the diffusion of  $\text{NO}_3\text{-N}$  from the overlying flood water into underlying soil. Several research workers (Stanford et al., 1975b; Bailey, 1976) also observed similar  $Q_{10}$  values for the  $\text{NO}_3$  reduction in mineral soils.

#### Fate of Flood Water $^{15}\text{NO}_3\text{-N}$ in the Soil-Water System

Data on the fate of flood water  $^{15}\text{NO}_3\text{-N}$  in the soil-water system for various treatments are presented in Tables 4, 5, and 6. Reduction of  $^{15}\text{NO}_3\text{-N}$  to either  $^{15}\text{NH}_4\text{-N}$  or organic  $^{15}\text{N}$  was influenced by the incubation temperature. In all treatments,  $^{15}\text{NO}_3\text{-N}$  recovered was either as organic  $^{15}\text{N}$  or  $^{15}\text{NH}_4\text{-N}$  with  $<0.3\%$  remaining as  $^{15}\text{NO}_3\text{-N}$ . At the  $8^\circ\text{C}$  incubation temperature, labeled N recovery was 9.8% of added N in the soil column with low oxygen-demand flood water (Table 4) and 19.9% of added N in the treatment with high oxygen-demand flood water with an underlying soil column (Table 5). At the same incubation temperature, 17.1% of the  $^{15}\text{NO}_3\text{-N}$  was recovered as  $^{15}\text{NH}_4\text{-N}$  and organic N from the high oxygen-demand flood water with no underlying column. At 18 and  $28^\circ\text{C}$  incubation temperatures,  $^{15}\text{NO}_3\text{-N}$  recovered as  $^{15}\text{NH}_4\text{-N}$  and organic  $^{15}\text{N}$  in low oxygen-demand flood water with an underlying soil column was 7.4 and 3.7% of added N, respectively. However, no appreciable differences in the reduction of added  $^{15}\text{NO}_3\text{-N}$  to  $^{15}\text{NH}_4\text{-N}$  and organic  $^{15}\text{N}$  were observed in the treatment with high oxygen-demand flood water with or without an underlying soil column incubated at 18 and  $28^\circ\text{C}$ . Labeled  $^{15}\text{NO}_3\text{-N}$  recovered as  $^{15}\text{NH}_4\text{-N}$  and organic N in both treatments at 18 and  $28^\circ\text{C}$  was about 7 to 10% of added  $^{15}\text{NO}_3\text{-N}$ .

In all treatments, labeled N recovery data indicate

**Table 4—Distribution of flood water  $^{15}\text{NO}_3\text{-N}$  in the system containing low oxygen-demand flood water with an underlying organic soil column at the end of 21-day incubation period.**

Labeled N fraction	Temperature, °C		
	8	18	28
— % added $^{15}\text{NO}_3\text{-N}$ —			
<u>Flood water</u>			
$\text{NO}_3\text{-N}$	0.3	0.1	0.0
$\text{NH}_4\text{-N}$	1.8	1.4	0.1
Organic N	0.5	0.3	0.2
Total	2.6	1.8	0.3
<u>Soil column</u>			
$\text{NO}_3\text{-N}$	0.1	0.0	0.0
$\text{NH}_4\text{-N}$	3.4	2.7	ND†
Soluble organic N	3.4	0.1	ND
Organic N	0.4§	2.8§	3.4‡
Total	7.2	5.6	3.4
Total N in soil and water	9.8	7.4	3.7
N-unaccounted for	90.2	92.6	96.3

† Not determined.

‡ Includes ammonium-N and soluble organic N.

§ Does not include soluble organic N.

that denitrification (expressed as *N-unaccounted for*) was the dominant process in the removal of  $\text{NO}_3\text{-N}$  from flood water. Presence of a C source in the flood water slightly increased  $\text{NO}_3\text{-N}$  reduction to  $\text{NH}_4\text{-N}$  or immobilization into microbial biomass. Nitrate-N reduction to  $\text{NH}_4\text{-N}$  or organic N was more pronounced at 8°C incubation temperature as compared to 18 and 28°C incubation temperatures. At the low temperature,  $\text{NO}_3\text{-N}$  that diffused from the flood water into the soil column remained in the soil column for longer periods of time, thus allowing the organisms to interact with the added  $\text{NO}_3\text{-N}$ . However, at 18 and 28°C incubation temperatures,  $\text{NO}_3\text{-N}$  that diffused from the overlying flood water into the underlying soil column was rapidly used by anaerobic organisms during respiration, thus allowing very little interaction with other organisms. Some of the added  $\text{NO}_3\text{-N}$  which was immobilized during the early part of the incubation period was probably mineralized and appeared as  $\text{NH}_4\text{-N}$ . Most of the immobilized  $^{15}\text{NO}_3\text{-N}$  was present as soluble organic N. Chen et al. (1972) observed 7 and 3% conversion of added  $^{15}\text{NO}_3\text{-N}$  to organic N and  $\text{NH}_4\text{-N}$ , respectively, in lake sediments. These authors attributed the presence of labeled organic N to assimilatory  $\text{NO}_3\text{-N}$  reduction, and they concluded that  $^{15}\text{NH}_4\text{-N}$  formed either by  $\text{NO}_3\text{-N}$  immobilization with subsequent rapid mineralization or by fixation of  $^{15}\text{N}_2$  formed via denitrification. Re-

