

ABSTRACT

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Nitrogen is a key nutrient in the eutrophication of coastal and estuarine systems. In shallow water systems, sediment recycling can be an important source of nutrients for phytoplankton growth. The balance between nitrogen recycling and denitrification regulates the importance of sediments as a nitrogen source. To assess controls on denitrification, we conducted intensive seasonal measurements of sediment water exchange and denitrification using sediment core incubations. Peak rates of denitrification were observed in fall and spring ($>100 \mu\text{mol N}_2\text{-N m}^{-2} \text{ h}^{-1}$) followed by a decrease to $10 \mu\text{mol N m}^{-2} \text{ h}^{-1}$ in summer. Although denitrification rates were stimulated by labile organic carbon additions from the water column, the overall efficiency of the process sharply declined as temperature increased and bottom water O_2 declined. Macrofauna activity was shown to enhance sediment transport of O_2 by >5 fold, increase organic matter decomposition and maintain a high rate of denitrification efficiency.

NITROGEN CYCLING AND CONTROLS ON DENITRIFICATION IN
MESOHALINE SEDIMENTS OF CHESAPEAKE BAY

By

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Preface

Nitrogen has been identified as key nutrient in the eutrophication of coastal and estuarine systems. These shallow water environments are impacted by many sources of nitrogen including groundwater discharge, land runoff, atmospheric deposition, and sewage. Once nitrogen enters the water column, given sufficient light and nutrients, growth of phytoplankton is stimulated. Many coastal and estuarine systems have been shown to be N limited in terms of phytoplankton production, and the over fertilization of these systems has caused a decline in ecosystem health. Phytoplankton blooms decrease water column light transparency and inhibit the growth of benthic algae and submerged aquatic grass through light limitation. In shallow water systems, dead phytoplankton cells rapidly sink and are decomposed in the sediment. This decomposition can be an important process that promotes and maintains hypoxic/anoxic bottom water conditions in many shallow water systems.

Dead phytoplankton cells that settle to the sediment decay and nutrients are regenerated through heterotrophic processes. The remineralized N that is formed during this decomposition can either be recycled back to the water column as dissolved inorganic nitrogen DIN ($\text{NO}_3^- + \text{NO}_2^- + \text{NH}_4^+$), dissolved organic nitrogen DON, or be denitrified to N_2 gas (Figure P-1). The NH_4^+ that is produced through ammonification can be released back to the water column or be transformed into NO_3^- through the aerobic chemoautotrophic process of nitrification. Heterotrophic denitrifying bacteria use NO_3^- as a terminal electron acceptor during the breakdown of organic matter and produce N_2 gas as an end product. Nitrification and

denitrification are often closely coupled, providing efficient removal of remineralized N. Nitrate that is not denitrified can also be transported back to the water column. Some DON can be lost directly to the water column or decomposed in the sediment. Measurements of DON flux from the sediment often do not show consistent trends and in many cases DON does not appear to be a significant source or sink for N.

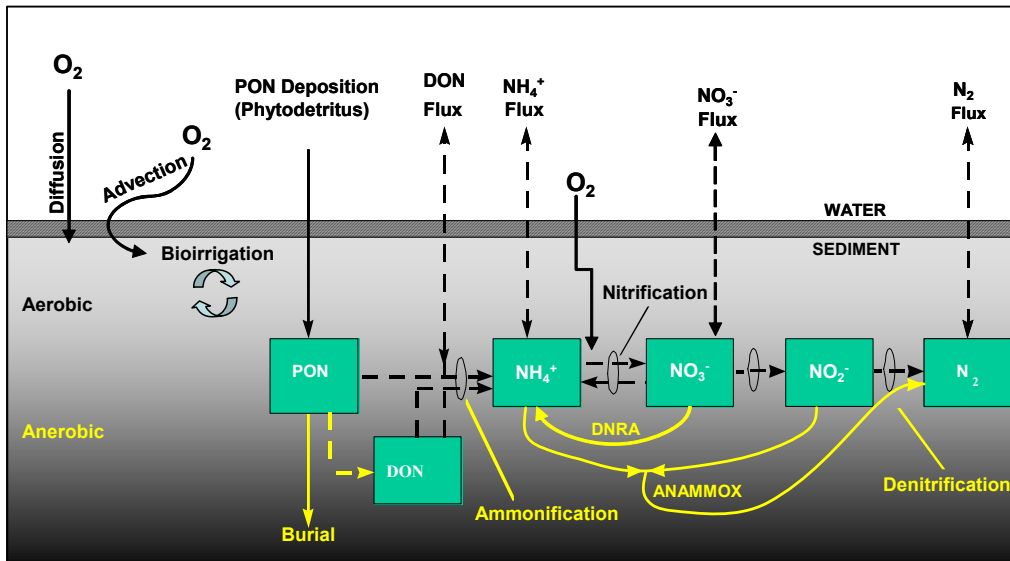


Fig. P-1. Conceptual diagram of the nitrogen cycle. Particulate organic matter settles to the sediment surface and is decomposed. Remineralized N can be buried, recycled back to the water column, or denitrified. Oxygen dependant nitrification can be closely coupled to denitrification leading to highly efficient removal of N as N₂ gas. In sulfidic sediments nitrification can be inhibited and DNRA may out compete denitrifiers for NO₃⁻. Bioirrigation can play an important role in the nitrogen cycle by oxygenating the sediment, preventing the buildup of H₂S, enhancing denitrification and ANAMMOX.

Herein we define denitrification as conventional heterotrophic denitrification, anammox and all other biogenic process that convert fixed-N to N₂.

Denitrification has been identified as the most important process removing nitrogen from many estuaries. The recycling of DIN from sediments can be an important source of N fueling phytoplankton blooms. Many factors can influence the balance between DIN release from the sediment and N removal via denitrification including the depth penetration of O_2 , organic carbon loading, bioirrigation, and pore water H_2S concentrations. Past studies of denitrification have been hampered by poor techniques that were time consuming or involved indirect measures of the process using chemical blocks. For this reason, few intensive studies of denitrification have been conducted and most studies report data from a number of locations in a system sampled a single time. In this study, we use membrane inlet mass spectrometry (MIMS) to obtain a direct measure of denitrification on flux incubations. The MIMS technique has the advantage of measuring all pathways to N_2 production including anammox (Thamdrup & Dalsgaard 2002) and oxidation of NH_4^+ by MnO_2 (Luther et al. 1997). The logistic efficiency of our incubation methods allows us to examine seasonal changes in the nitrogen cycle at multiple locations.

The progress of eutrophication often leads to reduced bottom water O_2 and an increased organic carbon load to the sediment. These two factors can cause biogeochemical changes in the sediment leading to; 1) a decrease in the depth penetration of O_2 ; 2) lower rates of coupled nitrification/denitrification and denitrification efficiency; and 3) a greater proportion of carbon metabolism via sulfate reduction. As O_2 penetration decreases there is a shift from oxic/suboxic metabolism to sulfate reduction and often an increase in pore water H_2S concentrations. Pore water sulfides have been shown to limit nitrification and

promote dissimilatory NO_3^- reduction to NH_4^+ (Figure P-1). The overall impact of these biogeochemical changes is a shift from sediments being a sink for fixed N through denitrification to a source of N as DIN is recycled back to the water column.

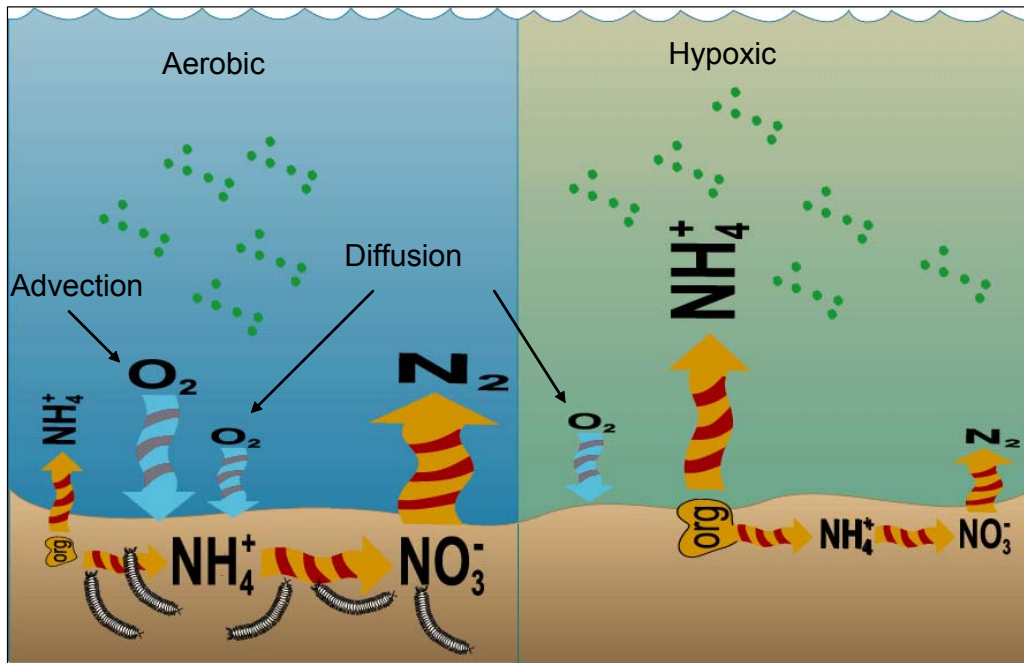


Fig. P-2 Conceptual diagram showing the influence of macrofauna on the nitrogen cycle. Under aerobic conditions (left panel) O_2 is transported into the sediment by both molecular diffusion and advection via bioirrigation. Under aerobic conditions sediments are more oxidized and organic matter is rapidly decomposed. Under low water column O_2 conditions (right panel) advection of O_2 may be lost and the depth of sediment O_2 penetration will decrease. This decrease in supply of O_2 will result in a greater proportion of DIN recycling.

Another consequence of reduced bottom water O_2 is the loss of habitat suitable for macrofauna (Figure P-2). Many species of macrofauna irrigate the sediment by flushing O_2 rich water through their burrows. The overall effect of bioirrigation is to increase the surface area of sediment where oxic and suboxic process can occur and to diminish the buildup of pore water sulfide. Bioirrigation

should have a positive impact on the removal of fixed N from the sediments by promoting higher rates of nitrification. Polychaetes can greatly enhance the rate of sediment organic matter decomposition and decrease the amount of carbon available for sulfate reduction.

In this study we will follow seasonal changes in nutrient flux and denitrification with emphasis on factors controlling the rate of denitrification and shifts in denitrification efficiency. Intensive sampling during the spring bloom will provide insight into how the sediments respond to labile organic matter settling to the bottom. We will also evaluate the rate of bioirrigation and the depth penetration of O₂ to determine how these factors may influence the nitrogen cycle.

Dedication

Kabrena Owens

My best friend and the love of my life

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I thank Pete Sampou for believing in my abilities and teaching me the basics of sediment biogeochemistry during my first years at Horn Point. I would like to express appreciation for those who assisted me in the laboratory and field work: Lora Pride, Erica Kiss, Jeff Alexander, Chris Chick and Teresa Coley. I especially thank Jack Seabrease for his help in the design and construction of flux chambers and sampling devices. I appreciate Jack's positive can do attitude that seems to be contagious. I thank my mother and father for unconditional love and encouragement in all my endeavors. Lastly, I would like to thank my wife and daughters who have been supportive and understanding when this work consumed nights and weekends and took priority over them.

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Chapter 1: Denitrification Efficiency in Chesapeake Bay Sediments

Abstract

Spatial and temporal variations in sediment-water flux of dissolved inorganic nitrogen (DIN), dissolved gases N_2 and O_2 were measured for the Choptank River, a subestuary of the Chesapeake Bay USA. Membrane Inlet Mass Spectrometry was used as a high precision direct measure of N_2 production in sediment core incubations. Peak rates of denitrification were observed in fall and spring ($>100 \mu\text{mol } N_2\text{-N m}^{-2} \text{ h}^{-1}$) followed by a sharp decline to $10 \mu\text{mol } N \text{ m}^{-2} \text{ h}^{-1}$ in summer. Coupled nitrification/denitrification was identified as the main pathway for the production of N_2 gas in the sediment. Although denitrification rates were stimulated by labile organic carbon additions from the water column, the overall efficiency of the process sharply declined as temperature increased and bottom water O_2 declined. Consequently, there was a transition from almost complete remineralization of nitrogen as N_2 gas in winter to nearly 100% recycling of NH_4^+ back to the water column in summer. Denitrification efficiency was shown to be sensitive to bottom water oxygen concentrations even in the absence of hypoxia or anoxia.

Introduction

Increased nitrogen loading to coastal ecosystems has been identified as a key factor in the eutrophication of coastal and estuarine ecosystems (Taylor et al 1995). Nitrogen is often cited as the limiting nutrient in coastal ecosystems (Fisher 2006) and considerable efforts have been dedicated to understanding the role of internal cycling processes in the fate of nitrogen. In ecosystems such as the Chesapeake Bay, the major N sinks include burial in sediments, denitrification, and export to the ocean (Boynton et al. 1995). Both fish harvest (Boynton et al. 1995) and the efflux of ammonium are much smaller terms in such budgets. Despite considerable effort studying the role of denitrification in coastal ecosystems, estimates of denitrification in many ecosystems suffer from 1) relatively poor spatial or temporal sampling regimes and 2) inadequate techniques for measurement (Cornwell et al. 1999). While new techniques and measurement approaches (i.e. Nielsen 1992; Kana et al. 1998) have provided better site-specific measurements, high quality measurements that encompass appropriate temporal and spatial scales are generally lacking.

The NO_3^- for denitrification can be supplied from the water column (D_W) via diffusion into the sediment or produced in the sediment via nitrification (D_N). Studies have suggested that a close coupling of these two processes must occur even though nitrification only occurs to the depth of O_2 penetration and denitrification is predominately a suboxic process (Cornwell et al. 1999, Codispoti et al. 2005). The spatial separation of these two processes suggests that (D_N) should be inefficient with some loss of nitrate to the overlying water. However, the presence of anoxic

microsites of denitrification may provide a close coupling of nitrification and denitrification allowing for a highly efficient removal of remineralized nitrogen (Jenkins and Kemp 1984, Kana et al. 1998).

The importance of bacterially mediated sediment nitrification and denitrification as an ecological process in coastal and estuarine sediments has been studied extensively with varying results. In some estuaries denitrification is responsible for removal of over 40% of DIN inputs yet in other estuaries denitrification may remove only minor amounts of nitrogen (Seitzinger et al. 1988). This variability stems from the observation that nitrogen recycling from sediments to the estuarine water column is highly dependent on nutrient input rates, sediment deposition rates, and sediment redox conditions; low oxygen leads to a decrease in the importance of D_N (Seitzinger 1988, Rysgaard et al 1994). Under low O_2 concentrations, high NH_4^+ efflux is often observed (Kemp et al. 1990). This seasonal increase in NH_4^+ flux is important in providing sufficient DIN to sustain summer planktonic production in many coastal systems (e.g. Fisher et al. 1978; Cowan and Boynton 1996). Controls on nitrification/denitrification also include rates of deposition of labile carbon on the sediment surface (Seitzinger 1985, Caffrey et al. 1993), activity of macrofauna (Svensson et al. 1996), temperature (Henriksen and Kemp 1988) and water column O_2 concentrations (McCarthy et al. 1990). In a single estuarine system, all of these controlling factors can have considerable spatial and temporal variability (Boynton and Kemp 1985).

Denitrification efficiency has been applied on a system wide scale as a measure of ecosystem function and has been shown to be dependent on the water

residence time of a system (Nixon et al. 1996). The efficiency of denitrification defines the balance of recycling versus loss of nitrogen as N_2 and is a critical measure of ecosystem function for shallow water systems.

A given atom of nitrogen may cycle from the water column to the sediment and back again numerous times and each pass through the sediment represents an opportunity for that atom of N to be denitrified. The longer a given atom of fixed nitrogen resides in the system the greater chance that it will be lost to the atmosphere as N_2 . In this paper, we will define the efficiency of denitrification as the probability of an atom of nitrogen being denitrified during one pass through the sediment.

Studies of sediment nitrification/denitrification using indirect measurements (acetylene block, N₂-serve, denitrification potentials) in the mesohaline Chesapeake Bay have shown that 1) hypoxic/anoxic conditions inhibit (D_N) and promote NH_4^+ recycling (Kemp et al. 1990), 2) denitrification potentials are higher in the tributaries than the mainstem and 3) denitrification rates exhibit high temporal and spatial variability (Twilley and Kemp 1986). This study presents a detailed examination of denitrification rates using a direct measure of N_2 flux from the sediment of an estuarine river and provides evidence for seasonal patterning in both the denitrification rate and the efficiency of coupled nitrification/denitrification relative to NH_4^+ regeneration.

Methods

Sampling Location

The Choptank River is a large subestuary of the Chesapeake Bay and its primarily agricultural watershed covers 251.7 km². Although nitrate inputs from the watershed (upriver direction) are large, tidally mixed nitrate and ammonium inputs from the mainstem Chesapeake Bay may be substantial in the lower river (Fisher et al. 1992). Our area of study included three paired stations in different basins of the mesohaline portion of the Choptank River (Figure 1.1). The stations in each basin were chosen to be similar in water depths near the axis of the channel. This portion of the Choptank River does not experience bottom water anoxia or hypoxia (Fisher et al. 2006) and oxidized surface sediments were observed at all sites and at all times throughout this study. The HP paired sites were in 12 m of water and consisted of very fine-grained sediment. The G13 paired sites were located in 5 m of water in an area of the estuary with no well defined channel with mixed fine grained and sandy sediment. The R10 sites were located in the channel in 13 m of water near the mouth of the estuary. R10 sediments were very fine grained and black at depth. Sampling sites were located using GPS and are listed in Table 1. We observed macrofauna at all sites; the benthic community was predominately composed of small polychaetes with the highest densities at the up river sites (G13, HP).

