APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Nov. 1986, p. 987–991
0099-2240/86/110987-05$02.00/0

Vol. 52, No. 5

Denitrification in Marl and Peat Sediments in the Florida Everglades

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Received 12 May 1986/Accepted 5 August 1986

The potential for denitrification in marl and peat sediments in the Shark River Slough in the Everglades National Park was determined by the acetylene blockage assay. The influence of nitrate concentration on denitrification rate and N_2O yield from added nitrate was examined. The effects of added glucose and phosphate and of temperature on the denitrification potential were determined. The sediments readily denitrified added nitrate. N_2O was released from the sediments both with and without added acetylene. The marl sediments had higher rates than the peat on every date sampled. Denitrification was nitrate limited; however, the yields of N_2O amounted to only 10 to 34% of the added nitrate when 100 μM nitrate was added. On the basis of measured increases in ammonium concentration, it appears that the balance of added nitrate may be converted to ammonium in the marl sediment. The sediment temperature at the time of sampling greatly influenced the denitrification potential (15-fold rate change) at the marl site, indicating that either the number or the specific activity of the denitrifiers changed in response to temperature fluctuations (9 to 25°C) in the sediment. It is apparent from this study that denitrification in Everglades sediments is not an effective means of removing excess nitrogen which may be introduced as nitrate into the ecosystem with supply water from the South Florida watershed and that sporadic addition of nitrate-rich water may lead to nitrous oxide release from these wetlands.

The Florida Everglades is a unique hydrographic, geologic, and biological environment which is important due not only to its vastness but also to the fact that it is a refuge for a number of endangered species (11). The Everglades environment is threatened by water availability problems in South Florida and the increasing possibility of the delivery of lower-quality water to the area (6).

The flow of water from Lake Okeechobee through the open glades is a natural feature of the Everglades ecosystem, which has been grossly modified by a system of canals and levees designed for flood control and water distribution (Fig. 1). The water distribution scheme, at present, is a system of canals, control structures, and pumping stations which supply water to the diked water conservation areas and the Everglades National Park (ENP). The northern reaches of this system are the site of heavy agricultural activity and correspondingly eutrophic waters (7). These waters, flowing south through the canals, are purified somewhat on route but still retain nutrient levels above those of Everglades marsh water.

In an undisturbed state, the nutrient levels in Everglades waters and sediments are low. Nitrate and inorganic phosphate concentrations are below 1 μM in surface waters and sediments (8; F. Parsons, Ph.D. dissertation, University of Miami, 1977). The water conservation areas serve as a partial buffer zone between the agricultural region bordering Lake Okeechobee and the ENP, but nutrient levels of up to 30 times background can be measured adjacent to the ENP at some sites. This canal water is the source water for the ENP. In addition, various water management alternatives under consideration, such as increased backpumping of canal waters into marsh areas (14), would increase nitrogen and phosphorus input into the water conservation areas (6) and possibly the ENP. It is therefore important to determine the possible effects of this influx of nutrients on the ecosystem of the Everglades.

Denitrification could potentially remove excess nitrate from water entering the protected areas of the Everglades. Because of this, we examined the capacity of microflora of peat and marl sediments from the Everglades for denitrification of excess nitrate added to the sediments.

MATERIALS AND METHODS

Sediment samples were taken from two sites in the ENP during the period from October 1984 through February 1985. The sites were chosen to represent the two major sediment types which occur in the freshwater portion of the Everglades: marl and peat (9). The marl site was accessible from a tram trail leading to an observation tower in the Shark River Slough. The peat site was reached by airboat. A 3-year record low temperature and large variation in water level at both sites occurred during the study period. Nearly complete drydown of the marl site occurred by late February.

The temperature and pH of the water and sediment at the sites were determined with a field pH-temperature meter (Corning model 4). The pH-temperature meter was not available on the first two sample dates, so the sediment temperature for these dates was estimated from the air temperature and the average difference between air, water, and sediment temperatures. The difference was calculated from data available from the National Park Service and our own observations. Dissolved oxygen in the water and sediment was measured with a YSI (model 57) oxygen meter. For estimation of oxygen in the interstitial water in the sediment, the dissolved oxygen probe (Y.S.I. model 5739) was protected with a fiberglass window screen. The probe was inserted ca. 10 cm into the sediment, and the interstitial water flowed through the screen to the electrode. Since no stirring or movement of the electrode was possible when it was placed in the sediment, the dissolved oxygen value was estimated as the value at which the meter drift changed from