**Table 6—Distribution of flood water  $^{15}\text{NO}_3\text{-N}$  in the system containing high oxygen-demand flood water without an underlying soil column at the end of 21-day incubation period.**

Labeled N fraction	Temperature, °C		
	8	18	28
— % added $^{15}\text{NO}_3\text{-N}$ —			
$\text{NO}_3\text{-N}$	0.1	0.1	0.0
$\text{NH}_4\text{-N}$	5.8	2.1	0.2
Organic N	11.3	7.0	10.2
Total	17.1	9.1	10.4
N-unaccounted for	82.9	90.0	89.6

**Table 5—Distribution of flood water  $^{15}\text{NO}_3\text{-N}$  in the system containing high oxygen-demand flood water with an underlying organic soil column at the end of 21-day incubation period.**

Labeled N fraction	Temperature, °C		
	8	18	28
— % added $^{15}\text{NO}_3\text{-N}$ —			
<u>Flood water</u>			
$\text{NO}_3\text{-N}$	0.1	0.1	0.0
$\text{NH}_4\text{-N}$	1.8	1.2	0.4
Organic N	0.4	1.1	2.0
Total	2.3	2.3	2.4
<u>Soil column</u>			
$\text{NO}_3\text{-N}$	0.1	0.0	0.0
$\text{NH}_4\text{-N}$	3.3	2.1	ND†
Soluble organic N	13.3	0.7	ND
Organic N	1.0§	1.8§	5.5‡
Total	17.6	4.6	5.5
Total N in soil and water	19.9	6.9	7.9
N-unaccounted for	80.1	93.1	92.1

† Not determined.

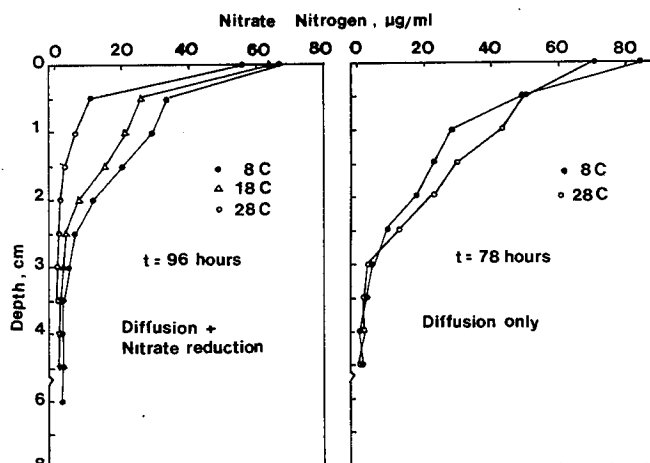
‡ Includes ammonium N and soluble organic N.

§ Does not include soluble organic N.

cently, Buresh and Patrick (1978) have shown that, under intense reducing conditions ( $E_h = -260$  mv), significant amounts of added  $\text{NO}_3\text{-N}$  were reduced to  $\text{NH}_4\text{-N}$  in a flooded soil incubated at 30°C. They concluded that  $\text{NO}_3\text{-N}$  was reduced to  $\text{NH}_4\text{-N}$  by a nonassimilatory pathway in which  $\text{NO}_3\text{-N}$  functioned as a terminal electron acceptor. Even though in our study organic soil columns were highly reduced ( $E_h = -250$  mv), very little flood water  $^{15}\text{NO}_3$  that diffused into the underlying soil column was reduced to  $\text{NH}_4\text{-N}$  and organic N at 28°C.

