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ABSTRACT

Sediment denitrification potential from two sites in the Elizabeth River estuary was studied over a nine-month period using the acetylene blockage method. Rates of microbial processes in this environment are of interest because of the high concentration of toxics present in some parts of the system. Highest rates were found in the highly polluted Southern Branch of the Elizabeth River with nitrate amended sediment ranging from 2-262 nmol N_2O/h per 20ml of sediment and exhibiting maximal rates during spring and fall. Rates in the Main Stem of the Elizabeth River were lower, with less than 1-85 nmol N_2O/h/20ml in nitrate amended sediment, and maxima in late fall. Unamended sediment from the Southern Branch denitrified in spring (2-131 nmol N_2O/h/20ml) and fall (1-124 nmol N_2O/h/20ml) only. Main Stem unamended sediment denitrified only minimally in the spring. Sediment denitrification potential was independent of temperature and dissolved oxygen in the water column. Comparison of phytoplankton abundance values and potential denitrification rates suggest that denitrification potential may be stimulated by phytoplankton bloom senescence. Comparison to other published studies shows sediment denitrification potential in the Elizabeth River to be within the range of values reported for other environments.

INTRODUCTION

Denitrification may serve as a mechanism for removal of excess nitrate in eutrophic aquatic environments. Generally denitrification rates are seen to be nitrate limited (Gordon et al. 1986; King and Nedwell, 1985; Oremland et al. 1984), so that in a eutrophic environment denitrification would be expected to increase. However, the presence of toxics could inhibit microbial processes in the sediment, including denitrification.

Pseudomonas and *Alcaligenes* species are considered the major contributors to denitrification in aquatic sediments. In addition, strains of *Bacillus, Corynebacterium, Micrococcus, Achromobacter,* and *Nitrosomonas* denitrify, indicating a wide diversity in bacterial denitrifiers (Knowles, 1982; Payne, 1973). Since denitrification is carried out by many sediment bacteria, it may be viewed as an indicator of the status of the sediment microbial population.

The Elizabeth River is an interesting environment for the study of microbial processes in a heavily industrialized region. Contamination from heavy metals and other toxic compounds such as polynuclear aromatics poses a serious problem to this estuary, and it is considered to be a system under stress (V.S.W.C.B. Gen. Inf.

Bull #557, 1984). The multitude of industries surrounding the river include shipbuilding, naval operations, waste treatment plants, coal facilities, chemical facilities, and power generating plants. The effects of such contamination and industrial activities on microbial processes in this system have not been assessed.

In this study we determined denitrification potentials at two sites in the Elizabeth River over a nine-month time period using the acetylene blockage method. To our knowledge this is the first such study in the Elizabeth River estuary. In order to determine factors controlling sediment denitrification potential, rates were compared to temperature, and dissolved oxygen in the water column. In addition, denitrification potentials at one site were compared to phytoplankton abundance in the water column.

METHODS

Sites and Sampling

Estuarine sediment was obtained from two sites in the Elizabeth River (Figure 1). Sediment was collected using a Ponar Grab (Wildco Instruments) and placed in sterile glass jars for transportation back to the laboratory. Water depth was between 0.9-1.8 m. One site was in the upper reaches of the Elizabeth River Southern Branch, adjacent to a nitrate fertilizer plant. This was an organic rich sediment. In a previous study, water column nitrate at the site was measured at 6.1 μM (Alden et al. 1988). Sediment from the second site was taken from the lower reaches of the Elizabeth River in the Main Stem, behind the docks of Norfolk International Terminal. The sediment from this site had patchy areas with relatively high sand content. Water column nitrate at this site was 0.5 μM (Alden et al. 1988).