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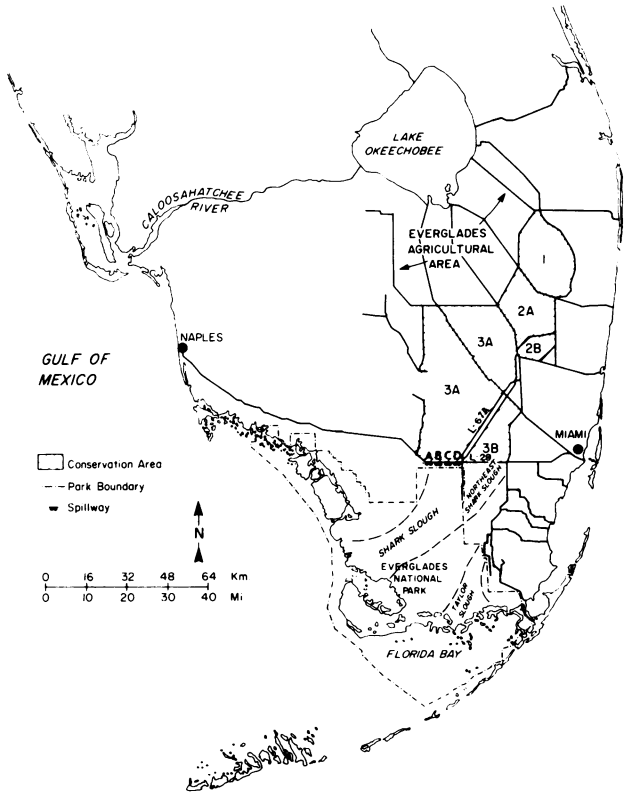

FIG. 1. Location of the Shark River Slough within ENP; water conservation areas (1, 2A, 2B, 3A, and 3B) and major South Florida canals.

a rapid decrease (due to electrode response time) to a slow drift (due to galvanic oxygen consumption by the electrode). The change in the rate of drift was readily apparent. Results with this method were compared with results from readings taken by the standard method in the water column and found to agree within 0.5 mg of O_2 per liter.

Sediment samples were taken in aluminum coring devices described previously (4). Five to eight cores were taken from each site on each day. Samples were transported to the laboratory in a cooler containing ice. Denitrification measurements were made within 3 h of sampling with the acetylene blockage assay (1, 21, 22). Sediments (upper 10 cm) were extruded from the cores, and the sediments from all the cores from each site were combined and placed into 500-ml Erlenmeyer flasks (100 ml per flask), which were purged with nitrogen and stoppered with a no. 10 rubber stopper. Flasks were prepared in duplicate for each treatment (e.g., control, added nitrate, added glucose). Data in the figures represent average values for the duplicate flasks. Acetylene, freshly generated from calcium carbide and water, was added to make a 10% atmosphere. Nitrate or other treatments were added by injection of 0.5 to 1 ml of concentrated solution into 100 ml of sediment slurry. The flasks were placed in an incubator-shaker (30°C, 100 rpm). Gas samples from the flasks (0.25 ml) were taken through the rubber stoppers with a 0.5-ml Glasspak syringe (Becton-Dickinson) and injected directly into a gas chromatograph for N_2O analysis. Rates were calculated by linear regression analysis of the data before N_2O evolution reached a plateau (1 to 3 h).

The gas chromatograph system (Varian model 3700) was equipped with electron capture detection. The gasses were separated with Porapak Q (Alltech Associates) at 90°C with methan-argon (1:9) as the carrier gas (20 ml/min). Acetylene was vented from the system to protect the nickel foil in the electron capture detector. Standards were prepared by serial dilution of N_2O (Liquid Carbonic Corp., Miami, Fla.) with nitrogen, and standard curves were prepared. Standards were injected prior to and after each experiment. Total N_2O evolved from the reaction was calculated with a dissolution constant determined by standard addition.

Pore water was separated from the sediment for nutrient analysis by centrifugation (12,000 × g, 30 min) and filtration (Gelman A/E glass fiber) of the supernatant. The filtrate was frozen until analysis. Nitrate and nitrate-nitrite analyses were run on a Technicon autoanalyzer by the cadmium reduction method (20). Ammonium was analyzed with an ammonium electrode (Orion model 95-10). Calibration of the electrode was made by both standard curve and standard addition (18) methods.

Sodium nitrate and phosphate were Baker analyzed reagent grade. Glucose was from Sigma Chemical Co. Nitrapyrin [2-chloro-6-(trichloromethyl) pyridine], an inhibitor of nitrification (19), was obtained from Dow Chemical Co. Nitrapyrin was dissolved in ethanol and added at a final concentration of 10 μM.