Table 1. Station locations

Site	Latitude	Longitude
HPA	38° 37.197N	76°08.061W
HPB	38°36.253N	76°07.553W
G13A	38°39.149N	76° 13.354W
G13B	38° 40.539N	76° 13.995W
R10A	38° 38.842N	76° 18.406W
R10B	38°39.212N	76°18.745W

Sample Collection

Sampling commenced in July 2000 and proceeded monthly through the fall. In 2001, sampling was less consistent but included the winter and spring seasons. Our sampling schedule covered a range of bottom water temperatures ranging from 5°C to 26°C. Cores were collected using an acrylic/PVC Soutar-design box corer that collects cores with minimal exposure of sediment to metal. This device is particularly good at collecting samples from areas with soft sediment. Only cores with an undisturbed sediment water interface were used for flux incubations. The box corer was sub-cored with 6.35 cm inner diameter acrylic liners to a depth of ~15 cm. The subcores were topped off with ~15 cm of bottom water, stoppered, and transported to the lab within 2 h of collection. Bottom water from each site was collected with a diaphragm pump. Sediment cores were held submersed in a bath of bottom water overnight with continual aeration and circulation of the overlying water with bath

water. Holding cores overnight promoted 1) the equilibration of temperature, oxygen, nitrogen, and argon in overlying and near-surface pore waters and 2) provided a better equilibration of gases in the acrylic used for the chambers.

Sediment-Water Exchange

Sediment cores were incubated under dark conditions in a temperature-controlled chamber at *in situ* ($\pm 2^\circ$ C) temperatures. Cores were capped with buna O-ring fitted stirring tops prior to incubation, taking care to exclude bubbles. During sample withdrawal, replacement bottom water was supplied through a port in the stirring top, with gravity feed providing the head pressure required to fill vials and syringes. A suspended magnetic stir bar was used to mix the overlying water in each core. Solute samples were filtered using a 25 mm diameter, 0.45 μ m cellulose acetate syringe filter (Nalgene #191-2045). Typically, 20 ml was filtered into vials for analyses of NH_4^+ , and NO_3^- . Dissolved gas samples were collected in ~ 7 ml ground glass stoppered test tubes that were filled with a dip tube; samples were preserved with 10 μ l of 50% saturated HgCl_2 solution. Dissolved gas samples were held under water at ambient bottom water temperature until analyses the following day. Samples were collected from the top 1 cm of sediment from each flux core at the end of the incubations for chlorophyll *a* analyses and overlying water volumes were determined from water column height above the sediment.

Time courses of solute and gas concentration were determined over the course of 4-8 hours, with 4 samples collected for each analyte. Oxygen concentrations were monitored during the incubations to insure that oxygen did not fall below 50 % of saturation. Linear regression of the data was used to determine the slope of the

time-concentration relationship and the flux rates were calculated on an areal basis. In a limited number of slope calculations a single data point was excluded from the regression. Blank cores had only minor changes in concentration at most sites. Corrections were made to account for water column effects when linear changes in the blank were observed for an analyte.

Gas, Solute and Sediment Analysis

Membrane inlet mass spectrometry (MIMS; Kana et al. 1994; 1998) was used for the analysis of dissolved N₂ and O₂ and Ar. Air saturated water was used for calibration of the N₂:Ar and O₂:Ar gas ratios which were used to estimate gas concentration. The overnight equilibration of sediments at *in situ* bottom water temperatures ensured that the overlying water and the pore water were close to thermal equilibrium, minimizing potential artifacts associated with the sensitivity of N₂:Ar gas ratios to temperature .

Nitrate + nitrite was analyzed via segmented flow analysis after Cd reduction and ammonium was manually analyzed with a phenylhypochlorite colorimetric technique (Parsons et al. 1994). Chlorophyll *a* was determined via HPLC (Van Heukelem et al. 1994) after extraction with acetone.

Statistical Analysis

We used multiple linear regression analysis to determine factors that might be important in controlling sediment oxygen demand (SOD), denitrification, and denitrification efficiency at the HP site. This site was chosen for statistical analysis

because of its close proximity to a monthly water quality monitoring site which provided a good proxy for algal biomass (water column chlorophyll *a*) and bottom water oxygen conditions in this part of the river. The HP site was sampled 3 additional times in August, October, and December of 2001 for a total of 11 flux and denitrification measurements. Since we sampled monthly, we used the water column chlorophyll *a* from the previous month for the regression analysis to compensate for settling of the bloom and incorporation of the algae into the sediment. All four replicate cores were entered into the model separately. The best fit model was chosen by examining combinations of different variables that gave the best adjusted R-squared, Mallows' C(p), Akaike's Information Criteria, and Bayesian Information Criteria for the smallest number of variables. Stepwise linear regression was used to remove variables that were not significant and exceeded an alpha value of 0.05. The residuals were examined on each analysis and found to be normally distributed without transformation. Bottom water properties (salinity, dissolved oxygen, nitrate, temperature, chlorophyll *a*) were entered as independent variables. We found that temperature and oxygen varied collinearly and could not be entered into the model simultaneously. These variables were found not to be significant for denitrification but were significant components for SOD and denitrification efficiency. We ran the model including temperature and bottom water oxygen separately to determine which model best predicted denitrification efficiency and found that oxygen explained most of the variability. Temperature has been shown to have an effect on sediment metabolism and was included in the SOD regression instead of bottom water O₂.

Results

Linear changes in N_2 greater than the blank were observed in most of our incubations but in 38% of incubations no change in N_2 was detected. Sample time course data showing replicate cores and blanks for N_2 and O_2 are shown in figure 1.2. The O_2 correction for our MIMS data was very small and difficult to measure with changes in the N_2/Ar ratio close to the analytical precision of the method (Kana and Weiss 2004). The flux data for SOD, N_2 , NH_4^+ , and $NO_3^-+NO_2^-$ at our paired stations within a basin were similar with less than 5% of fluxes having a statistical difference (analysis of variance ANOVA). Our data indicates that the sediments were fairly homogeneous within each basin at least over the scale of several hundred meters and at similar water depth. (Figure 1.3-1.6). The HP basin stations had the highest sediment oxygen demand (SOD) ranging from 900 to 4800 $\mu\text{mol m}^{-2} \text{h}^{-1}$; G13 and R10 had ranges of 400 to 3800 $\mu\text{mol m}^{-2} \text{h}^{-1}$ and 400 to 3000 $\mu\text{mol m}^{-2} \text{h}^{-1}$ respectively (Figure 1.3). SOD rates peaked in early May at all sites well before the temperature maximum in July. A second peak in SOD occurred in late summer early fall at the HP and G13 sites. In November SOD rates were low in all three basins.

The highest N_2 flux rates coincide with the cooler months of the year and rates drop off sharply in the summer (Figure 1.4). The highest rates observed were in the HP basin at ca. 160 $\mu\text{mol } N_2-N \text{ m}^{-2} \text{h}^{-1}$ with the other basins exhibiting highest rates near 100 $\mu\text{mol } N_2-N \text{ m}^{-2} \text{h}^{-1}$. G13 was the only basin to have denitrification rates above 50 $\mu\text{mol } N_2-N \text{ m}^{-2} \text{h}^{-1}$ during July and August (Figure 1.4). No measurable N_2

flux was detected in July or October at sites R10 or G13 and rates were $< 25 \mu\text{mol N}_2\text{-N m}^{-2} \text{ h}^{-1}$ during July and August at all sites.

Ammonium fluxes followed the same pattern as temperature at the HP and R10 paired stations with a maximum in August and minimum in February and November (Figure 1.6). The flux of NH_4^+ increased in May along with SOD at the HP and R10 basins stations. The R10 transect had the highest NH_4^+ fluxes at $\sim 450 \mu\text{mol m}^{-2} \text{ h}^{-1}$ followed by HP with $\sim 250 \mu\text{mol m}^{-2} \text{ h}^{-1}$. The pattern of NH_4^+ flux at the G13 basin sites showed minimum rates in February and November but there was no clear seasonal pattern from April through October, averaging about $100 \mu\text{mol m}^{-2} \text{ h}^{-1}$. Bottom water reached a temperature maximum in June and began to decline by late August while dissolved oxygen showed an inverse pattern with a minimum of $3\text{-}6 \text{ mg l}^{-1}$ in July (Figure 1.7).

The mesohaline portion of the Choptank River typically has low bottom water NO_3^- concentrations year round of $<8 \mu\text{mol L}^{-1}$ (Malone et al. 2003). High sediment NO_3^- uptake occurred in May when bottom water NO_3^- concentrations peaked (Figure 1.5). Station G13 and HP had low sediment uptake rates of NO_3^- ($<60 \mu\text{mol m}^{-2} \text{ h}^{-1}$) between February and May. R10 had the highest NO_3^- uptake and the highest bottom water concentration in May when NO_3^- levels were unusually high ($16 \mu\text{M}$). Stations HP and R10 had NO_3^- fluxes of about $40 \mu\text{mol m}^{-2} \text{ h}^{-1}$ directed out of the sediment in October/November.

Sediment chlorophyll *a* has been used as an indicator of labile carbon loading to the sediment (Cowan et al. 1996). This indicator works best in sediments where the major source of labile organic carbon is from phytoplankton and benthic primary

production is light inhibited. No seasonal changes in sediment chlorophyll *a* were found during this study despite high temporal variability in spring and fall water column chl *a* concentrations (Malone et al. 2003). The sediment chlorophyll *a* averaged $15.6 \pm 4.7 \text{ mg m}^{-2}$, $13.9 \pm 6.4 \text{ mg m}^{-2}$ and $35.6 \pm 18 \text{ mg m}^{-2}$ for stations HP, G13, and R10 respectively (Figure 1.8). Station R10 had sediment chlorophyll *a* concentrations that were two to three fold higher than the other sites, suggesting that organic carbon loading may be higher at this location (Figure 1.8).

Multiple Linear Regression Analysis

The initial independent variables used in all our multiple linear regressions included bottom water parameters; salinity, temperature, dissolved oxygen, nitrate, and chlorophyll *a*. Oxygen and temperature covaried and could not be used in the analysis simultaneously. The best model fit for SOD included 4 variables, water column nitrate, temperature, sediment chlorophyll *a*, and bottom water chlorophyll *a*. Stepwise regression showed that bottom water chlorophyll *a* and temperature were significant at the 0.05 level. Bottom water chlorophyll *a* had a positive linear relationship with SOD and explains 75% of the variation in SOD (Figure 1.9) over this 1 year study ($\text{O}_2 \text{ flux } \mu\text{mol m}^{-2} \text{ h}^{-1} = 109.55 * (\text{chl } a \text{ } \mu\text{g l}^{-1}) + 28.94 * \text{Temperature} + 404.84$).

The best fit model for denitrification rate included the same four variables as SOD. Interestingly, bottom water O_2 and temperature were not found to be significant in the model. After stepwise regression only salinity, chlorophyll *a*, and

nitrate remained in the model as significant. Our model results represent the denitrification data very well explaining 78% over the variability in the rate (Figure 1.10). The linear relationship can be expressed as: $\text{denitrification} = 3.28 * (\text{chl a } \mu\text{g l}^{-1}) + 17.59 * \text{salinity} + 1.53 * \text{nitrate} - 188.8$

We calculated denitrification efficiency (DE) as the fraction of sediment nitrogen flux lost to denitrification versus being returned to the water column as a nutrient source for further use by algae. DE was calculated by dividing the N_2 flux by the sum of the efflux of NH_4^+ , $\text{N}_2\text{-N}$ and the $\text{NO}_3^- + \text{NO}_2^-$ flux :

$$\text{DE} = \text{N}_2\text{-N flux} / (\text{N}_2\text{-N flux} + \text{NH}_4^+\text{flux} + \text{NO}_3^- + \text{NO}_2^-\text{flux})$$

Denitrification efficiency was found to be linearly related to the single variable bottom water oxygen (Figure 1.11). Temperature covaried with oxygen and was not included in the regression. The equation for this relationship is $\text{DE} = -0.23164 + 0.0857 * \text{O}_2 \text{ mg L}^{-1}$; explaining 73% of the variability in DE.

Discussion

Temporal Patterns and the influence of oxygen

The maximum rates of denitrification measured were considerably higher in this study compared to previous measurements in Chesapeake Bay. Kemp et al.'s (1990) measured maximum rates in spring in the mesohaline portion of the bay, measured using the acetylene block method, were only ~ 20% of the average spring

rates reported here. An underestimate would be expected considering the inhibitory effects of acetylene on nitrification and lower bottom water O₂ concentrations in this part of the bay. Ambient spring time rates (77-89 N₂-N μmol m⁻² h⁻¹; Jenkins and Kemp 1984) estimated for the mesohaline Patuxent River using ¹⁵N isotopic tracer techniques were very close to our peak rates in spring. Our study, together with the two previous studies, shows a dramatic suppression of denitrification rates during summer. The near absence of summer denitrification in previous studies was attributed to bottom water anoxia/hypoxia inhibiting sediment nitrification. In this study a similar pattern of denitrification inhibition is shown in summer for the Choptank River despite the bottom water being oxic (Figure 1.7). In our study denitrification rates are much more dynamic than would be predicted by changes in bottom water O₂ concentrations alone and O₂ was not a significant variable explaining denitrification rates in our regression analysis. Denitrification rates decreased to very low levels in summer despite having well oxygenated bottom water > 4 mg L⁻¹. The decline in summer rates in our study indicates that other factors are responsible for driving denitrification under oxic conditions. Our model results for denitrification efficiency suggest that the process of coupled nitrification/denitrification at our sites is sensitive to small changes in bottom water O₂. The deposition of the spring bloom along with rising temperature drives an increase in SOD, decreases the depth of O₂ penetration, and decreases the zone where nitrification can occur. This decrease in nitrification shifts the sediment nitrogen efflux from N₂ to NH₄⁺ and decreases denitrification efficiency. The eutrophication

feedback associated with the inhibition of denitrification is likely to begin before hypoxic/anoxic conditions develop in the water column.

Denitrification Efficiency

One of the advantages of the MIMS technique is the ability to simultaneously measure N_2 fluxes combined with $NO_3^- + NO_2^- + NH_4^+$ fluxes and account for all the inorganic forms of nitrogen on the same flux core. Ignoring fluxes of organic nitrogen, we can construct a budget of the nitrogen fluxes and calculate the overall efficiency of the denitrification process. To determine denitrification efficiency we summed the DIN effluxes and calculated the percentage that was lost as N_2 versus NH_4^+ (Figure 1.12). Despite the fact that these sites were always exposed to aerobic bottom water there is a distinct seasonal pattern in denitrification efficiency. In the winter and fall, denitrification efficiency was high (50% to 75%), declining sharply through the spring and having negligible rates during the summer. The proportion of NH_4^+ flux (Figure 1.12) showed the inverse pattern accounting for nearly all the inorganic fixed nitrogen released from the sediment. The process of denitrification at these sites is heavily dependent on sediment nitrification since water column nitrate concentrations were low most of the year. An exception to this pattern was found at station R10 where there was sufficient water column NO_3^- delivered primarily from the Susquehanna River in April and May to supply the majority of the denitrification based on fluxes of $NO_3^- + NO_2^-$ into the sediment. During the winter and fall when temperature drives sediment metabolism to a minimum, and dissolved O_2 to an annual maximum, O_2 penetration into the sediment would be maximized. Deeper O_2

penetration translates into more sediment volume for nitrification. As temperature rises in spring and organic matter supply increases, nitrification is reduced due to lower O₂ availability. Figure 1.13 shows a plot of the individual core fluxes of O₂ vs N₂ with a line representing the Redfield ratio of O₂/N (6.6:1) for remineralized phytoplankton biomass. Assuming that the main source of labile carbon to the sediment was derived from algae, we would expect decomposition to yield approximately 1 mole of N for 6.6 moles of O₂ consumed or CO₂ produced. Points that fall on the line represent cores where all the remineralized nitrogen was denitrified while points to the right of the line represent fluxes where nitrogen was either sequestered in the sediment or released to the water column as NH₄⁺. The general pattern shows a pronounced divergence from the 100% line as SOD increases, indicative of a transition from denitrification to NH₄⁺ release. There appears to be 3 different groupings of the data: 1) during fall and winter when metabolism is low nearly all remineralized nitrogen was being lost via denitrification, 2) during the spring bloom deposition when metabolism reached maximum rates the denitrification yield was 16.8-38.5%, and 3) during the summer the denitrification efficiency approached 0%. The sediments appear to be the most efficient in denitrifying during the late fall and winter when organic carbon supply from the water column is low and NO₃⁻+NO₂⁻ is high.

The addition of labile organic matter in the spring tends to stimulate denitrification but drive efficiency down allowing a greater proportion of the remineralized nitrogen to be returned to the water column as NH₄⁺. This trend has been shown in other studies (Newell et al. 2002, Enoksson 1993) where increasing

organic carbon additions result in a greater proportion of remineralized N being returned to the water column as NH_4^+ . A similar trend was also found in warm-temperate Australian lagoons where declining denitrification efficiency was observed across an increasing eutrophication gradient (Eyre et al. 2002). High denitrification efficiencies were found for Port Phillip Bay in the range of 75-85% for sediments with organic matter degradation rates of 625-1,041 $\mu\text{mol O}_2 \text{ m}^{-2} \text{ h}^{-1}$ but at other sites efficiencies decreased to 0 at degradation rates of 4167 $\mu\text{mol O}_2 \text{ m}^{-2} \text{ h}^{-1}$ during summer (Heggie et al. 1999). The temporal range in organic matter degradation at our sites (400-4800 $\mu\text{mol O}_2 \text{ m}^{-2} \text{ h}^{-1}$) was similar to the Port Phillip Bay study but our denitrification efficiencies were much higher (16.8-38.5%) during periods of highest organic loading. High loading sites in the Port Phillip Bay study had sulfide in the porewaters which has been shown to inhibit nitrification (Joye & Hollibaugh 1995) and stimulate dissimilatory NO_3^- reduction to NH_4^+ (An & Gardener 2002). Sulfate reduction may still be an important respiratory pathway but reduced metabolites like H_2S must oxidized rapidly before they can buildup in the pore water. The absence of pore water H_2S at our sites even under the highest loading conditions could explain the higher denitrification efficiencies. The presence of pore water H_2S could be an important control of sedimentary N-cycling, tipping the balance toward NH_4^+ recycling.