In the laboratory, the sediment was homogenized and diluted with surface water samples (4:1, sediment:water, vol:vol) from each respective site. The slurry was then dispensed in 20 ml portions (graduated cylinder) into sterile 125 ml Erlenmeyer flasks which were then sealed with rubber stoppers and gassed with N_2 for 5 min to obtain anaerobic conditions. Duplicate flasks were prepared for each condition. Acetylene (Union Carbide) was added through the rubber stoppers (which had wells cored out of the top 2/3 portion) by injection to the headspace gas using a 20 ml syringe (Stylex) for a final concentration of 10% (Taylor, 1983). In the last two experiments acetylene was freshly generated in a separate flask by the reaction of calcium carbide and water and added as above. Nitrate additions in the form of KNO_3 (10 mM solution) were made by injection into the slurry to obtain a 100 μM concentration in each flask. Sediments were incubated within 3 hours of collection in an incubator-shaker set at 100 rpm and 26°C. Incubation time (time = 0) began with the addition of potassium nitrate (executed immediately after addition of acetylene) in nitrate amended flasks, or, in flasks with ambient nitrate concentrations immediately after addition of acetylene.

For phytoplankton determination, two composite water samples of 15 liters each were taken above and below the pycnocline, using an intake hose and shipboard pump, at a mid-channel station in the Southern Branch, monthly from February through December 1989 (Figure 1). A 500 ml water sample was then taken from each composite sample and preserved with Lugols solution for phytoplankton analysis. A settling and siphoning procedure followed to obtain a
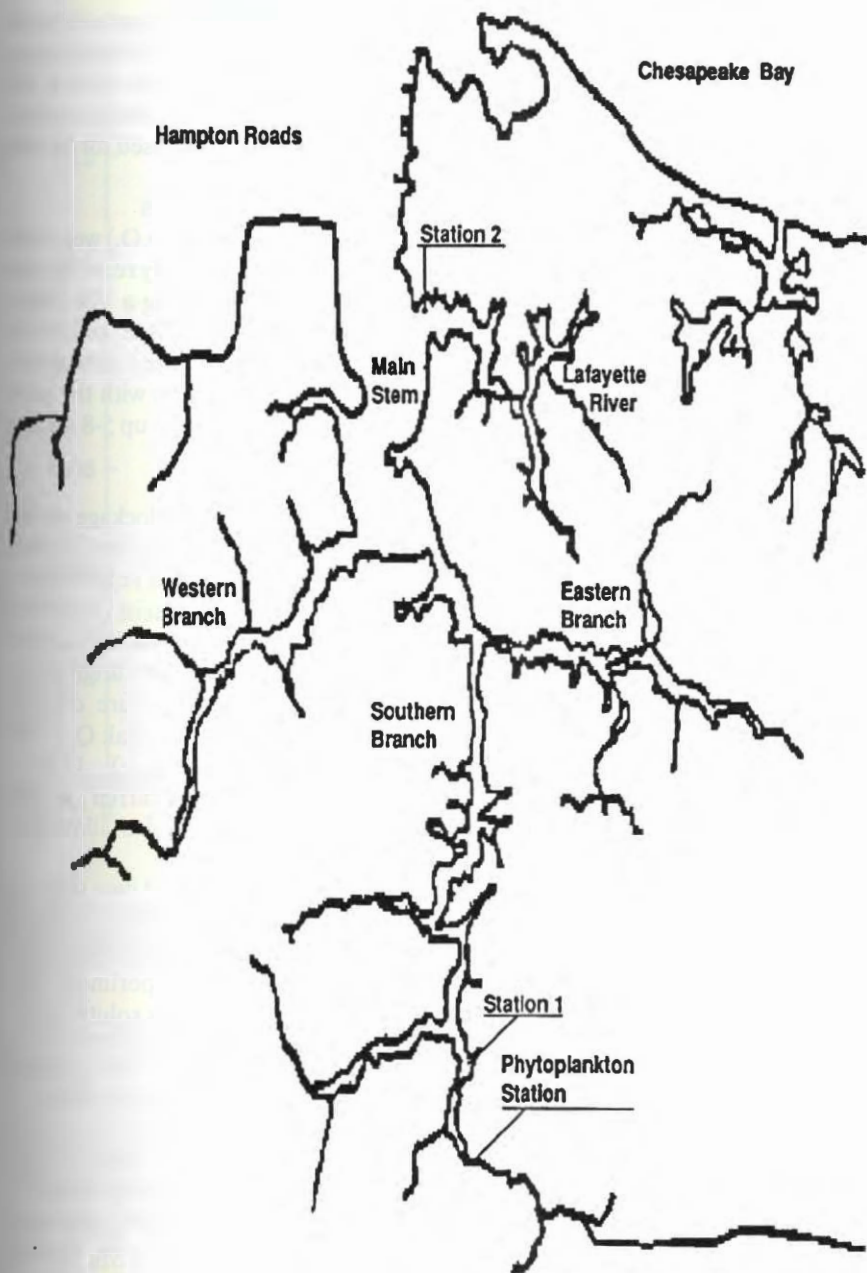
FIGURE 1. Location of sediment sampling sites for the denitrification assay, collection station for phytoplankton analysis, and major tributaries.