RESULTS

During the study period the sediment temperature varied from 9 to 25°C in the marl sediment and 16 to 21°C in the peat. The pH in the marl was 6.9 to 7.5, while in the peat it ranged from pH 6.2 to 6.7. The peat was only slightly acidic, which is characteristic of Everglades peats. The dissolved oxygen in the marl sediment was 3 to 4 mg/liter until the last sampling date in January, when it dropped to 0.8 and remained low until the final sampling date. This drop in oxygen content corresponded to the settling of the cyanobacterial periphyton mat onto the surface of the sediment as the surface water depth dropped to zero. Dissolved oxygen in the peat sediment ranged from 0.7 to 1.8 mg/liter. The nitrate levels in the sediment pore water ranged from 0.07 to 0.50 μM. There were no apparent trends in nitrate level from site to site or over time.

Both peat and marl sediments exhibited N_2O production when nitrate was added to the sediments (Fig. 2). The flasks accumulated N_2O even in the absence of added acetylene when 100 μM nitrate was added, but the N_2O level was not maintained in the flasks without added acetylene (Fig. 3). Nitrapyrin did not retard the rate of accumulation of N_2O in the absence of acetylene.

The marl sediment had a higher denitrification rate than peat sediments on all dates. The kinetics of the process were also different in the two sediment types. In the marl sediments denitrification was linear as a function of time. In the peat sediments the rate generally increased with time in nearly exponential fashion. N_2O generation in the presence of acetylene was never detected without added nitrate in either sediment.

There was an increase in the rate of denitrification when added nitrate levels were increased from 25 to 100 μM (1.0 to 1.8 nmol/min per 100 ml at 20°C and 2.5 to 3.3 nmol/min per 100 ml at 30°C). Increasing the assay temperature from 20 to 30°C approximately doubled the rate of denitrification in marl sediments, which were 20°C when collected, from 1.0 to 2.5 nmol/min per 100 ml at 25 μM and 1.8 to 3.3 nmol/min per 100 ml at 100 μM.

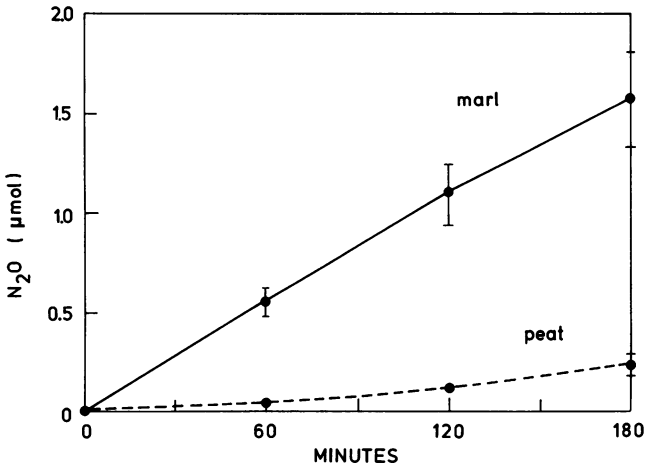

FIG. 2. Production of nitrous oxide from nitrate over time by peat and marl sediments from the Shark River Slough. The error bars are the standard error for replicate flasks. $NaNO_3$ (100 µM) was added to the sediments (100 ml) as a nitrate source. The sediment was incubated at 30°C.

The yield of N_2O from added nitrate (100 µM) was only about 15% in the peat and 34% in the marl (Table 1). Addition of an organic carbon source (glucose) after denitrification of the added nitrate (100 µM) had ceased did not affect N_2O production. Addition of more nitrate caused additional N_2O to accumulate and increased the yield to 80% (Table 1). Addition of 100 µM phosphate (pH 7.0) had no effect on the rate or on N_2O yield.

The initial concentration of NH_4^+ in the pore water of the marl sediment was about 70 µM. After incubation of the sediment in the incubator-shaker (24 h), the porewater ammonium concentration increased to 560 µM in the absence of any added nutrients. When 100 µM nitrate was added, the concentration increased to 692 µM. This difference was more than enough to account for the balance of the added nitrate after denitrification (80 µM). The difference was significant ($P < 0.05$, student's t test, $n = 5$). We did not observe significant increases in ammonium concentration in the peat sediment either with or without added nitrate.