Denitrification efficiency is a measure of how well a system eliminates nitrogen nutrients. High rates of sediment denitrification can be sustained by eutrophic systems with adequate nitrate supply but in the absence of coupled nitrification/denitrification a high proportion of the remineralized nitrogen is returned

to the water column as NH_4^+ . Our linear regression results show that bottom water oxygen concentration was a strong indicator of the proportion of nitrogen removed via denitrification; higher O_2 levels enhanced the fraction of nitrogen removed via denitrification. Low water column O_2 in summer lead to nearly complete recycling of remineralized nitrogen back to the water column as NH_4^+ . These results suggest denitrification efficiency can be sensitive to even small decreases in water column O_2 . This result is somewhat surprising when we consider that bottom water oxygen concentrations remain above hypoxic levels for the study period. Water column O_2 levels were close to saturation during the majority of the year (except summer) yet the model suggests that DE is sensitive to changes in oxygen saturation induced by seasonal temperature increases. The efficiency of denitrification could decline under a warmer climate scenario, enhancing the recycling of sediment nitrogen back to the water column.

Temporal versus Spatial Variability

The stimulation of denitrification and declining denitrification efficiency caused by settling water column blooms has implications for the frequency at which such processes must be studied. The short half-life of sedimented fresh algal derived organic carbon (4 weeks - Enoksson 1993; 2-3 weeks - Garber 1984)) causes rapid biogeochemical changes in the sediment altering nitrogen recycling rates and denitrification. These rapid recycling rates represent large sources of recycled nitrogen that might not be included in nutrient budgets if sampling frequencies are less than monthly. The cross channel sites showed very little spatial variability

indicating relatively uniform N cycling processes over spatial scales of 10-100 meters, at least at a given depth. Depth of water was different at each of our paired sites and flux rates did vary considerably between sites (Figure 1.3-1.6). Due to funding constraints, many studies prioritize spatial over temporal coverage hoping to gain as much system wide information as possible. In this study spatial variability within a single basin was much less important than the temporal variability at a given site. Future studies may benefit from making a one-time assessment of spatial variability to determine the initial site locations and with a more directed focus of remaining resources on temporal coverage.

Water Column and Sediment Chlorophyll a

The seasonal rates of denitrification at the three sites have a similar pattern with peaks in spring and late summer to early fall (Figure 1.4). These peaks were associated with corresponding peaks in SOD and NH_4^+ flux and were likely the result of water column bloom deposition. The multiple linear regression results for SOD suggest that fresh organic matter in the form of algae was responsible for most of the seasonal change in SOD and that freshly deposited material to the sediment was processed rapidly with little storage. The highest flux of organic matter to sediment occurred during the spring bloom but the elevated SOD that resulted only persisted for several weeks. This rapid turnover of organic matter is beneficial with respect to nitrification because more of the organic matter is processed directly through aerobic rather than anaerobic pathways. There was no smell of H_2S in these sediments even during the lowest bottom water oxygen concentration in summer and almost no

measurable phosphate flux (data not shown). This rapid turnover of organic matter likely promotes the retention of porewater P through Fe oxide binding near the sediment/water interface.

Figure 1.14 shows the annual average bottom water chlorophyll *a* along with the average denitrification rates for our 3 transects. The peaks in denitrification occurred about a month after the peaks in bottom water chlorophyll *a* suggesting a lag time for material to settle and become incorporated into the sediment. A study on the degradation of sinking particulate organic carbon (POC) in the oxygen deficient waters of the eastern tropical North Pacific has shown that denitrifiers preferentially consume N-rich amino acids but would not degrade refractory POC (Van Mooy et al. 2002). This suggests that denitrifiers may be limited by the quality of the organic substrate. The denitrifiers in our sediments appear to be stimulated by highly labile organic substrate derived from both the spring and fall phytoplankton blooms. Our multiple linear regression results suggest that water column chlorophyll *a*, salinity, and nitrate are the most important factors for denitrification. Since the lower Choptank River does not have a large salinity range and nitrate concentrations were low for most of the year, the most important factor controlling the overall rate of denitrification was the supply of fresh organic matter from the water column. Interestingly, the model suggests that an increase in salinity would promote higher rates of denitrification; the low salinity range would suggest that salinity-driven differences in adsorption are very low. Water column O₂ was not found to be a significant factor controlling the overall rate of denitrification.

The process of nitrification appears to be stimulated as a result of bloom deposition as well, probably by providing higher pore water NH_4^+ concentrations to the nitrifiers. Nitrification and denitrification were closely coupled in our sediments with nitrification rates explaining 66% of the variability in denitrification rates (Figure 1.15). Nitrification rates were estimated by subtracting our nitrate flux rate from the N_2 flux for individual cores.

Surface sediment chlorophyll *a* showed no seasonal trends despite a very pronounced spring bloom (Figure 1.8). Trimmer et al. (1999) also found enhanced denitrification and SOD resulting from spring bloom deposition in Irish Sea sediments with no change in sediment chlorophyll *a* concentrations. In our study this could be attributed to our frequency of sampling since chlorophyll *a* has been shown to degrade very quickly under oxic conditions with a half-life <7 days (Sun et al. 1991). This would suggest that our sampling scheme might not have captured peak bloom deposition. Sediment chlorophyll *a* has been used as a surrogate for labile organic supply to the sediment below the photic zone and generally correlates well with SOD and NH_4^+ release. The lack of a seasonal pattern in this study suggests that sediment chlorophyll *a* may not preserve a monthly-integrated record of phytoplankton deposition in oxic sediments and inter site differences may only represent focusing of material at a given site. The longer half-life of sediment chlorophyll *a* in anoxic sediments of 50-60 days (Sun et al. 1993) should preserve a longer-term record of planktonic deposition and may account for its greater utility in deep mid-bay sediments (Cowan and Boynton 1996).

The NH_4^+ fluxes at R10 were nearly twice that of the other sites in May, August and September and were out of proportion with SOD (Figures 1.4, 1.6). Using a Redfield ratio of (O_2/N) 6.6 we would predict a summer NH_4^+ efflux rate of $\sim 230 \mu\text{mol m}^{-2} \text{h}^{-1}$ which is about half of the rate we measured. R10 appears to have the highest carbon loading and may have a higher proportion of anaerobic metabolism than the other sites. Stations HP and G13 both had maximum NH_4^+ fluxes in summer when almost 100% of the remineralized nitrogen was returned to the water column (Figure 1.13). The determination of nutrient remineralization rates from stoichiometric relationships to O_2 may result in an under estimate because the summer burial of solid phase sulfide minerals derived from anaerobic remineralization would not be accounted for. Moreover, the fall reoxidation of these minerals would result in an overestimation of metabolism (Giblin 1984; Sampou and Oviatt 1991; Cornwell and Sampou 1995).

Conclusions

High rates of denitrification were found in the mesohaline portion of the Choptank River in late spring as remnants of the spring bloom settled to the sediment fueling high rates of metabolism. The supply of labile organic carbon (phytoplankton) to the sediment stimulated rates of denitrification, SOD, and NH_4^+ release. The supply of NO_3^- via nitrification appears to be the main controlling factor limiting the rate of denitrification in these sediments. Labile carbon substrate may also limit denitrification especially during summer. The correlation between bottom

water O₂ and denitrification efficiency is likely the result of a depression in the sediment O₂ penetration depth at higher metabolic rates and a subsequent depression in nitrification. These sediments become less efficient at removing nitrogen via denitrification with higher carbon loading and decreasing water column O₂. This study demonstrates how dynamic the nitrogen cycle can be even in sediments with well-oxygenated overlying water and how sensitive denitrification is to changes in labile carbon loading. The intensity of phytoplankton blooms may have an impact on the proportion of remineralized nitrogen that is denitrified. Increased algal bloom intensity brought on by eutrophication could reduce sediment oxygen penetration depths thereby reducing nitrification and hence driving down sediment denitrification efficiency. Our study suggests that a positive eutrophication feed back mechanism from the sediments may be invoked prior to the onset of anoxic or hypoxic water column conditions.

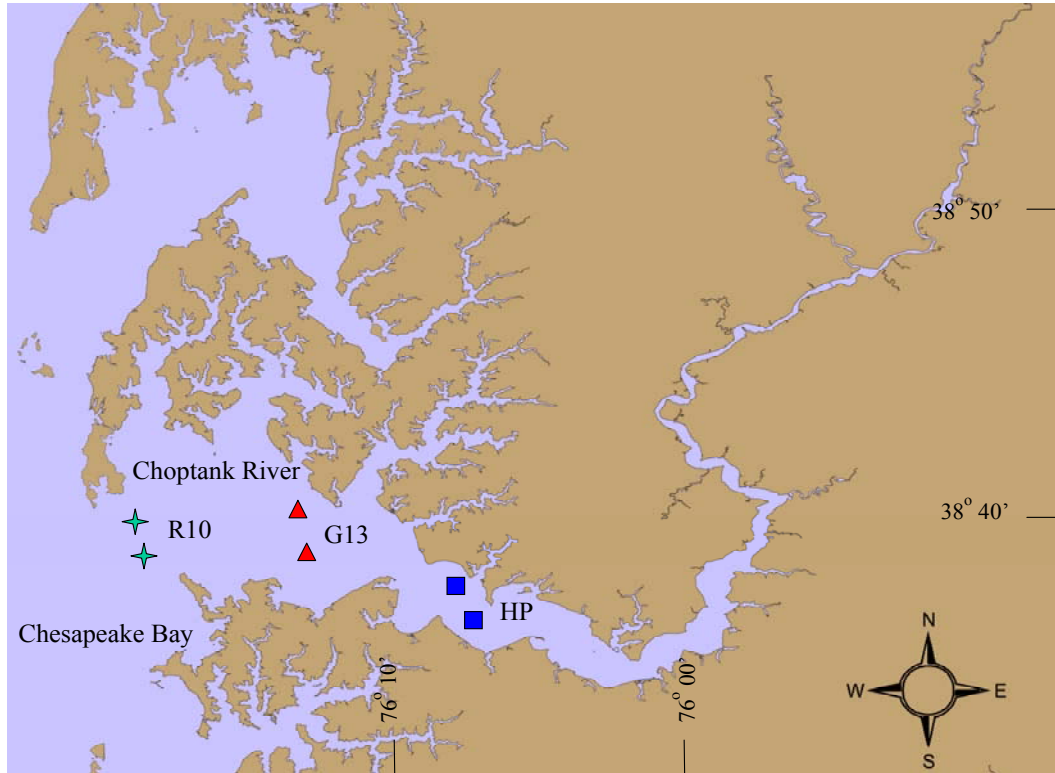


Figure 1.1 Map of the Choptank River - a sub estuary of Chesapeake Bay.

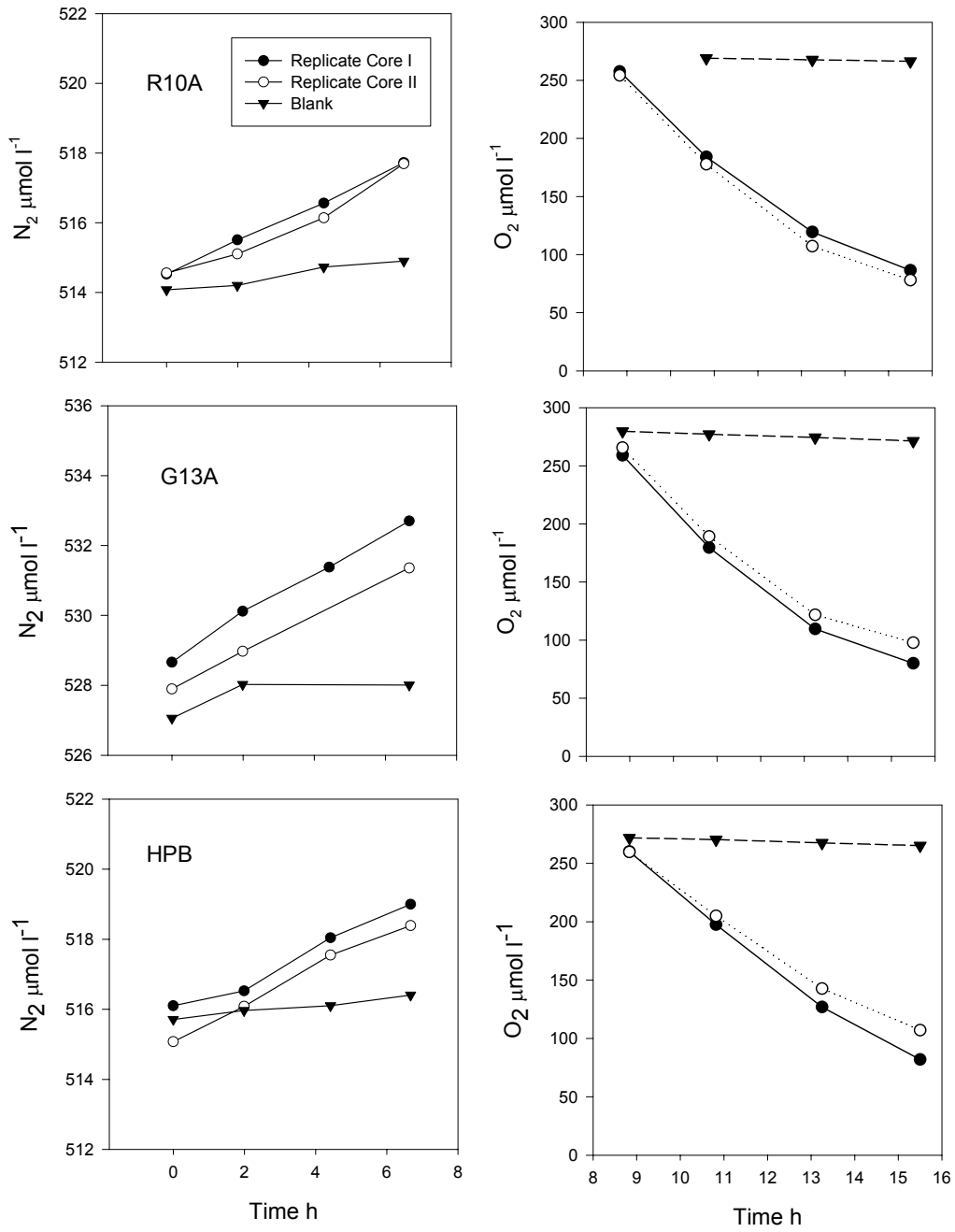


Figure 1.2 Sample data of time course changes in N_2 and O_2 measured with the MIMS system. Cores I and II are replicates from one site in May.

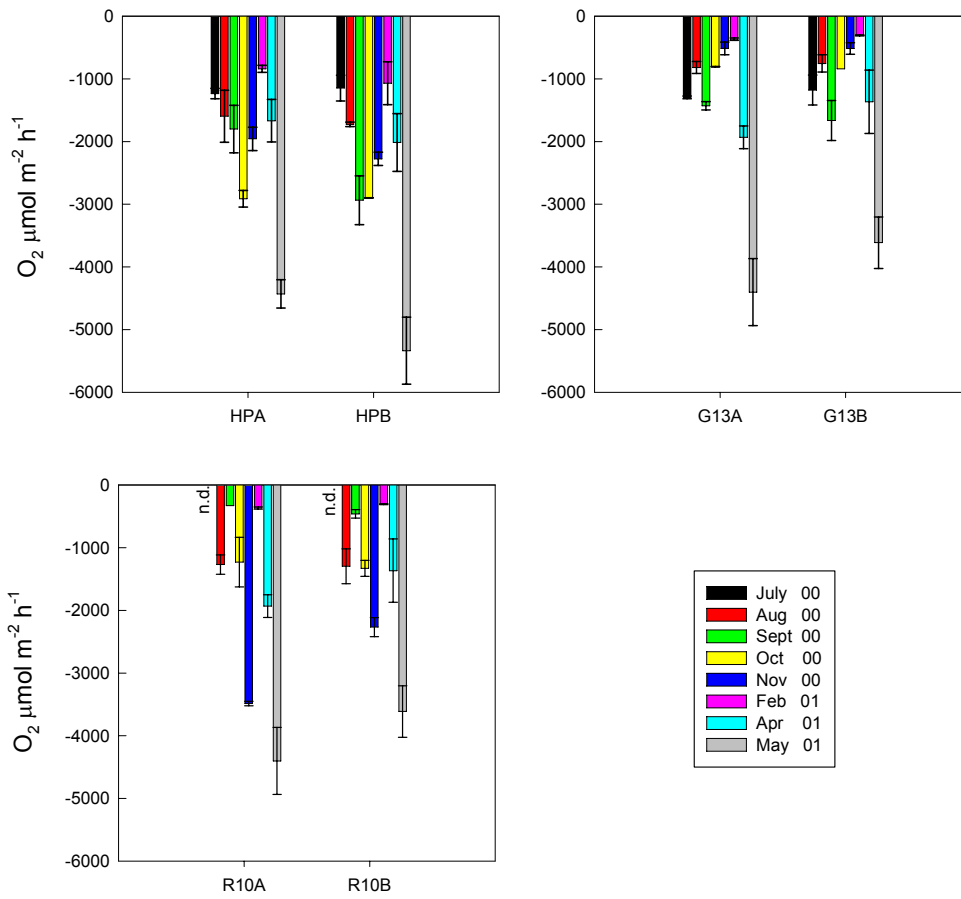


Figure 1.3 Sediment-water exchange data for July 2000 through May 2001 for SOD. Each bar represents the average of replicate cores collected at each station. A and B represent distinct stations sampled within a given basin. Missing bars indicate no measurable flux. No flux data (n.d.) was collected in July at site R10.