20 ml concentrate that was transferred to a settling chamber for examination with an inverted plankton microscope. The entire sample was scanned at 125x for counts of larger net species. A random field and minimum count basis was used at 315x for microplankton and 500x for nanoplankton to obtain an 85% accuracy estimate for these two categories. Mean values of replicate samples were used for the final counts.

Temperature, Salinity, and Dissolved Oxygen Measurements

Measurements of temperature, salinity, and dissolved oxygen (D.O.) were made at the sites before or following collection. Temperature and salinity readings were taken with a YSI S-C-T Meter (model 33) and D.O. readings using a YSI Oxygen Monitor (model 54A) fitted with a Clarke electrode. Both surface and bottom readings were taken for each measurement. Surface readings were taken with the probe immediately below the water surface and bottom readings with the probe directly above the bottom. This was achieved by pulling the probe up 5-8 cm after contact with the bottom. Only bottom readings were analyzed.

Denitrification Measurements and Calculations

Nitrous oxide determinations were made using the acetylene blockage method (Dodds and Jones, 1987; Gordon et al. 1986; Jorgensen, 1986, King and Nedwell, 1985). Corrections for N_2O in solution were made by injecting a representative amount of N_2O to the gas phase of a flask with deactivated sediment (autoclaved and corrected for water loss) and monitoring the subsequent decrease in headspace nitrous oxide. Headspace nitrous oxide concentrations were measured using a Varian 3600 gas chromatograph fitted with a [63]Ni electron-capture detector. Samples of the gas phase (0.1 ml) were injected into a 1.84m Porapak Q column set at $60^{\circ}C$ using a 0.5ml Glaspak syringe (Becton and Dickinson). Detector temperature was at $300^{\circ}C$ and injector temperature at $250^{\circ}C$. The carrier gas (95% argon 5% methane) was set at a flow rate of 30 ml/min. A valve allowed for acetylene venting to prevent damage to the detector.

Nitrous oxide concentration in the headspace gas was measured over time, and rates were calculated using linear regression analysis. Standards were prepared by dilution of 1% N_2O into flasks purged with N_2. The smallest concentration was prepared by serial dilution in the same manner as above. Two experiments were carried out in which second nitrate additions or glucose (injected as solution) were made to sediments in which denitrification had slowed or stopped.

RESULTS

Southern Branch Station

Temperature readings ranged from 11°-$16^{\circ}C$ in April and December to $32^{\circ}C$ in late July. Dissolved oxygen levels were lowest in August (2.9 ppm) and highest in April and December (7.0, 8.9 ppm, respectively). Denitrification rates were independent of temperature ($r = -0.22$) and oxygen ($r = 0.43$) in the overlying water. Figure 2 shows a typical result of the denitrification assay.