#### Distribution of Flood Water $\text{NO}_3\text{-N}$ in the Underlying Soil Column

Distribution of the flood water  $\text{NO}_3\text{-N}$  (initial  $\text{NO}_3\text{-N}$  concentration =  $100 \mu\text{g N/ml}$ ) that diffused into the underlying soil column at the end of 96 hours is shown in Fig. 5 for 8, 18, and 28°C incubation temperatures. Nitrate-N movement from the overlying flood water is



**Fig. 5—Distribution of flood water  $\text{NO}_3\text{-N}$  as influenced by temperature.**

governed by diffusion from the overlying flood water into the underlying soil column, and microbial reduction of  $\text{NO}_3\text{-N}$  in the soil column. At  $28^\circ\text{C}$ , the  $\text{NO}_3\text{-N}$  distribution profile was very shallow, indicating rapid reduction of  $\text{NO}_3\text{-N}$  that diffused into the soil column, whereas, at  $8^\circ\text{C}$ ,  $\text{NO}_3\text{-N}$  diffused deeper into the soil layer because of slower microbial reduction of  $\text{NO}_3\text{-N}$  at cooler temperatures. The  $\text{NO}_3\text{-N}$  distribution profile at  $18^\circ\text{C}$  was approximately midway between the  $\text{NO}_3\text{-N}$  distribution profiles measured at 8 and  $28^\circ\text{C}$ . The  $\text{NO}_3\text{-N}$  concentrations at the soil and flood water interface were 56, 64, and  $67\ \mu\text{g}\ \text{NO}_3\text{-N}/\text{ml}$  for incubation temperatures of 28, 18, and  $8^\circ\text{C}$ , respectively.

To demonstrate the effect of temperature on the diffusion of  $\text{NO}_3\text{-N}$  from the flood water into the underlying soil column,  $\text{NO}_3\text{-N}$  movement was measured at 8 and  $28^\circ\text{C}$  incubation temperatures in sterile conditions, where microbial activity was prevented. The data presented in Fig. 5 indicate that  $\text{NO}_3\text{-N}$  diffusion was slightly influenced by temperature, with deeper  $\text{NO}_3\text{-N}$  movement at 28 as compared to  $8^\circ\text{C}$ . Nitrate-N concentrations at the soil-water interface were 71 and  $84\ \mu\text{g}/\text{ml}$  for 28 and  $8^\circ\text{C}$ , respectively. These data indicate that, when microbial reduction was prevented,  $\text{NO}_3\text{-N}$  disappearance due to diffusion was slower. However,  $\text{NO}_3\text{-N}$  movement from the flood water into the soil was enhanced when microbial reduction of  $\text{NO}_3\text{-N}$  was active in the soil column. These data also show that when biological activity is low in the soil column (e.g., soils with low organic matter),  $\text{NO}_3\text{-N}$  can possibly diffuse into deeper soil layers of the soil column.

## SUMMARY AND CONCLUSIONS

Use of flooded organic soils for  $\text{NO}_3\text{-N}$  removal from agricultural drainage waters was evaluated under simulated soil column conditions. Flood water  $\text{NO}_3\text{-N}$  removal rates were measured as a function of initial  $\text{NO}_3\text{-N}$  concentration of the flood water, temperature, and energy source. Nitrate loss from flood water was described by first-order kinetics. Labeled  $\text{NO}_3\text{-N}$  was used to differentiate between denitrification and  $\text{NO}_3\text{-N}$  reduction to  $\text{NH}_4\text{-N}$  and organic N.

The following conclusions were drawn from this study: (i) flooded organic soils can be used as an effective sink for  $\text{NO}_3\text{-N}$  removal from drainage waters; (ii) flood water  $\text{NO}_3\text{-N}$  removal rate was influenced by temperature with average  $Q_{10}$  values ranging from 1.4 to 2.1 for 18 to  $28^\circ\text{C}$  and 2.0 to 2.5 for 8 to  $18^\circ\text{C}$ ; (iii) creating high oxygen demand in the flood water increased the  $\text{NO}_3\text{-N}$  removal rates; (iv) nitrate-N removal rate was increased with increase in the concentration of the flood water  $\text{NO}_3\text{-N}$ ; (v) more flood water  $\text{NO}_3\text{-N}$  was reduced to  $\text{NH}_4\text{-N}$  and organic N at  $8^\circ\text{C}$  as compared to 18 and  $28^\circ\text{C}$ ; and (vi) at low temperature ( $8^\circ\text{C}$ )

flood water  $\text{NO}_3\text{-N}$  diffused into deeper soil layers compared to high temperature ( $28^\circ\text{C}$ ).

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