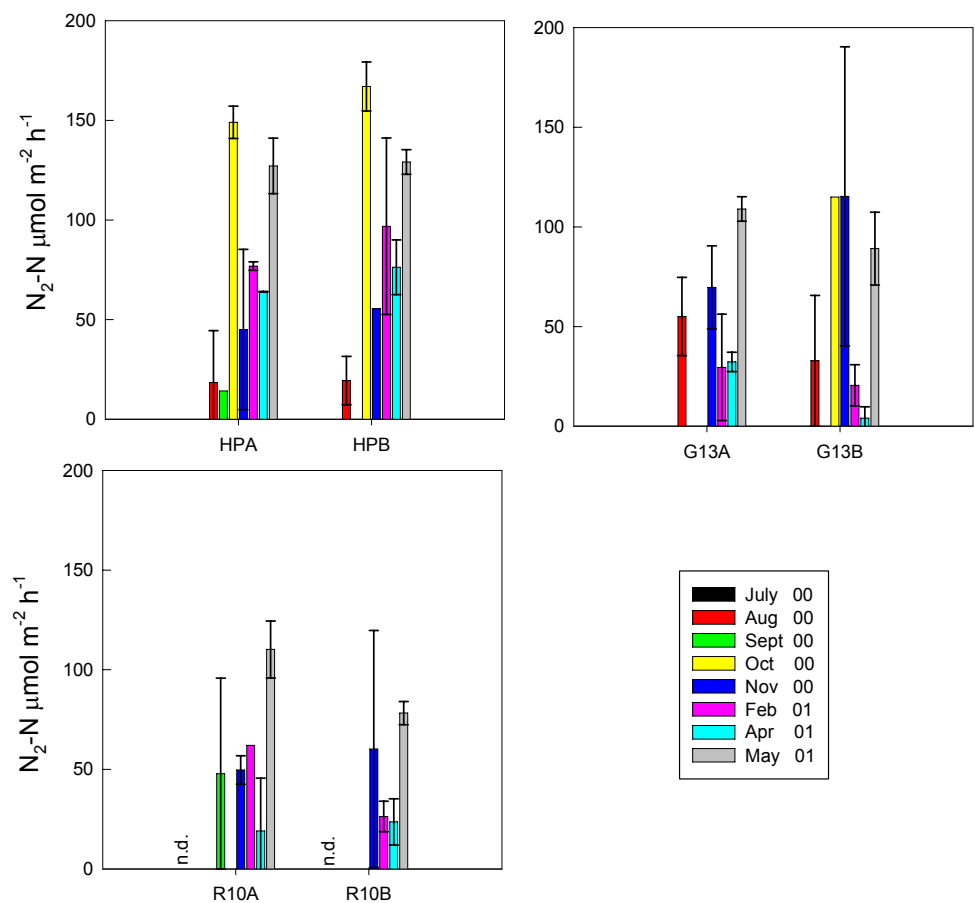


Figure 1.4 Sediment-water exchange data for July 2000 through May 2001 for N_2 . Each bar represents the average of replicate cores collected at each station. A and B represent distinct stations sampled within a given basin. Missing bars indicate no measurable flux. No flux data (n.d.) was collected in July at site R10.

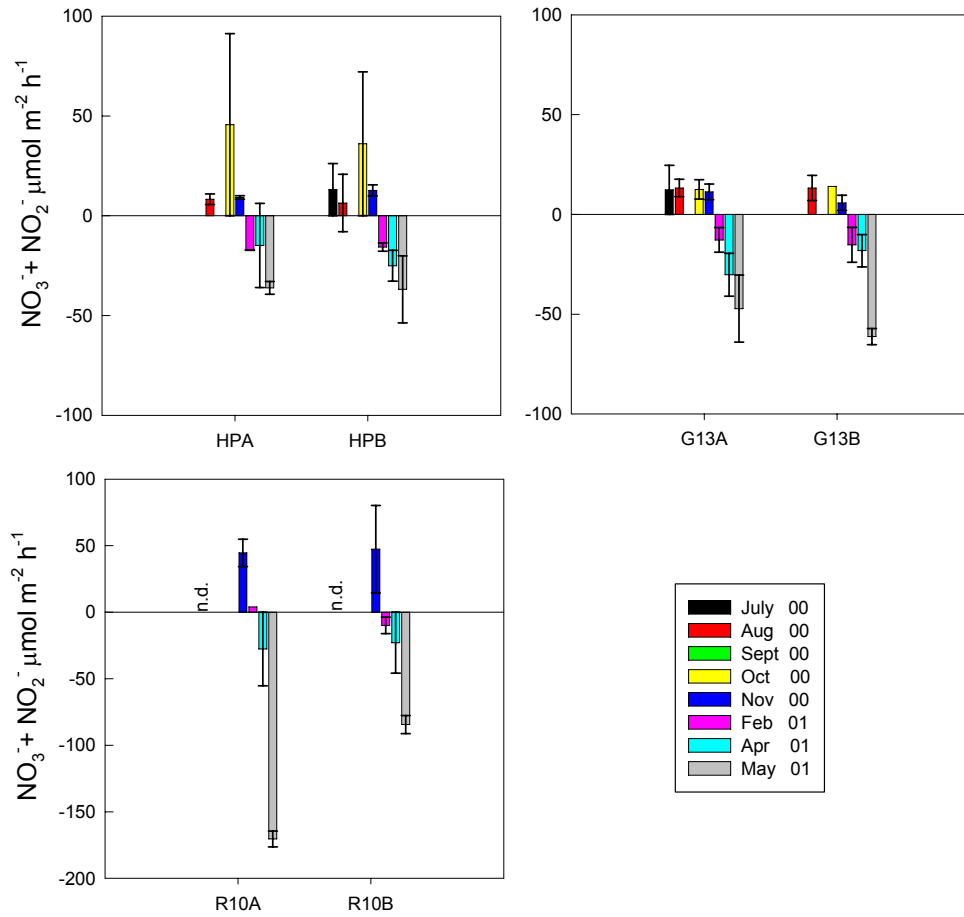


Figure 1.5 Sediment-water exchange data for July 2000 through May 2001 for $\text{NO}_3^- + \text{NO}_2^-$. Each bar represents the average of replicate cores collected at each station A and B represent distinct stations sampled within a given basin. Missing bars indicate no measurable flux. No flux data (n.d.) was collected in July at site R10.

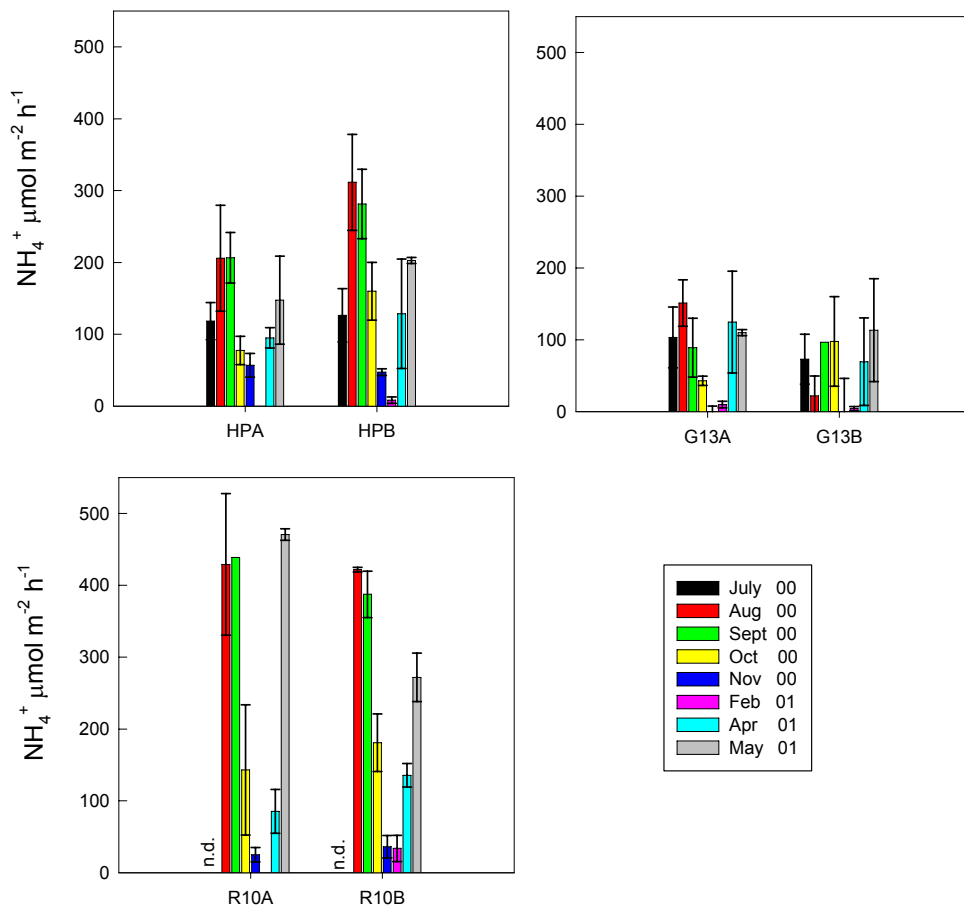


Figure 1.6 Sediment-water exchange data for July 2000 through May 2001 for NH_4^+ . Each bar represents the average of replicate cores collected at each station. A and B represent distinct stations sampled within a given basin. Missing bars indicate no measurable flux. No flux data (n.d.) was collected in July at site R10.

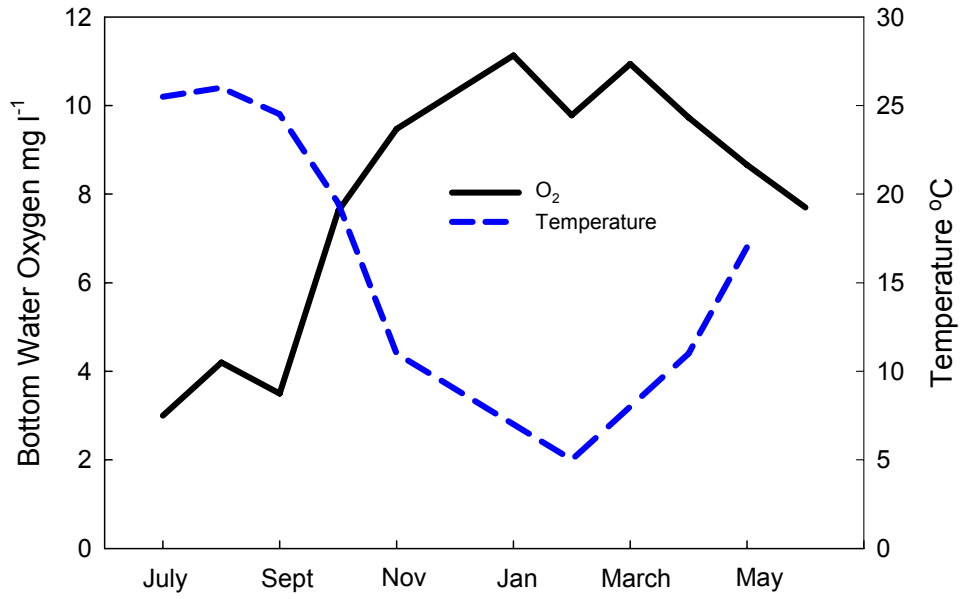


Figure 1.7 Monthly bottom water temperature and O₂ concentrations for site HP.

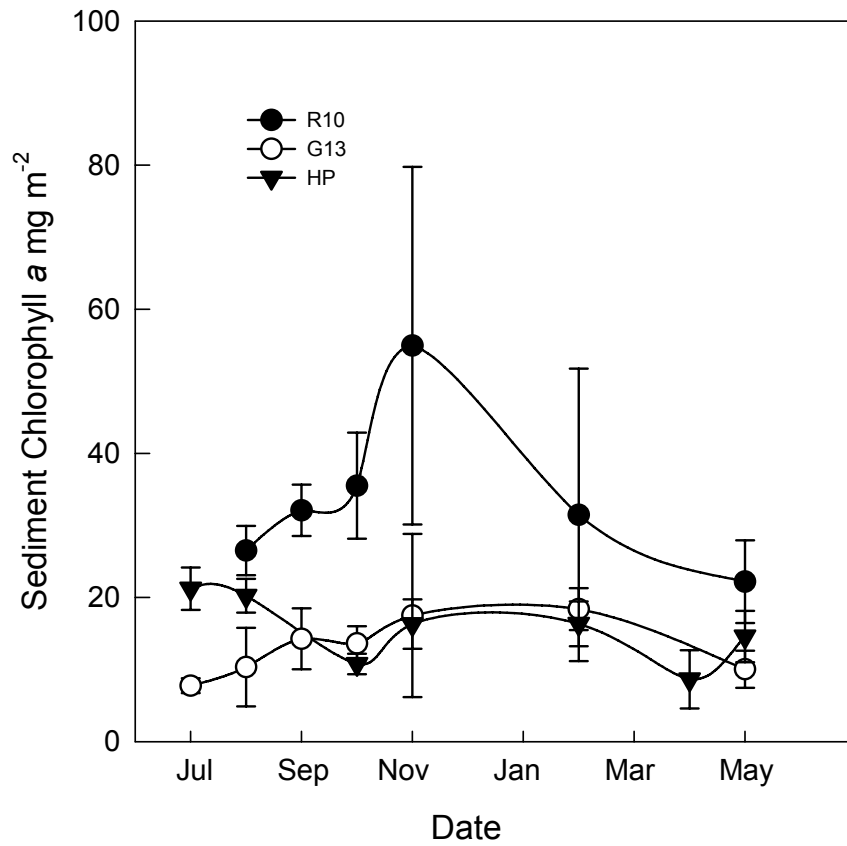


Figure 1.8 Sediment chlorophyll *a* from the top 1.0 cm of sediment. Error bars represent standard deviation of 4 replicate samples.

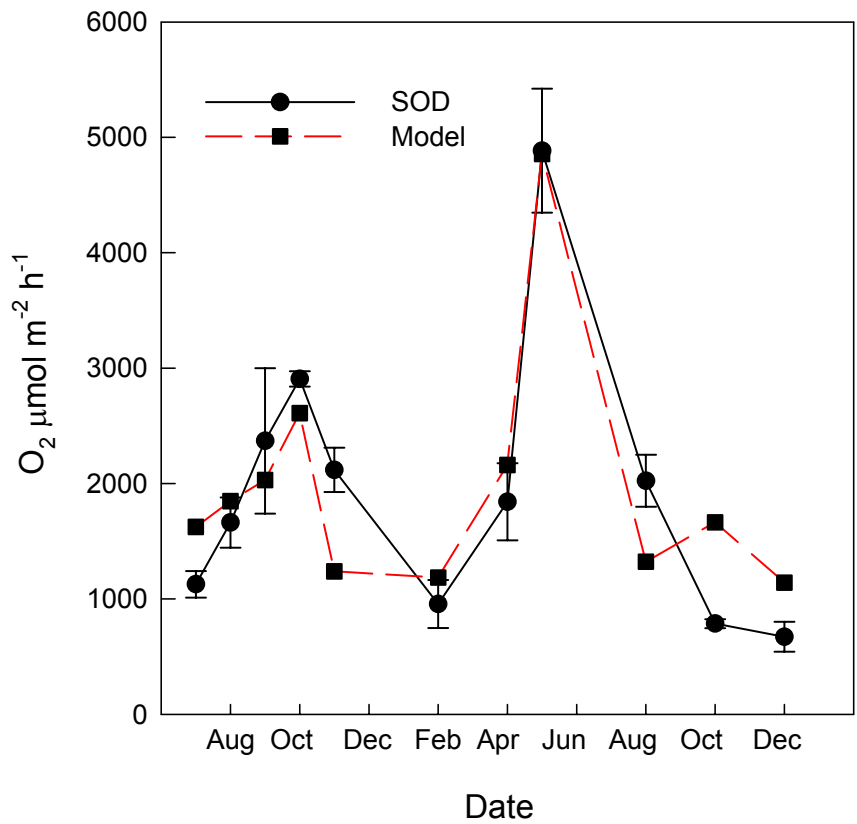


Figure 1.9 Multiple linear regression model of sediment oxygen demand for station HP. Average data (solid line) and model results (dashed line) plotted by month from July 2000 through December 2001. Error bars represent standard deviation of replicate core fluxes.

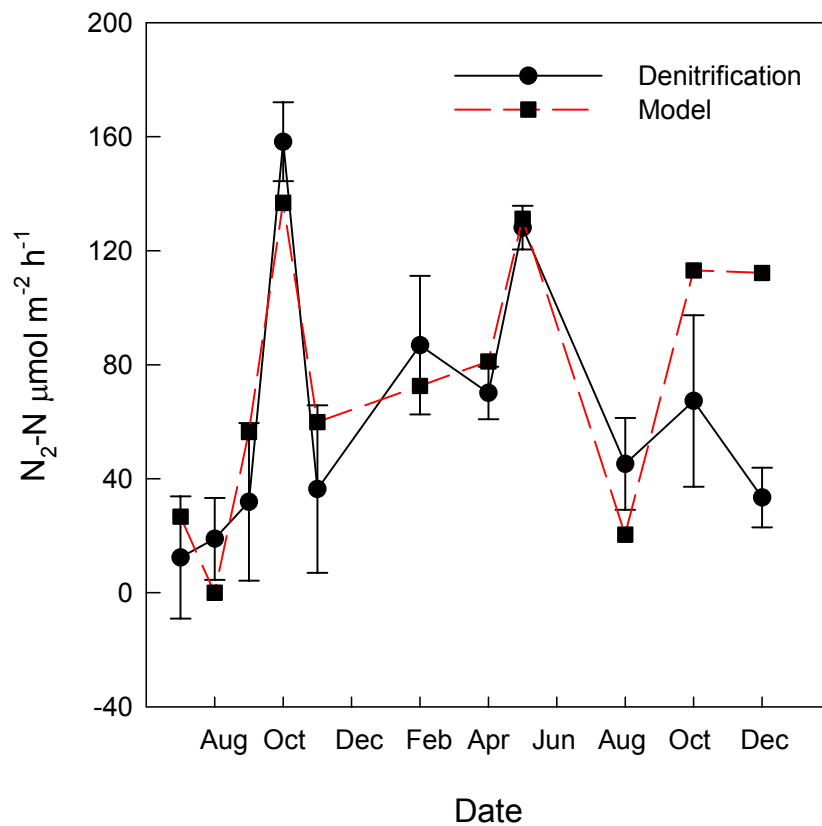


Figure 1.10 Multiple linear regression model of denitrification for station HP. Average data (solid line) and model results (dashed line) plotted by month from July 2000 through December 2001. Error bars represent standard deviation of replicate core fluxes.