Potential rates exhibited maxima in spring and in autumn (Figure 3). Unamended sediments denitrified in late spring (131 nmol N_2O/h per 20ml sediment) and early autumn (124 nmol N_2O/h/20ml), with lower rates than amended sediments, except on Sept. 27 when ambient and amended sediments denitrified at approximately equal rates. The maxima for amended sediments, in nmol N_2O/h
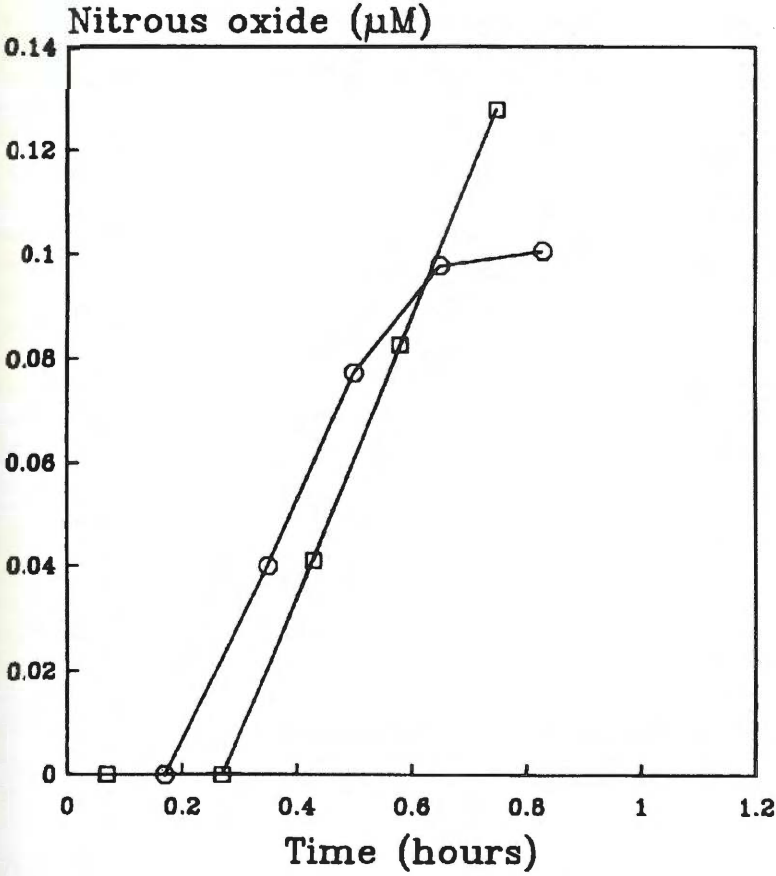
FIGURE 2. Results of a typical denitrification assay (week 26) from (CIRCLE) the Southern Branch and (SQUARE) Main Stem nitrate amended sediment. KNO_3 (0.2 ml, 10 mM solution) was added to 20 ml sediment. Results represent mean of duplicate flasks incubated at $26^{\circ}C$.

per 20ml sediment, were 262 on May 24, 117 on Sept. 27, 107 on Oct. 11, and 159 on Dec. 6. The rates ranged from 1-131 nmol N_2O/h/20ml in unamended sediment and 2-262 nmol N_2O/h/20ml in amended sediment. Sediments in which denitrification had slowed or stopped resumed denitrification following a second addition of nitrate. Sediments receiving glucose did not respond.

The dominant contributor to phytoplankton biomass in this region was the diatoms (Bacillariophyceae). Data showing diatom abundance and denitrification potential are represented in Figure 4.