Throughout the study period a correlation was apparent between marl sediment temperature at the time of sampling and the rate of denitrification measured in the lab under standard conditions (100 µM added nitrate, 30°C; Fig. 4). This correlation was not observed in the peat, but the range of temperature in the peat sediment was not as great. As the marl site dried down (arrow, Fig. 4), the denitrification rates increased with temperature (the temperature and rate lines converged) until the final sampling date (days 103 to 131, Fig. 4). The times when the rate was higher, when normalized to temperature, corresponded to the large drops in sediment oxygen concentration on the sampling dates.

TABLE 1. Influence of added nitrate and subsequent addition of glucose or nitrate on nitrate recovered as N_2O

Sediment	Initial nitrate added (µM)	Subsequent addition[a]	% Yield[b]
Peat	10	None	10
	100	None	16
	100	Glucose (3 mM)	14
Marl	10	None	18
	100	None	34
	100	Glucose (3 mM)	34
	100	Nitrate (100 µM)	80

[a] Added within 1 h after N_2O evolution had stopped.
[b] Measured as micromoles of N_2O per 100 ml of sediment.

DISCUSSION

Marl and peat sediments from relatively unpolluted regions of the ENP denitrified nitrate that was added in concentrations equal to the highest values encountered in the Everglades watershed. Removal of nitrate from the system as N_2O in our experiments, however, only accounted for 10 to 34% of the added nitrate. This is in contrast to observations in other freshwater marsh areas, where 90 to 95% of the nitrate was denitrified (2). Thus, this study indicates that in Everglades sediments, excess nitrogen that may be added as nitrate with inflowing water is probably not efficiently removed by denitrification but remains in some form and thus can alter the nutrient chemistry of the sediments.

It has recently been shown in similar anoxic experiments that nitrate is converted to ammonium, with the ratio be-

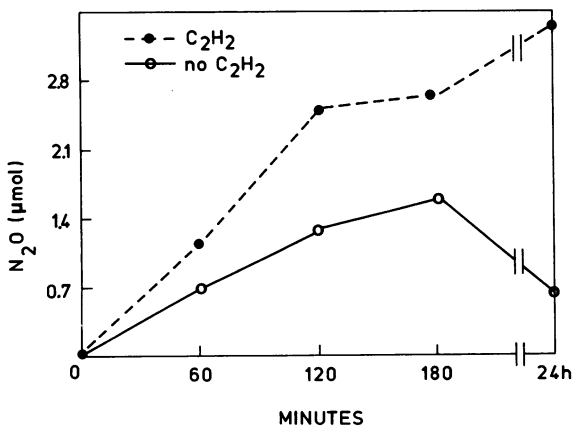

FIG. 3. Production of N_2O in the marl sediment with (●) and without (○) acetylene added to the atmosphere of the flask. $NaNO_3$ (100 µM) was added to 100 ml of sediment slurry, and the flasks were incubated at 30°C.

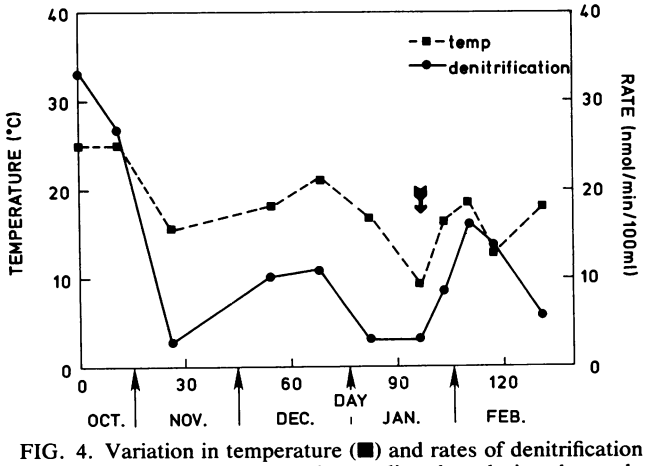

FIG. 4. Variation in temperature (■) and rates of denitrification (●) in the marl sediment on each sampling date during the study period. The bold arrow indicates the date on which there was no longer surface water.

tween denitrification and ammonium production determined by nitrate concentration (12). Our results concerning ammonium production in Everglades sediments, although not conclusive, are consistent with a large contribution of dissimilative nitrate reduction to total nitrate reduction in these sediments.