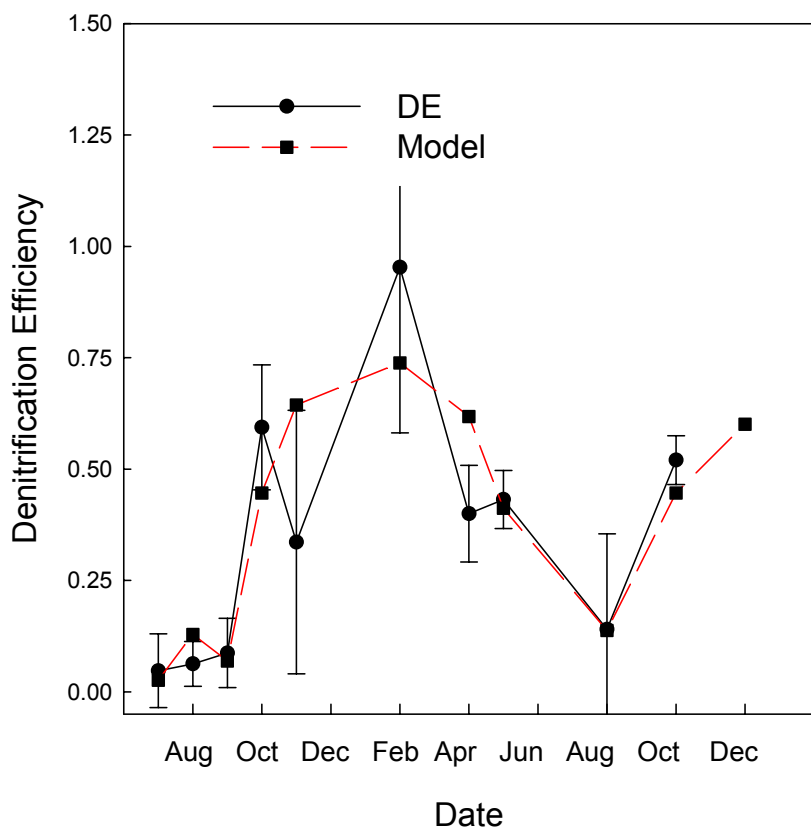


Figure 1.11 Multiple linear regression model of denitrification efficiency for station HP. Average data (solid line) and model (dashed line) plotted by month from July 2000 through December 2001. Error bars represent standard deviation of replicate core fluxes.

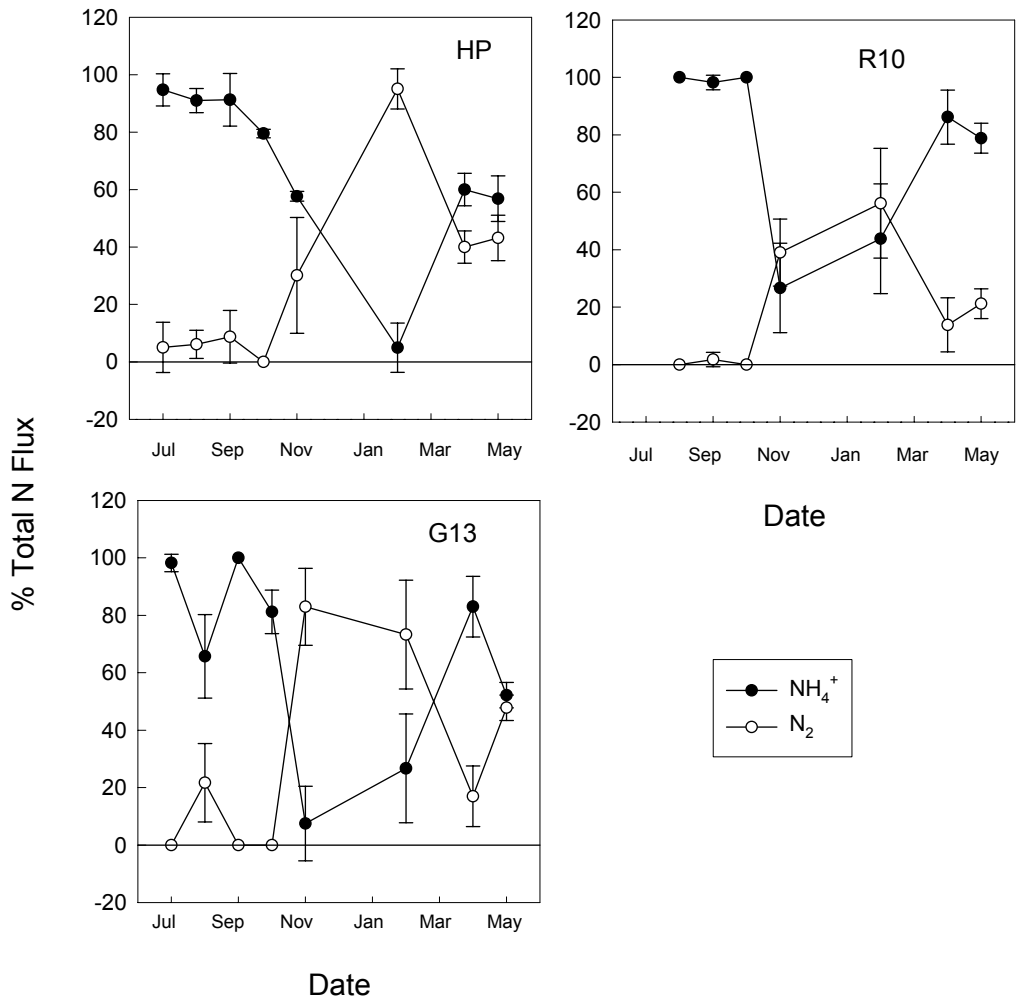


Figure 1.12 Percentage of the total nitrogen efflux from the sediment as NH_4^+ (filled circles) or N_2 (open circles). All cores for a given transect have been pooled (n=4). Error bars represent standard deviation of replicate cores.

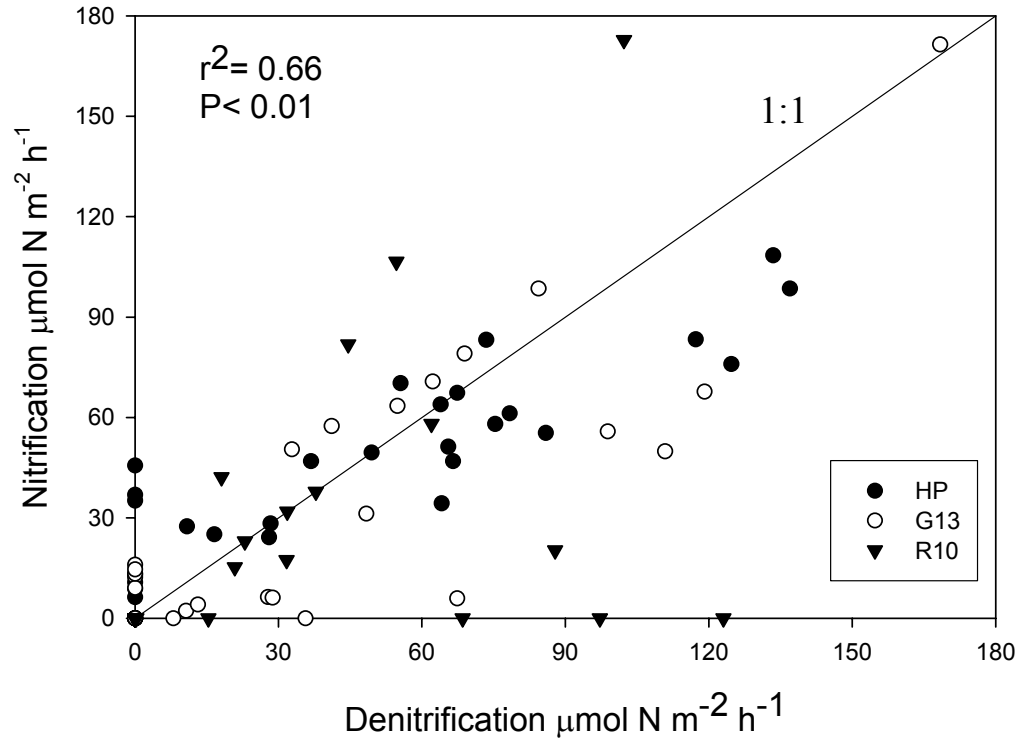


Figure 1.13 Correlation between nitrification and denitrification for individual core fluxes. The 1:1 line represents cores where sediment nitrification supplies all the NO_3^- for denitrification. Points below the line represent cores where some NO_3^- was supplied from the water column.

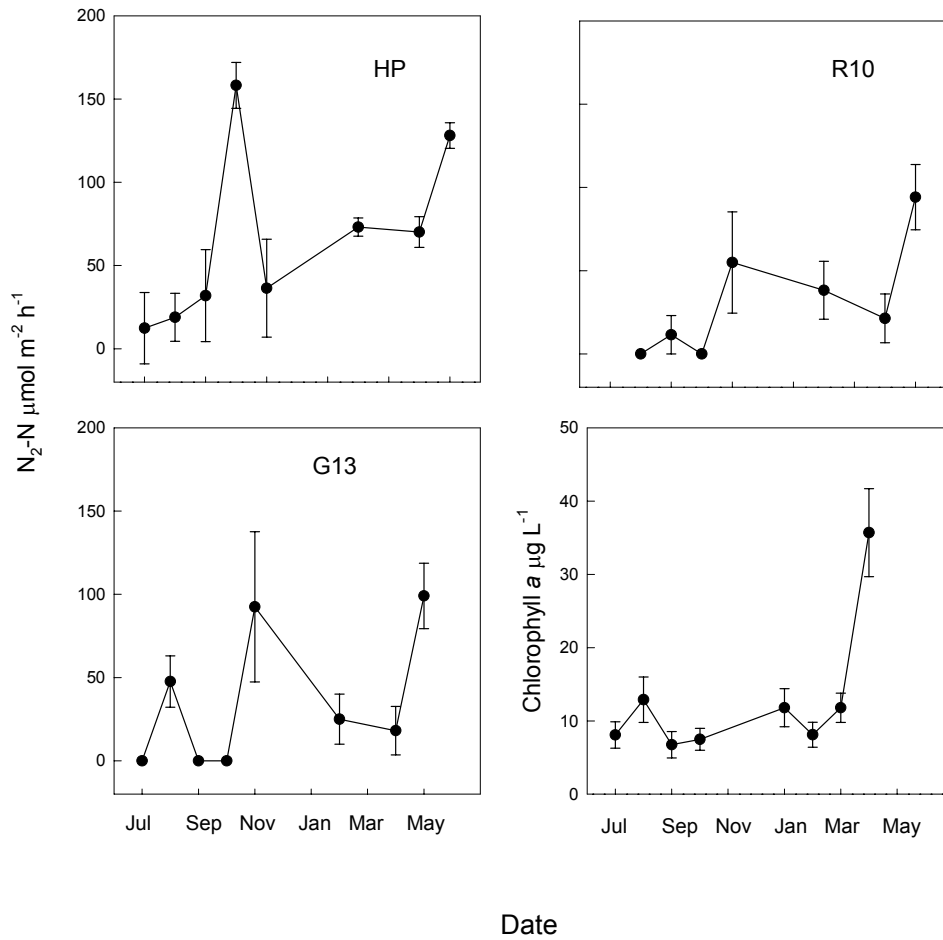


Figure 1.14 Pooled N_2 flux data from each transect plotted over an annual cycle. Bottom right panel shows the average bottom water chlorophyll *a* concentration over the same time period. Error bars for N_2 represents the standard deviation of all cores sampled on a given date and transect (N=4). Chlorophyll *a* error bars represent the standard deviation of all 3 sites.

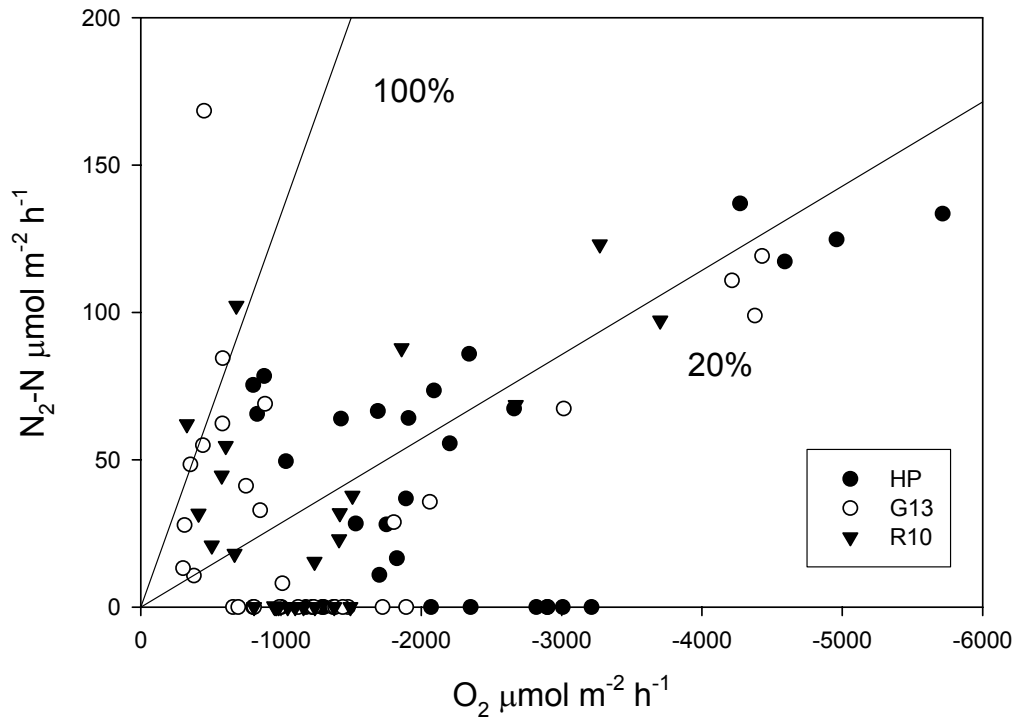


Figure 1.15 Correlation between N_2 and SOD for individual flux cores. The 100% line represents a Redfield ratio of $O_2:N$ of 6.6. Points falling near this line represent cores where all remineralized N was denitrified. The 20% line represents core fluxes where most of the remineralized N was returned to the water column as NH_4^+ .

Chapter 2: Seasonal Changes in Sedimentary Denitrification Efficiency:
The effects of Bioirrigation and Reduced Bottom Water Oxygen

Abstract

Estuaries are constantly in a state of change, often leading to shifts in water column oxygen on short time scales that can alter the fate of remineralized nitrogen. Intensive temporal scale studies of sediment denitrification are often not conducted due to the expense and effort required. Consequently, sampling strategies may not capture the effects that these perturbations may have on nitrogen cycling. We conducted a seasonally intensive study of nitrogen fluxes and denitrification at one site in the Choptank River estuary. Bioirrigation was measured immediately following core incubations using a bromide tracer. The diffusive flux of O₂ was calculated from microelectrode O₂ profiles and compared with core O₂ fluxes as a second measure of irrigation. Bioirrigation rates were ranged from ~2 to 15 times molecular diffusion in Spring and early Summer to negligible rates by August. Following the spring bloom, bioirrigation increased, indirectly accounting for a large proportion of the sediment oxygen demand. Low bottom water oxygen (~3 mg L⁻¹) in summer was associated with a loss of bioirrigation, polychaete abundance, and very low denitrification efficiency. Denitrification efficiency decreased from ~ 90% in early Spring to < 5% in August. The depth penetration of O₂ ranged from ~4 mm to 0.5 mm and was found to be a good indicator of denitrification efficiency under both high and very low irrigation rates.

Introduction

Eutrophication of coastal systems has led to increased intensity of algal blooms (Kemp et al. 2005), loss of submersed vegetation (Orth and Moore 1984, Stevenson et al. 1993), toxic algal blooms (Anderson et al. 2008), and water column hypoxia/anoxia (Hagy et al. 2004). Nitrogen has been identified as a key nutrient limiting phytoplankton growth in many estuarine systems (Gobler et al. 2006, Bernhard and Peele 1997, Fisher et al. 1992). In shallow water systems benthic and pelagic processes can be closely coupled (Nixon 1981) with sediments an important source of nutrients to the water column (Cowan & Boynton 1996). Sediments can be a source or a sink of dissolved inorganic nitrogen (DIN) to the water column depending on the efficiency of denitrification. Denitrification is a heterotrophic anaerobic process that transforms NO_3^- into N_2 gas removing fixed nitrogen from the system. The NO_3^- supply for denitrification can be supplied from the water column (Dw) or from the process of nitrification (Dn) in sediments. Denitrification has been identified as the most important nitrogen removal process in many estuaries (Nixon et al. 1996). Several factors can alter denitrification efficiency: 1) low bottom water O_2 concentrations can inhibit nitrification (Rysgaard et al. 1994); 2) bioirrigation transport of O_2 into the sediment can enhance nitrification (Mayer et al. 1995; Nielsen et al. 2004); 3) dissimilatory nitrate reduction to ammonia (DNRA) can short circuit Dw and convert NO_3^- into NH_4^+ (An & Gardener 2002); and 4) organic carbon loading to the sediment can decrease the O_2 penetration and limit nitrification (Caffrey et al. 1993). Denitrification efficiency has been applied on a system wide

scale as a measure of ecosystem function and has been shown to be dependant on the water residence time of a system (Nixon et al. 1996). Nitrogen may cycle from the water column to the sediment and back again numerous times and with each pass through the sediment there is a chance for N to be denitrified. The longer the N residence time in the system, the greater the probability that N will be lost to the atmosphere as N₂ gas. In this study, we focus on the efficiency of denitrification as an instantaneous rate in the sediment. Denitrification efficiency defined in this manner defines the balance between recycling versus denitrification and may be a critical measure of ecosystem function for many shallow water systems.