Main Stem Station

Temperature and D.O. readings for this site were similar to the Southern Branch station, though for two dates (6/14, 7/12) D.O. readings are not available due to equipment failure. Denitrification rates in Main Stem sediment do not correlate to water column temperature ($r = -0.13$) or D.O. ($r = -0.08$).
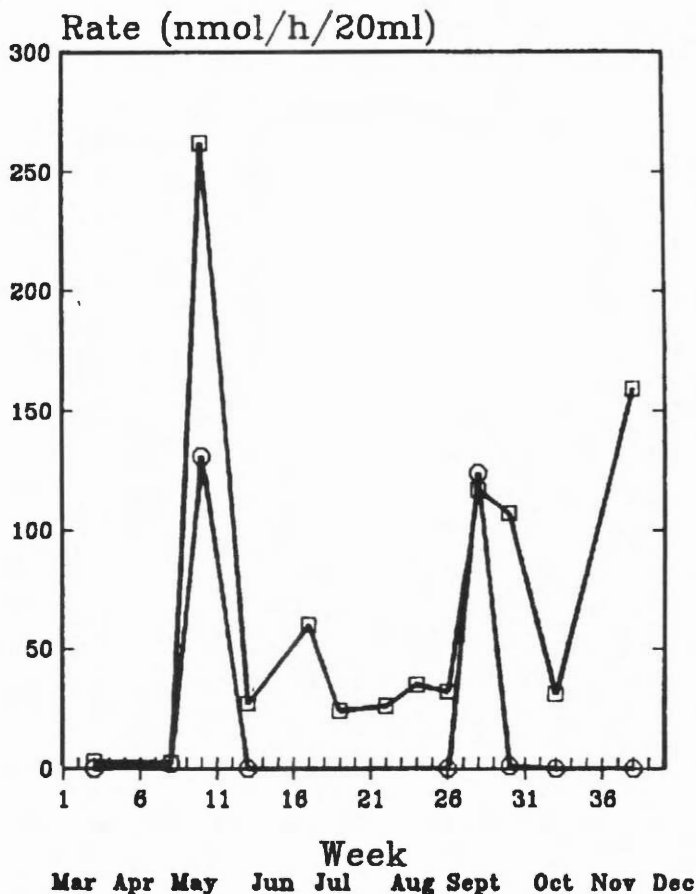
FIGURE 3. Variation in rates of nitrous oxide production during the study period starting with week 1 (March 19) in the Southern Branch. Legend is as follows: (CIRCLE) flasks with ambient nitrate concentrations, (SQUARE) flasks with added nitrate.

The overall potential rates for Main Stem sediment were lower than those for the Southern Branch. Only nitrate amended sediments denitrified (Figure 5), except on May 11 when unamended sediment produced N_2O at a minimal rate. The sediment had a gradual increase in denitrification potential with the progression of summer, then a relatively marked increase during autumn. The maxima for this site occurred in early fall (85 nmol N_2O/h per 20ml sediment on Sept. 27, 77 nmol N_2O/h/20ml on Oct. 11) and early December (58 nmol N_2O/h/20ml on Dec. 6). Potential rates (amended sediment only) ranged from less than 1-85 nmol N_2O/h/20ml. As in Southern Branch sediment, subsequent addition of glucose had no effect, whereas addition of nitrate caused the sediment to resume denitrification. The sediment did not denitrify on May 24, even with added nitrate.
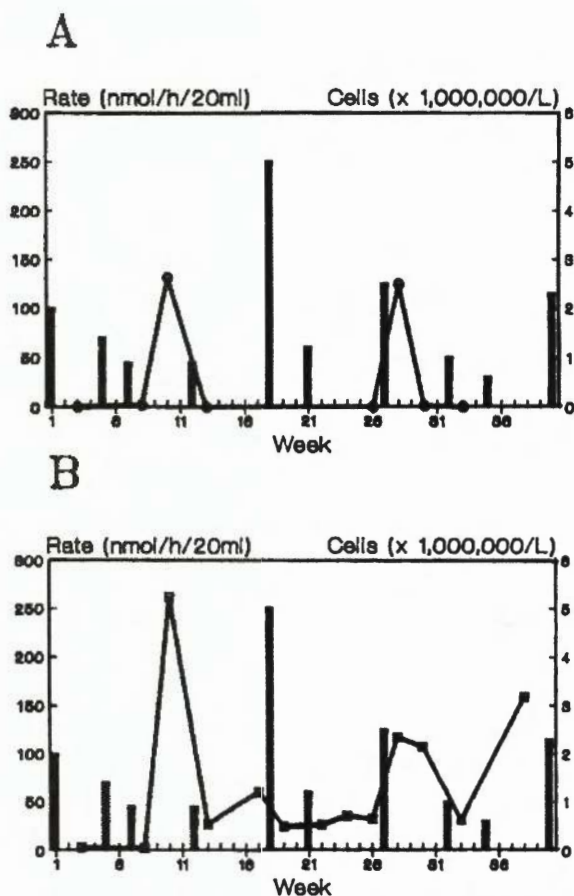
A