The rates of denitrification measured with added nitrate in these freshwater Everglades sediments were comparable to those measured by Oremland et al. (15) in intertidal sediments from San Francisco Bay. When the systems were saturated, the N_2O production from the systems were as follows: Everglades marl, 3 to 33 nmol/min per 100 ml of sediment; Everglades peat, 1 to 5 nmol/min per 100 ml; San Francisco Bay, 4 to 12 nmol/min per 100 ml. In contrast to San Francisco sediments, no N_2O production was detected at ambient nitrate concentrations in the Everglades sediments, probably because ambient nitrate levels are 10 to 180 times lower in the Everglades sediments than those measured by Oremland et al. (15). It is interesting that equivalent or greater potentials for denitrification were found in the areas with lower ambient nitrate concentrations.

The assay system was limited by nitrate availability, since second nitrate additions caused denitrification to resume. Second additions also increased the yield of N_2O. This could be due to adaptation or growth of the denitrifiers or to destruction of competing organisms by acetylene. Acetylene has been reported to inhibit clostridia (17). Some clostridia reduce nitrate to ammonia (5), and thus the acetylene may have inhibited a pathway which competes with denitrification. Glucose or phosphate addition did not influence the N_2O yield, suggesting that the denitrifiers were not limited in carbon source or phosphate. Nitrate limitation and the absence of phosphate limitation is opposite to the nutrient limitation of macrophytes in the Shark River Slough observed in marsh fertilization experiments (D. R. Walker, M. D. Flora, R. G. Rice, and D. J. Scheidt, manuscript in preparation). These experiments showed a marked increase in macrophyte production when phosphate was added to Everglades sediments and no increase when nitrate was added.

The production of N_2O in the absence of acetylene was not due to the production of N_2O by nitrifying bacteria operating at low oxygen levels (10), since nitrapyrin did not decrease N_2O production. The transient accumulation of N_2O in the absence of acetylene is thus probably due to kinetic differences between the individual steps of the pathway of denitrification (3). This effect could result in N_2O release from Everglades sediments if nutrient-rich waters were added sporadically to the system. This is a real possibility, since increased flow rates in the canals in the supply system caused by opening gates upstream leads to measurable increases in nutrient levels in waters in the southern areas of the watershed. Opening of upstream gates is used as a method of water level control.

The responses of the two sediment types to increases in nitrate concentrations were different. The marl sediment generally exhibited linear kinetics with time. Although the rates were lower in the peat, they increased with time. It appears that the sediment microflora in the marl have fully induced denitrification systems prior to nitrate addition, whereas in the peat the system is induced in response to nitrate. The induction of nitrate reductase is controlled by both oxygen and nitrate levels (13, 16). Anoxia can derepress nitrate reductase synthesis; however, nitrate may also be required. The induction of denitrification in the peat is probably not attributable to differences in oxygen concentrations in the sediment, since these were generally lower in the peat. The differences may be due to different rates of nitrate input into the two sites. Such wide variability in rates of nutrient cycling in different sediment types in the Everglades should be considered in planning discharge of canal waters into the park.

The influence of temperature which we observed in the laboratory studies was not sufficient to explain the variations in denitrification potential observed in relation to temperature of the sediment at the time of sampling. It would appear therefore that either the numbers or the specific activity of the population of denitrifiers in the marl sediment changes in response to temperature variations. This could be due to a direct temperature effect or to an effect of temperature on some other environmental parameter controlling denitrification rates. The variation of the denitrification potential in the marl sediment was further complicated by the drying of the site in winter. Once the periphyton mat was deposited on the sediment, oxygen concentrations decreased. This could have been due to decreased exposure of the sediment to oxygen or to oxygen demand caused by decomposition of organic matter in the mat. In any case, the denitrification potential, normalized to temperature, was high during this period. The large change in denitrification potential (15-fold) with a reasonably small temperature fluctuation (16°C) is somewhat surprising and may be due to greater than expected temperature sensitivity in bacteria in this subtropical environment.

In conclusion, only 10 to 35% of excess nitrate added to Everglades sediments was removed from the system by denitrification, and the balance of the added nitrogen remained in the system, probably as ammonium. In its natural state the denitrification potential in the marl sediment changed greatly in response to moderate temperature fluctuations. Both temperature variation and hydroperoid seem to play a role in determining the denitrification potential of the sediment. In addition we suggest that sporadic addition of nitrate-rich waters to the Everglades sediments may lead to N_2O emission from the area. While further, in situ studies of denitrification and nitrous oxide release from Everglades sediments are clearly required, consideration of these implications is important in planning the requirements for nutrient levels in supply water for Everglades wetlands.

ACKNOWLEDGMENTS

We thank Barrie Taylor for the use of equipment, helpful suggestions, and review of the manuscript. We also thank John Martin for help with the denitrification assay.

This work was supported by a grant from the Everglades National Park, South Florida Research Center.

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