Many species of macrofauna irrigate the sediment by flushing O₂ rich water through their burrows. The overall effect of bioirrigation is to increase the volume of sediment where oxic and suboxic process can occur and to diminish the importance of sulfate reduction and the buildup of pore water sulfide (Heilskov & Holmer 2001). Bioirrigation has been shown to have a positive impact on the removal of fixed N from the sediments by promoting higher rates of nitrification (Mayer et al. 1995) and denitrification (Gilbert et al. 2003, Tuominen et al 1999). The degree to which macrofauna alter the nitrogen cycle has been linked to both density and species composition (Karlson et al. 2007). The presence of macrofauna can fundamentally change the diagenetic processing in sediments not only through irrigation and reworking of sediment but also by increasing the turnover rate of organic matter (Levin 1986, Grassle & Grassle 1974). Bottom water hypoxia or anoxia can result in decreased macrofaunal abundance and a loss denitrifying capacity. This loss of

denitrification may result in increased internal N loading to the system (Karlson et al. 2007).

In this paper, we examine seasonal nutrient fluxes, denitrification rates and the efficiency of denitrification in the Choptank River estuary. The main objectives of this paper are to: 1) present evidence for seasonal shifts in bioirrigation rates; 2) provide insight into the effects of bioirrigation on the nitrogen cycle; and 3) investigate factors controlling denitrification efficiency, (i.e., sediment O₂ penetration depth).

Methods

Sampling Location

The Choptank River watershed covers 252 km² and is heavily dominated by agriculture. Primary production is nitrogen limited in the river except in early spring when high nitrate concentrations from upstream sources persist over much of the river (Fisher et al. 2006). Our study site was located in the mesohaline portion of the Choptank River (Figure 2.1). This study site was chosen for several reasons: 1) previous studies had shown this site to be representative of the sediment type and bottom water oxygen conditions of a large portion of the lower Choptank River; 2) we had records of nutrient exchange and denitrification data sampled at varying intervals since July 2000 (Owens Chapter 1); and 3) this site was in close proximity to a monthly water column monitoring site maintained by the Maryland Department of the Environment. This portion of the Choptank River does not experience bottom water anoxia or hypoxia but historical data shows that bottom water oxygen

concentrations are declining as a result of continued eutrophication (Fisher et al. 2006). Oxidized surface sediments were observed in all cores throughout this study. The site (HPA) chosen for this study was located near the channel in the mesohaline portion of the river (38° 37.197N 76°08.061W) in ~9 m of water and consisted of very fine-grained sediments. Brown oxidized surface sediments were observed at all sampling times and there was no evidence of free porewater H₂S at any depths in our sediment cores. We observed only small infauna at this site predominately in the form of small polychaetes.

Sample Collection

Sampling was initiated in March 2006 with monthly sampling through June and two additional samplings in August and October 2006. Our sampling schedule covered a range of bottom water temperatures between 5°C and 29°C. Cores were collected using an acrylic/PVC Soutar-design box corer that collects cores with minimal exposure of sediment to metal. This device worked well collecting intact sediments with minimal resuspension from the very fine grained bottom deposits found at our sampling site. Only cores with an undisturbed sediment water interface were used for our measurements. The box corer was sub-cored with 6.35 cm inner diameter acrylic liners to a depth of ~15 cm. The subcores were topped off with ~15 cm of bottom water, stoppered, and transported to the lab within 2 h of collection. Bottom water from each site was collected with a diaphragm pump. Sediment cores were held submersed in a bath of bottom water overnight with continual aeration and circulation of the overlying water with bath water. Holding cores overnight

promoted: 1) the equilibration of temperature, oxygen, nitrogen, and argon in overlying and near-surface pore waters; and 2) provided a better equilibration of gases in the acrylic used for the chambers.

Sediment-Water Exchange

Sediment cores were incubated under dark conditions in a temperature-controlled chamber at *in situ* ($\pm 2^\circ \text{C}$) temperatures. Cores were capped with buna O-ring-fitted stirring tops prior to incubation, taking care to exclude bubbles. During sample withdrawal replacement bottom water was supplied through a port in the stirring top, with gravity feed providing the head pressure required to fill vials and syringes. A carboy of bottom water elevated above the cores supplied bottom water through manifolds which distributed water to each core. A suspended magnetic stir bar was used to mix the overlying water in each core. Solute samples were filtered using a 25 mm diameter, 0.45 mm cellulose acetate syringe filter. Typically, 20 mL were filtered into vials for analyses of NH_4^+ , and NO_3^- . Dissolved gas samples were collected in ~7 ml ground glass stoppered test tubes that were filled with a dip tube; samples were preserved with 10 μl of 50% saturated HgCl_2 solution. Dissolved gas samples were held under water at ambient bottom water temperature until analysis the following day.

Time courses of solute and gas concentration were determined over the course of 4-8 hours with 4 samples collected for each analyte. Oxygen concentrations were monitored during the incubations to insure that oxygen did not fall below 50 % of

saturation. Linear regression of the data was used to determine the slope of the time-concentration relationship and the flux rates were determined on an areal basis. In a limited number of cases, single data points were excluded from the regression. Blank cores had only minor changes in concentration at most sites. Corrections were made to account for water column effects when linear changes in the blank were observed for an analyte.

Microelectrode oxygen measurements

Dissolved pore water O₂ concentrations were determined using a Clark type O₂ microsensor (www.unisense.com) with 50µm tip (Revsbech 1989). Triplicate microelectrode profiles of O₂ were made on each of the three replicate cores. Overlying water in the core was homogenized with a low flow of air through an air stone. Measurements were made at 100 µm intervals starting ~0.4 mm above the sediment water interface and continued down to the depth where O₂ was depleted. All microelectrode measurements were made at ambient bottom water temperatures.

Bioirrigation measurements

Estimates of infaunal irrigation rates were made by comparing discrepancies in modeled and actual measured profiles of an added conservative bromide tracer (Mackin et al. 1988; Martin & Banta 1992). Immediately following the flux core incubations water lift pumps were placed in each core. The cores were kept

submerged with lift pumps providing both aeration of the water and complete mixing of the water overlying the cores with the bottom water in the tank. A concentrated solution of NaBr was added to the tank water to reach a final concentration of ~5 mM. The Br⁻ tracer was allowed to diffuse into the sediment overnight (17-24 hours) before cores were removed and sectioned for pore water. Surface sections down to 2 cm were sectioned at 0.5 cm intervals and deeper strata were sectioned at coarser intervals down to a total depth of ~ 8 cm. Pore water was extracted by centrifugation and analyzed with a Dionex ICS 2000 ion chromatograph. Theoretical bromide tracer profiles were modeled using Fick's first law of diffusion and compared with the actual concentrations of bromide found in the pore water. Modeled pore water concentrations of Br⁻ were calculated from Fick's first law of diffusion $J_s = -\phi D_s (dc/dx)$ where J_s is the flux mol cm⁻² s⁻¹, ϕ is porosity, D_s is the molecular diffusion coefficient cm² s⁻¹, and dc/dx the concentration gradient in mol cm⁻³ cm⁻¹. Diffusion coefficients within sediments were estimated from diffusion coefficients in a particle free solution at *in situ* temperature and the conversions of porosity to tortuosity squared using

$$\text{Boudreau's law } (\theta^2 = 1 - \ln(\phi^2)), \text{ (Boudreau 1997)}$$

The pore water O₂ measured with microelectrodes was used in conjunction with our measured O₂ flux (core incubations) as a second measure of bioirrigation. We used our microelectrode profiles of pore water dissolved oxygen to calculate the diffusive flux of O₂ across the sediment water interface. This was calculated using the equation: Flux = $-\phi D_s (dC/dx)_{x=0}$ where ϕ is porosity, C pore water concentration, x depth and D_s molecular diffusivity corrected for tortuosity (Berg et

al. 2001). A porosity of 0.88 was used for all calculations of diffusive O₂ flux. The calculated flux O₂ from the microelectrode profiles was compared to actual flux measured during our flux core incubations to quantify any enhancement of solute transport beyond that which could be explained by simple molecular diffusion.

Gas, Solute and Sediment Analysis

A "dissolved gas analyzer" (DGA; Kana et al. 1994; 1998) was used for the analysis of N₂ and O₂ in flux samples. This analyzer consisted of a quadrupole mass spectrometer with a silicone membrane inlet, which exchanged the gases between the water and the instrument's vacuum. Saturated water standards were used for calibration of the N₂:Ar and O₂:Ar gas ratios which were used as gas concentration estimates. The overnight equilibration of sediments at *in situ* bottom water temperatures ensured that the overlying water and the pore water were close to thermal equilibrium, minimizing potential artifacts associated with the sensitivity of N₂:Ar gas ratios to temperature. Nitrate + nitrite was analyzed via segmented flow analysis after Cd reduction and ammonium was manually analyzed with a phenylhypochlorite colorimetric technique (Parsons et al. 1994).

Infaunal sampling

Previous work at the HPA site had shown that replicate core fluxes were very similar over spatial scales of 10 -100m for a given water depth. All the measurements made at a given sampling date including the infaunal sampling were made using the same cores. Sediment cores were collected (day 1), conditioned overnight and flux measurements completed the next day. Once the flux incubations were complete, bromide was added and allowed to diffuse into the sediment overnight (day 2). The following morning (day 3) microelectrode pore water O₂ profiles were measured on all cores and a small sub core was sectioned from each flux core for pore water Br⁻. The remaining sediment in each core was then sieved through a 100µm mesh for infaunal sampling. This method allowed us to measure all parameters on the same cores and minimized problems caused by core to core variability.

Results

Sediment water exchange

Our lowest rates of sediment oxygen demand (SOD), $508 \pm 34 \mu\text{mol O}_2 \text{ m}^{-2} \text{ h}^{-1}$ were measured in March when water temperatures were 5 ° C (Figure 2.2). The SOD rates increased through the spring with a peak rate of $4198 \pm 72 \mu\text{mol O}_2 \text{ m}^{-2} \text{ h}^{-1}$ measured in June. SOD decreased 3 fold between June and August and remained low into the fall. The flux of NH₄⁺ was directed out of the sediment and followed a

similar pattern to SOD with peak rates in June of $490 \pm 95 \mu\text{mol N m}^{-2} \text{ h}^{-1}$ (Figure 2.3). The peak in NH_4^+ flux was more pronounced than for SOD; NH_4^+ flux increased 6 fold from May to June and then decreased nearly 9 fold from June to August. The peak bottom water temperatures of 29°C were not reached until August. Measured $\text{NO}_3^- + \text{NO}_2^-$ fluxes were variable ranging from $60 \pm 9 \mu\text{mol N m}^{-2} \text{ h}^{-1}$ directed out of the sediment to $-34 \pm 9 \mu\text{mol N m}^{-2} \text{ h}^{-1}$ into the sediment (Figure 2.4). Fluxes of $\text{NO}_3^- + \text{NO}_2^-$ were directed out of the sediment only in early spring and fall. The flux of dissolved N_2 across the sediment water interface was directed out of the sediment on all individual core measurements made during this study (Figure 2.5). The rates of N_2 flux were variable with the highest rates of efflux of $\sim 170 \mu\text{mol N}_2\text{-N m}^{-2} \text{ h}^{-1}$ measured in April and June. In August, the rate of dissolved N_2 efflux had declined to very low levels and was only detectable in one of the triplicate flux cores.

Bottom water O_2 and Polychaete Abundance

Bottom water O_2 concentrations at our sampling site remained near 100 % saturation from March to April and declined from May through August (Figure 2.6). The lowest bottom water O_2 concentration of 3.05 mg l^{-1} (43% saturation) was measured in August. The dominant infaunal species found at our sampling site was a small spionid polychaete $\sim 0.5 \text{ mm}$ in width and ranged from $\sim 1\text{-}1.5 \text{ cm}$ in length. Polychaete densities were high in the spring ranging from 2.8×10^4 to 1.1×10^4 individuals m^{-2} but declined by an order of magnitude in summer and remained low into the fall (Figure 2.6). No data on polychaete densities was collected in June.

Sediment microelectrode O₂ measurements

Large changes in the sediment depth penetration of O₂ were found during the course of this study (Figure 2.7, 2.8). Sediment pore water O₂ penetration reached nearly 4 mm into the sediment in March when both temperature and SOD were low. The penetration depth of O₂ decreased in April and May and reached the lowest level in August. Our microelectrode profiles of pore water O₂ were measured with O₂ saturated overlying water. Under O₂ saturating conditions, the depth penetration decreased from 4 mm to about 2 mm from March to August (Figure 2.7). The actual depth penetration of O₂ would have been much lower *in situ* as the bottom waters became undersaturated with O₂ from May through October (Figure 2.8). We estimated the *in situ* O₂ penetration depth by taking the difference in the overlying water between O₂ saturating conditions and actual bottom water O₂ and applied this difference to the measured profiles. The *in situ* depth penetration of O₂ was then estimated from our adjusted profiles. During the course of our study, the *in situ* depth penetration of O₂ changed from a 4 mm max in March to less than a 0.5mm in August (Figure 2.8).

The microelectrode O₂ profile data was used to calculate the diffusive flux of O₂ through the sediment water interface (Figure 2.9). The diffusive flux of O₂ increased with temperature but only increased ~2 fold during this study. The core O₂ flux and the diffusive flux were similar in March and August but the core flux increased more than 8 fold from March to April as a result of advective transport induced by bioirrigation.

Bioirrigation was also measured by modeling the transport of Br^- into the sediment. We adjusted the theoretical (diffusion only) profiles to match our measured Br^- profiles and estimated an advection factor. Plots of Br^- concentration with depth are shown in figure 2.10 for June and August. Highest rates of bioirrigation were measured in June and were reflected in the large discrepancy between theoretical and measured profiles of Br^- . An advection factor > 1 would represent enhanced solute transport via bioirrigation. The advection factors calculated from the Br^- tracer and the comparison of SOD and diffusive flux from O_2 sediment profiles were similar for most of the months sampled (Figure 2.11). The June data showed a large disparity between the 2 approaches with Br^- tracer giving a 3 fold higher advection factor. The June sampling also had by far the highest rates of bioirrigation; rates were 5 to 15 times that of diffusion depending on which method was used.

Denitrification Efficiency

Denitrification efficiency is a good measure of how effective the sediments are in removing nitrogen as N_2 gas through the process of denitrification. We calculated denitrification efficiency by dividing the denitrification rate by the sum of the dissolved inorganic nitrogen ($\text{NO}_3^- + \text{NO}_2^- + \text{NH}_4^+ + \text{N}_2$) efflux. Water column nitrate concentrations were in the range of 2-20 μM during our study and at times the water column was an important source of nitrate for denitrification. The sediments at our study site were very efficient at denitrification in March and April with nearly all DIN flux in the form of N_2 gas (Figure 2.12). Efficiency decreased after April and

reached a low of < 3% by August and then increased to 36% by October. In June and August NH_4^+ was the dominant form of DIN flux from the sediment.

Discussion

Seasonal changes in bioirrigation

The two methods used in this study to measure bioirrigation have been shown to give comparable results (Berg et al. 2001). In our study, the March and June Br⁻ method gave statistically (analysis of variance ANOVA) lower values for enhanced transport of solutes via bioirrigation compared to the microelectrode/core flux method. The largest disparity was measured in June when rates of bioirrigation peaked for the year. Small opportunistic polychaetes have been shown to take advantage of fresh organic material by increasing growth rate and population densities over short time scales (Levin 1986, Grassle & Grassle 1974). Spring peaks in small polychaete abundance have also been observed in other studies and thought to be a response to fresh algal detritus settling to the bottom as a result of the spring bloom (Cheng et al. 1993, Rossi & Lardicci 2002).

Our previous work at this site (2000-2001) had shown a tight benthic pelagic coupling with the spring bloom stimulating high rates of SOD. If we apply our linear regression SOD model from our previous study, we find that the model predicts the general pattern of SOD except for the winter rates (Figure 2.12). Our previous SOD model ($\text{O}_2 \text{ Flux} = 131.2 * \text{Chl a } \mu\text{g l}^{-1} + 682.5$) did not include temperature and as a consequence over predicted SOD during the intense 2006 winter bloom. These

results suggest that temperature is a component driving SOD and that there was probably some sediment storage of the winter bloom that was decomposed later in the spring as temperature increased. These model results also indicate that the activity of macrofauna were responsible for the tight benthic-pelagic coupling.

In our study, we observed a 10 fold decrease in polychaete abundance from May to August. This type of boom and bust cycle is common for opportunistic species of polychaetes and the summer decline in abundance may result from a lack of nutrition in summer (Rossi & Lardicci 2002, Linton & Taghon 2000). Spionid polychaetes have been shown to have good survival during short term hypoxic events lasting up to 2 weeks but longer term effects of reduced oxygen are likely to have an adverse effect on survival (Llanso 1991). The lack of bioirrigation in our study during summer is likely due to both decreased abundance of polychaetes and modified feeding behavior in response to lower bottom water oxygen concentrations (Llanso 1991). The highest abundance of polychaetes in March did not correspond to high rates of irrigation, suggesting that worm activity may have been suppressed due to temperature ($\sim 5^{\circ}\text{C}$). In the absence of abundance data, we can only speculate as to why irrigation rates were high in June. Increased feeding activity, increased abundance, or increased irrigation due to lower O_2 concentrations could be possible explanations for the June spike in irrigation rates. Visual inspection of the cores during sectioning for Br^- in June suggested that small polychaetes were still the dominant species.