B

FIGURE 4. Denitrification potential in Southern Branch sediment (line) and Bacillariophyceae numbers (bars), for (a) sediment with ambient nitrate levels, and (b) sediment with added nitrate.

DISCUSSION

Overall the rates for the Southern Branch were greater than those for the Main Stem, with much variability of potential denitrification rates during the study period. In contrast to Gordon et al. 1986, rates did not show the expected variability with respect to seasonal changes. This is consistent with the findings of other studies (Anderson, 1977; Caveri and Phelps, 1977). The rates did not increase with increasing water temperatures and decreasing levels of dissolved oxygen as expected. The Southern Branch even exhibited high potential rates during low temperatures and high D.O. levels (May 24, Oct. 11, Dec. 6; D.O. and temperature data not shown). The maxima in spring and fall both occurred at intermediate levels (18-25°C, 5-6 ppm D.O.) Rates for both Southern Branch and Main Stem sediment were usually nitrate limited, the exception occurring on Sept. 27 (Southern Branch), when amended and unamended sediments denitrified at nearly equal rates, indicating saturation of the system.
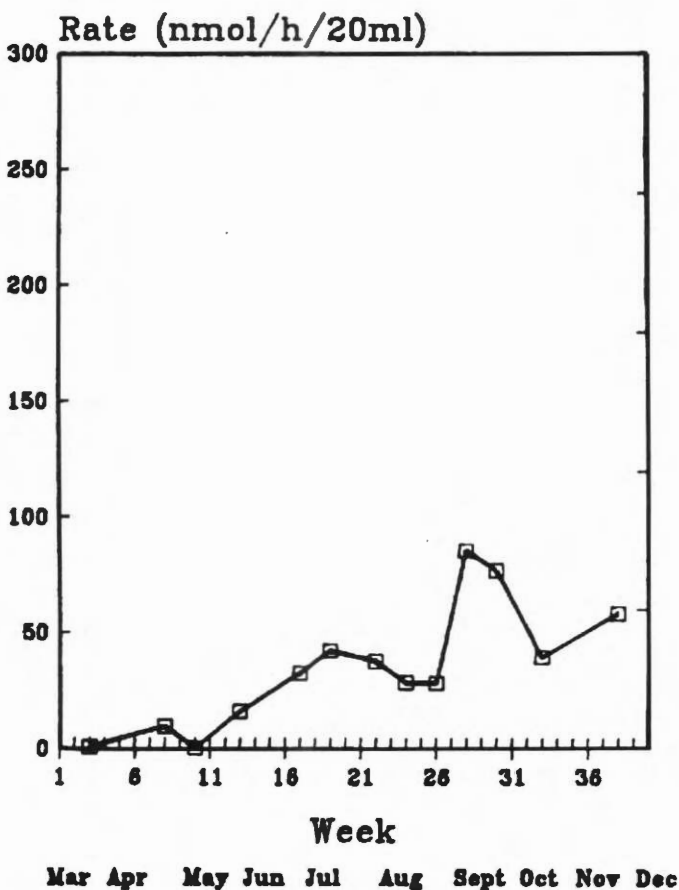
FIGURE 5. Variation in rates of nitrous oxide production in nitrate amended Main Stem sediment, starting with week 1 (March 19). Sediment with ambient nitrate levels denitrified only minimally in the spring (not shown).

The rates from Southern Branch sediment were similar to those reported by Oremland et al. 1984 in San Francisco Bay sediment. In nitrate amended sediments the potential rates were 32-190 nmol $N_2O/h/20ml$ (San Francisco Bay) and 2-262 nmol $N_2O/h/20ml$ (Southern Branch). For sediments with ambient nitrate concentrations having undergone comparable treatments, the rates were 5-80 nmol $N_2O/h/20ml$ (San Francisco Bay) and 1-131 nmol $N_2O/h/20ml$ (Southern Branch).

The potential rates from the study by Gordon et al. in Everglades peat sediment were comparable to the rates from Main Stem sediment (nitrate amended reported in this study. The rates were 12-60 nmol $N_2O/h/20ml$ (Everglades peat and 1-85 nmol $N_2O/h/20ml$ (Main Stem). Rates reported for marl sediment in the Everglades, with rates ranging 36-396 nmol $N_2O/h/20ml$, surpassed those of the eutrophic Southern Branch (2-262 nmol $N_2O/h/20ml$).

Gordon et al. (1986) reported increased denitrification rates when water levels receded and a periphyton mat came to rest on the sediment. This was suggested

to be due to input of organic material from the deposition of the cyanobacterial mat. We suspected therefore that the productivity of phytoplankton, which constitute the major autotrophic component of the Elizabeth River (O'Reilly and Marshall 1988), could influence the denitrification potential of the sediment.

The spring and fall maxima of both amended and unamended sediments appear between peaks in phytoplankton abundance in the Southern Branch region (Figure 4). A possible explanation for this effect is low availability of nitrate during bloom periods. Another possibility is that as the phytoplankton blooms recede and particulate organic matter is deposited on the sediment, N mineralization and nitrification in combination contribute to increasing sediment nitrate levels, thus increasing denitrification potential.

Denitrification potential is also dependent on the activities of other nitrate-utilizing biochemical pathways. Studies have found that denitrification competes with nitrate ammonification (dissimilatory reduction) and nitrate assimilation, which deplete nitrate levels (Jorgensen, 1986; Rher and Klemme 1989; Wyer and Hill, 1984). Studies on marine sediments indicate that nitrate ammonification is maximal in late summer when denitrifying processes are minimal (Jorgensen, 1986), and that equal reduction of nitrate to ammonium and nitrous oxide may occur (King and Nedwell, 1985). These competing pathways may have a small effect on denitrification potential with elevated nitrate levels, but they become significant competitors in nitrate limiting conditions. Low denitrification potentials observed during the summer in this study may therefore reflect successful competition for available nitrate by other nitrate utilizing pathways.

In Main Stem sediment, the overall diminished rates (compared to the Southern Branch) could be attributed to the generally lower nutrient content of the station and the higher flushing characteristics of this area. This site also displayed patchy areas of extremely sandy sediment, possibly explaining the absence of denitrification activity on May 24.

In summary, Southern Branch spring and fall maxima in denitrification potential appear to correspond with the decline of phytoplankton blooms in the water column. These data suggest an interaction between denitrification potential at this site and phytoplankton production. Comparison of rates to other environments in the Everglades National Park and in San Francisco Bay indicates that sediment denitrification potential in this stressed water system is within the range of reported values for both polluted and pristine environments. Denitrification potential in the sediments is, therefore, maintained in the presence of toxics in the sediment. Further studies are needed in order to determine the effect of contamination in this estuary on other microbial processes and the general influence of phytoplankton productivity on sediment denitrification rates and potential in estuarine systems.

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