Bioirrigation and Nutrient Cycling

The cycling of fresh organic matter in the sediments has been shown to be greatly enhanced through the activity of macrofauna (Heisklov & Holmer 2001, Bianchi et al. 2000). The exact mechanism of organic matter degradation enhancement by macrofauna is under debate but there is some evidence that increased exposure to O₂ alone can enhance decomposition (Harvey et al. 1995). In our study, the large spike in SOD rates in June could be the result of enhanced polychaete feeding and resulting transport of O₂ into the sediment. Through the activity of these animals the sediment was rapidly depleted of fresh organic matter and SOD rates decline within a month and remain low until fall. Studies have shown that sediment chlorophyll *a* can preserve a short term record of phytoplankton deposition to the sediment (Cowan & Boynton 1996). Our previous work at the HP site showed low sediment chlorophyll *a* through spring and summer after the spring bloom. The efficient feeding activity of macrofauna may have enhanced the decomposition of chlorophyll *a*.

High rates of irrigation can short circuit the process of coupled nitrification/denitrification through rapid transport of NH₄⁺ from burrows into the water column (Mermillod-Blondin et al. 2004). This irrigation effect is demonstrated in our study; under moderate rates of irrigation in spring denitrification efficiencies remain high but under very high rates of irrigation in June denitrification efficiency decreased to ~30% with most of the DIN returning the water column as NH₄⁺. The high denitrification efficiencies found in Port Phillip Bay sediments over a wide range of bioirrigation rates suggests that irrigation has little effect on efficiency (Berelson et

al, 1998). In our study the decline in denitrification efficiency in spring was also associated with the settling of the spring bloom and this increase in fresh organic matter may have contributed to less efficient denitrification due to a decreased penetration of O₂ and a shrinking of the zone where nitrification could occur (Caffrey et al. 1993). The summer decline in both the abundance of polychaetes and irrigation was also associated with the lowest denitrification efficiency measured in our study. These results suggest that when present in sufficient abundance, these small opportunistic polychaetes promote the removal of nitrogen via denitrification.

Irrigation and rapid organic matter turnover via macrofauna promote denitrification for the following reasons: 1) There is little long term labile organic matter storage in the sediment that could drive a bottom up enhancement of hypoxia later in the summer; 2) The increased transport of O₂ into the sediment promotes higher rates of denitrification through both coupled nitrification/denitrification and enhanced transport of nitrate from the water column ; and 3) Sediments are more oxidized inhibiting the buildup of H₂S and promoting a deeper diffusional depth of O₂. Studies of fish-farm sediment with added *Capitella sp* and *Nereis diversicolor* have shown both an enhancement of aerobic metabolism and a decrease in sulfate reduction compared to azoic sediments (Heilskov & Holmer 2001). The sediments below these fish pens were very reducing with H₂S present near the sediment-water interface. In sediments that are more oxidized, macrofauna could have a greater impact on the balance between aerobic versus anaerobic metabolism and may actually prevent free sulfide from building up in pore water by advecting O₂ and promoting the reoxidation of reduced metabolites. A tipping point or threshold is likely to exist

driven by decreasing bottom water O_2 or increasing organic matter load that could drive the sediments into a state where free sulfide could persist in pore water. The result of crossing this threshold would be a substantial increase in the recycling of remineralized N through the inhibition of nitrification (Joye and Hollinbaugh 1995), dissimilatory NO_3^- reduction to NH_4^+ (An & Gardner 2002), and a decrease in habitat for macrofauna due to H_2S poisoning.

Denitrification Efficiency

Our study indicates that DE was strongly influenced by both bottom water O_2 concentrations (Figure 2.13) and the depth penetration of O_2 (L) into the sediment (Figure 2.14). The multiple regression analysis from our previous work had shown that the main factor controlling the balance between NH_4^+ release and denitrification was the concentration of bottom water O_2 . When applied to the 2006 data, our regression predicted ~75% of the variability in DE (Figure 2.12). These model results indicate that the relationship between the depth of O_2 penetration and DE is robust and might be used as a predictive tool in other coastal systems.

The majority of the change in L in our study can be attributed to changes in bottom water O_2 . The *in situ* estimated L explains ~67% of the variability in DE even though most of time the transport of O_2 through bioirrigation was at least equivalent to the diffusional flux of O_2 . There are several possibilities that could explain the strong dependence of DE on L: 1) deeper O_2 penetration leads to greater surface area for nitrification; 2) the rapid turnover of labile organic matter by macrofauna maintains a low level of labile organic carbon in the sediment promoting

greater L year round; and 3) decreasing L may lead to less irrigation through modified macrofaunal behavior or population decline. This study gives some strong evidence that O₂ penetration depth could be a useful tool in predicting the efficiency of denitrification in other systems.

Implications

The results of this study have implications for nitrogen cycling in coastal systems. The loss of bioirrigation through sediment disturbance or low bottom water O₂ could have major impacts on the nitrogen cycle leading to higher rates of NH₄⁺ recycling back to the water column. Our results indicate that macrofauna can; 1) enhance O₂ advection, 2) enhance the decomposition of labile organic matter, and 3) maintain sediment conditions that promote a high level of denitrification efficiency. We cannot definitively conclude that the 10 fold decline in polychaete abundance during summer in this study was caused by low bottom water O₂ but the data show that under low O₂ (~3 mg l⁻¹) conditions and in the absence of irrigation, coupled nitrification/denitrification was greatly inhibited. The progression of eutrophication often leads to reduced bottom water O₂ concentrations and at some point a loss of ecosystem function. Thresholds of hypoxia (~2 mg l⁻¹) used for management purposes are currently being revised. New information suggests that macrofauna behavior and abundance begin to change above the 2 mg l⁻¹ (hypoxia) threshold (Vaquer-Sunyer and Duarte 2008). Our study demonstrates the importance of defining O₂ thresholds that will maintain a high level of denitrification efficiency

through maintaining healthy infaunal communities and a deeper aerobic zone within sediments.

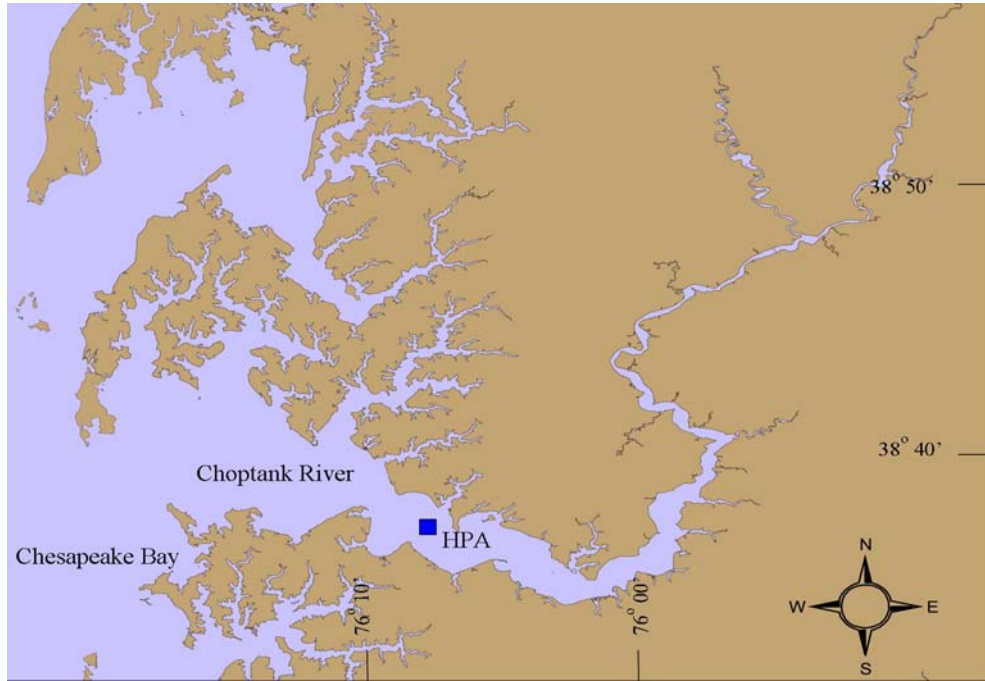


Figure 2.1 Map of the Choptank River a sub estuary of the Chesapeake Bay.

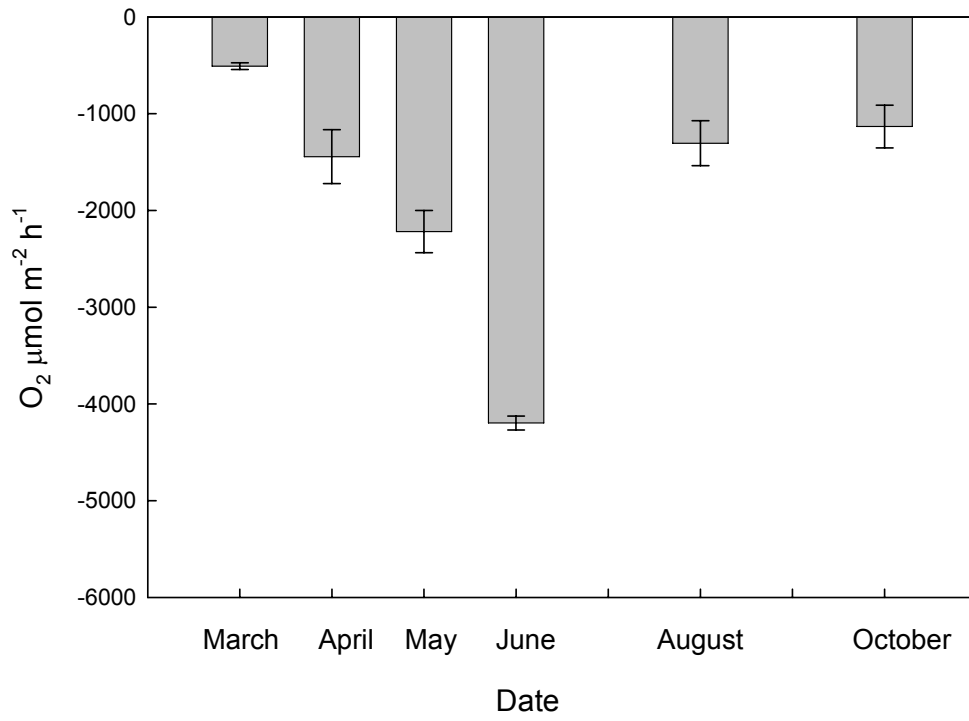


Figure 2.2 Sediment O_2 flux from March through October 2006. Error bars represent standard deviation of 3 replicate cores.

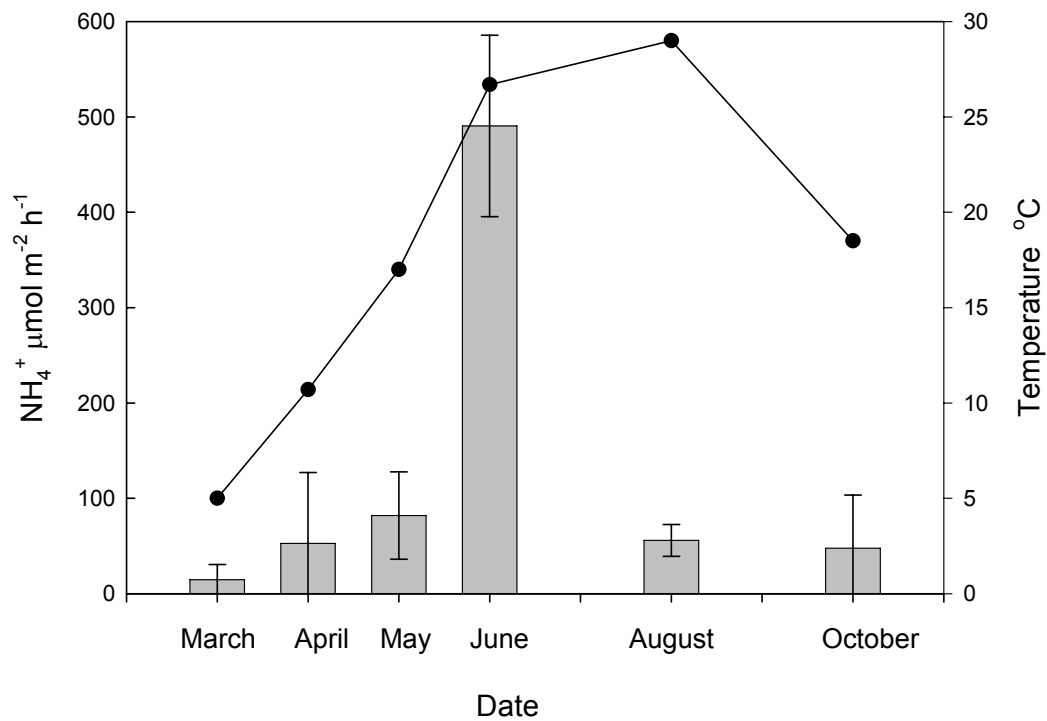


Figure 2.3 Sediment NH_4^+ flux from March through October 2006 plotted on left axis. Bottom water temperature plotted on the right axis. Error bars represent standard deviation of 3 replicate cores.

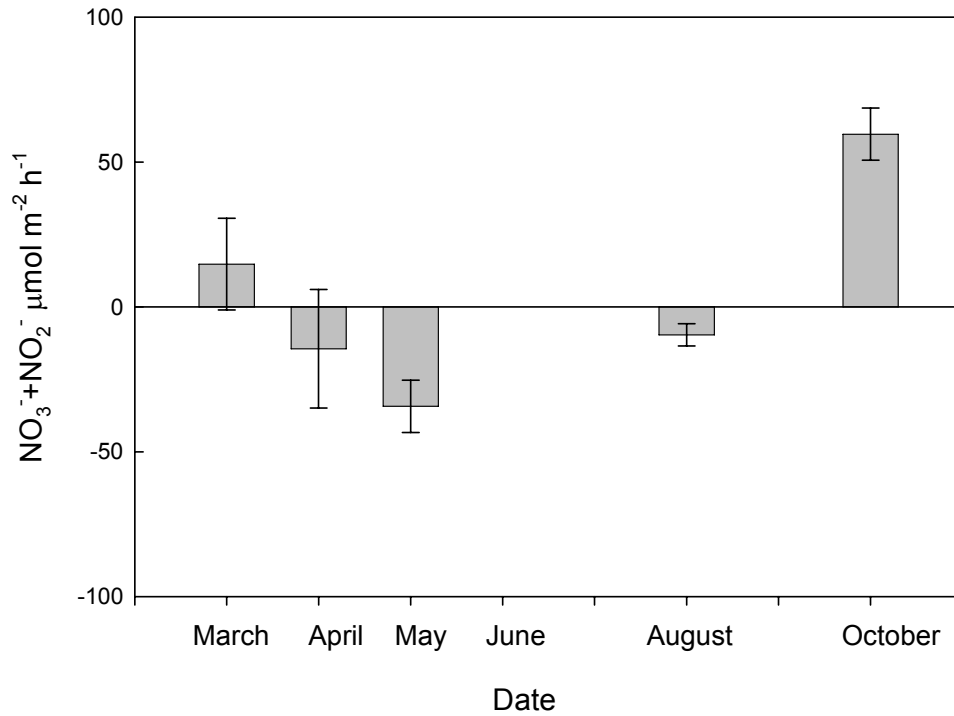


Figure 2.4 Sediment $\text{NO}_2^- + \text{NO}_3^-$ flux from March through October 2006. Error bars represent standard deviation of 3 replicate cores.

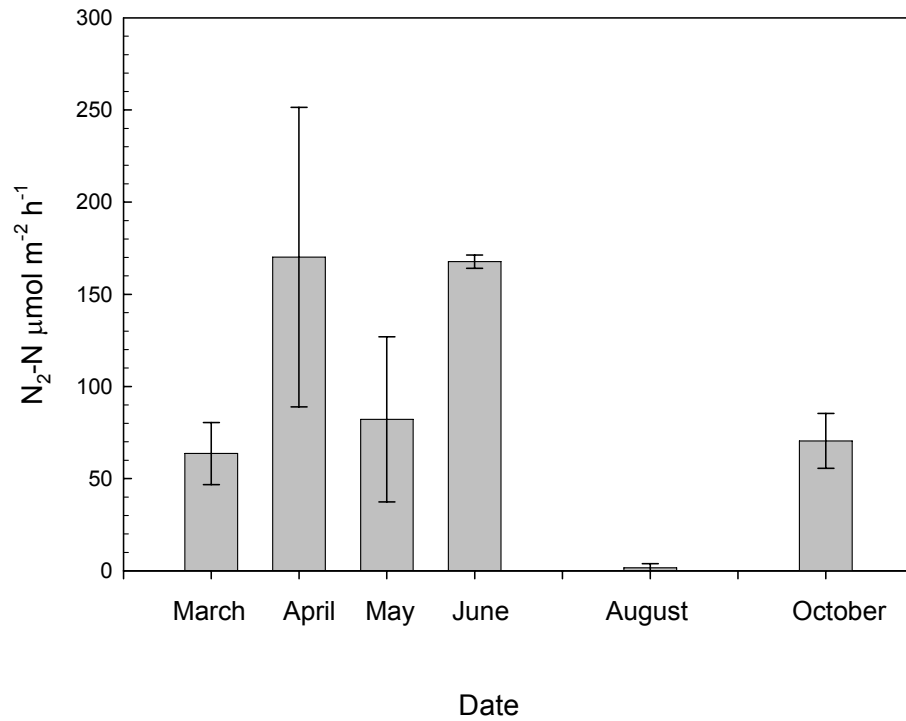


Figure 2.5 Sediment N_2-N flux from March through October 2006. Error bars represent standard deviation of 3 replicate cores.

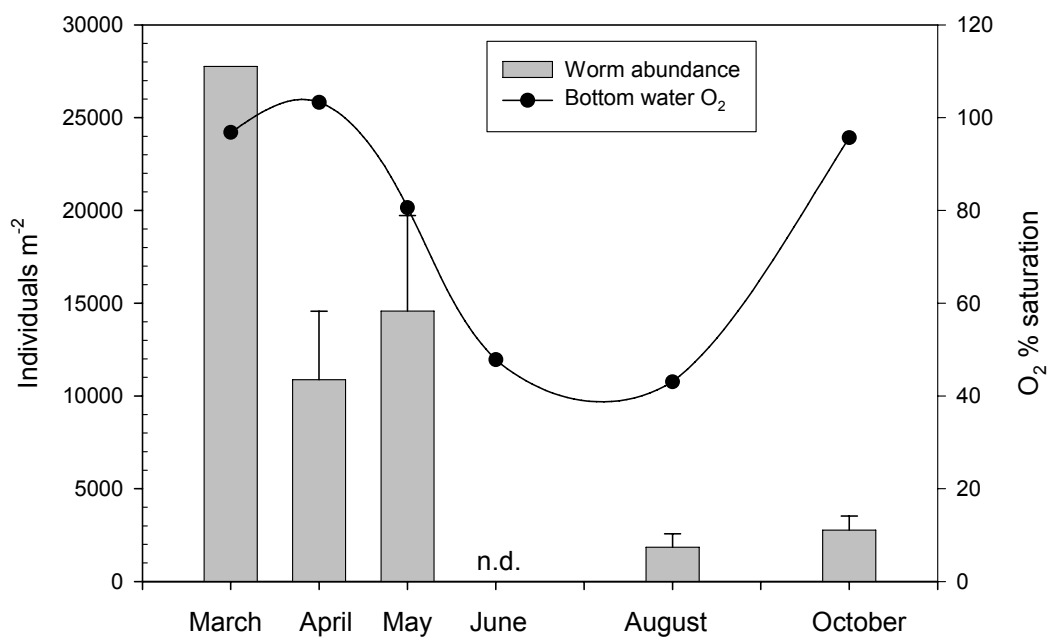


Figure 2.6 Polychaete abundance from March through October 2006. No abundance data was collected in June. *In situ* bottom water O₂ percent saturation plotted on the right hand axis. Error bars represent standard deviation of 3 replicate cores. March data n=1

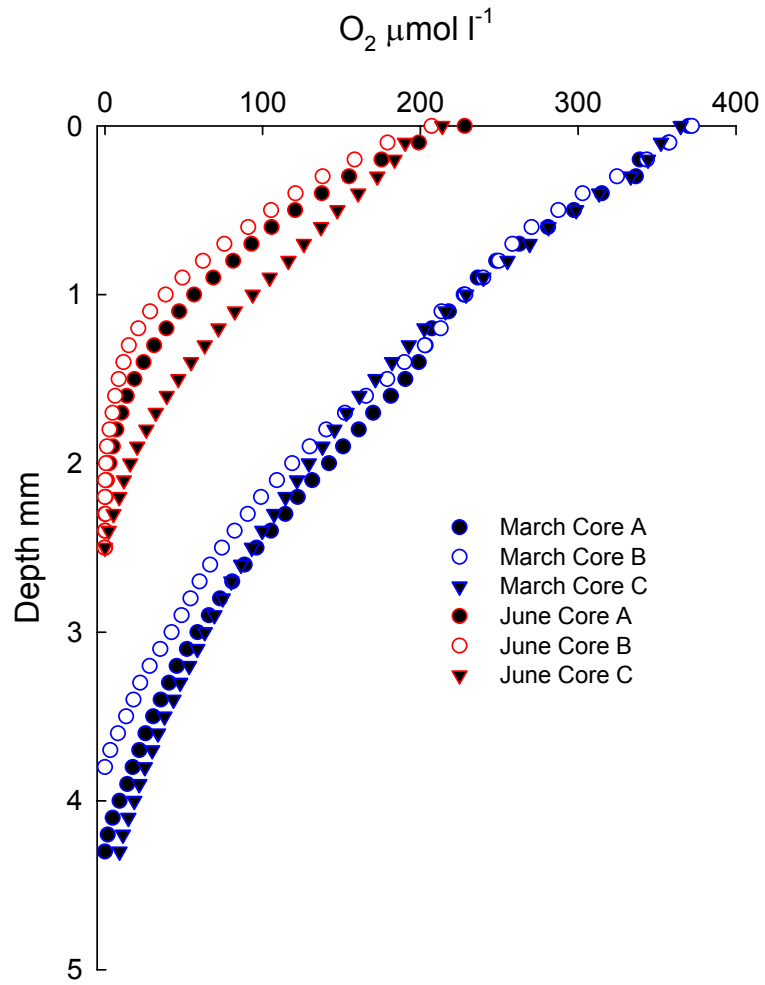


Figure 2.7 Microelectrode O_2 profiles from March and June. One profile for each replicate core measured in March (blue) and June (red)

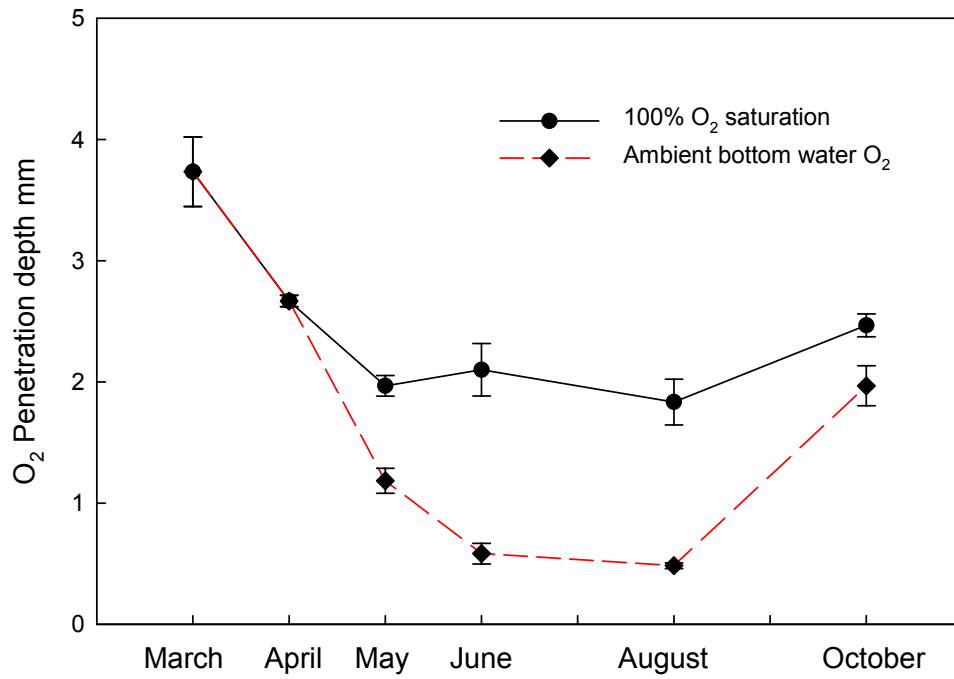


Figure 2.8 Solid line represents the O₂ penetration depth in mm under 100% saturated O₂ conditions in the laboratory. The dashed line represents the estimated depth of O₂ under actual water column O₂ concentrations. Error bars represent standard deviation of 3 replicate cores.

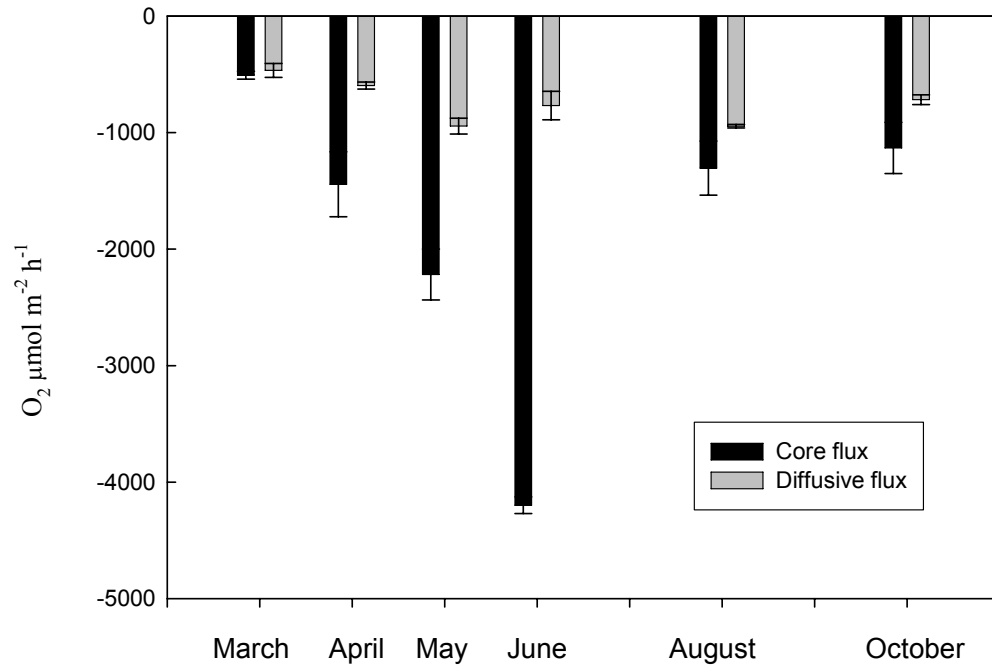


Figure 2.9 The black bars represent the flux of O₂ from our core incubations (diffusive and advective). The grey bars show the flux of O₂ from molecular diffusion only. The difference between the bars is the advective transport of O₂ via bioirrigation. Error bars represent standard deviation of 3 replicate cores.

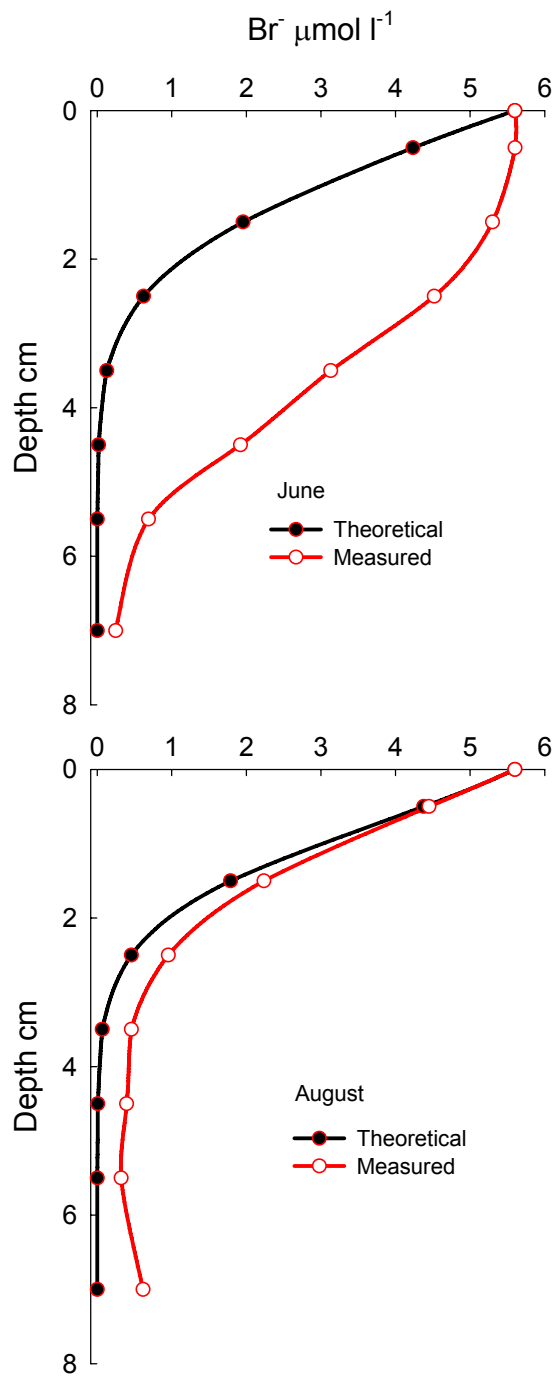


Figure 2.10 Bromide profiles from June (top panel) and August (bottom panel). The theoretical Br^- diffusion profile (diamonds) plotted with the measured profile (squares).

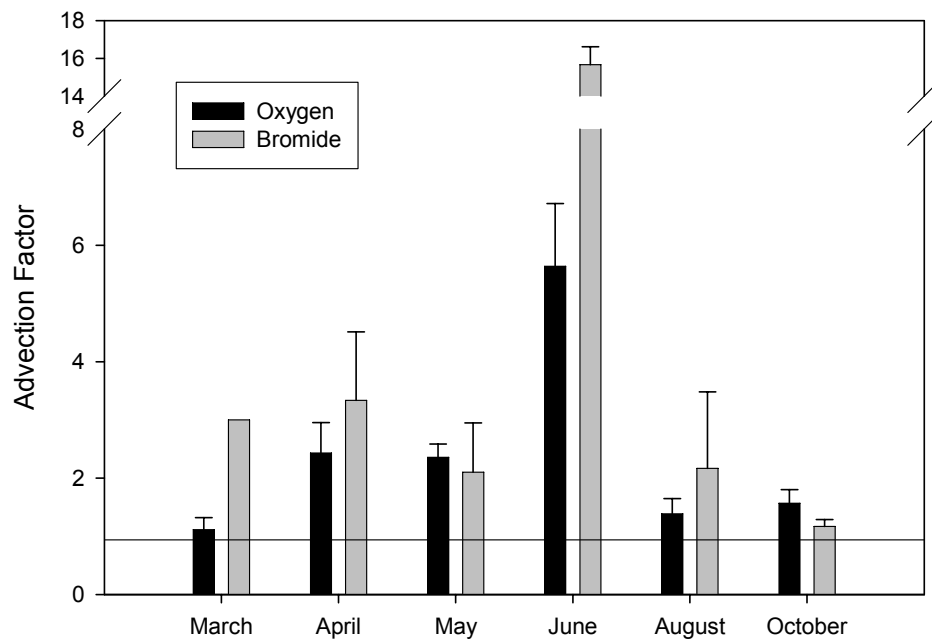


Figure 2.11 Comparison of our 2 methods for measuring bioirrigation. The black bars represent the advection factor for the Br^- tracer. The white bars show the advection factor calculated from the comparison of whole flux core incubations and diffusional transport of O_2 measured with microelectrodes. Only March and June data show statistically different advection numbers between methods. An advection factor of 1 represents transport only by diffusion. Error bars represent standard deviation of 3 replicate cores.

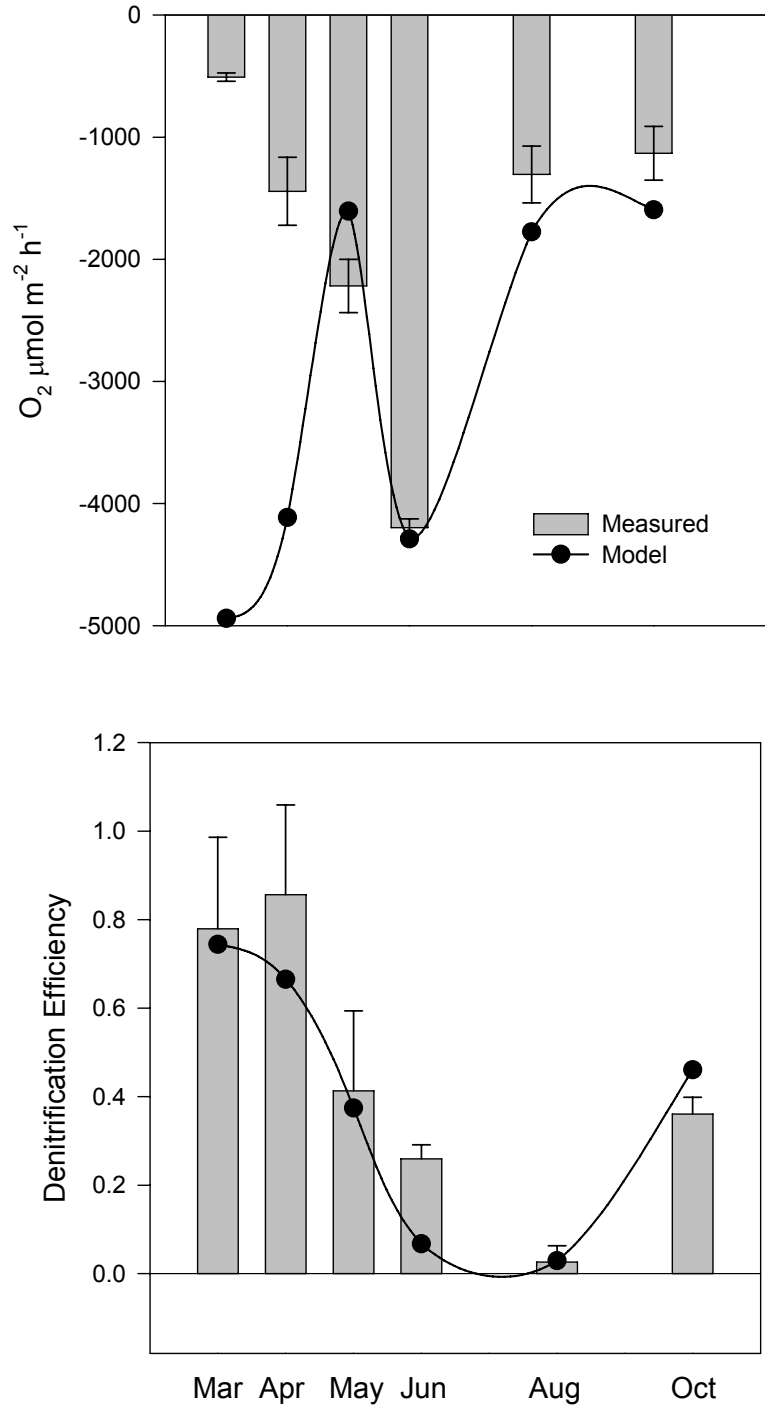


Figure 2.12 Sediment O₂ demand and denitrification efficiency for the period March through October 2006. Error bars represent standard deviation of 3 replicate cores. Closed circles represent model predictions based on a multiple linear regression model developed from seasonal data collected at the same site in 2000-2001.

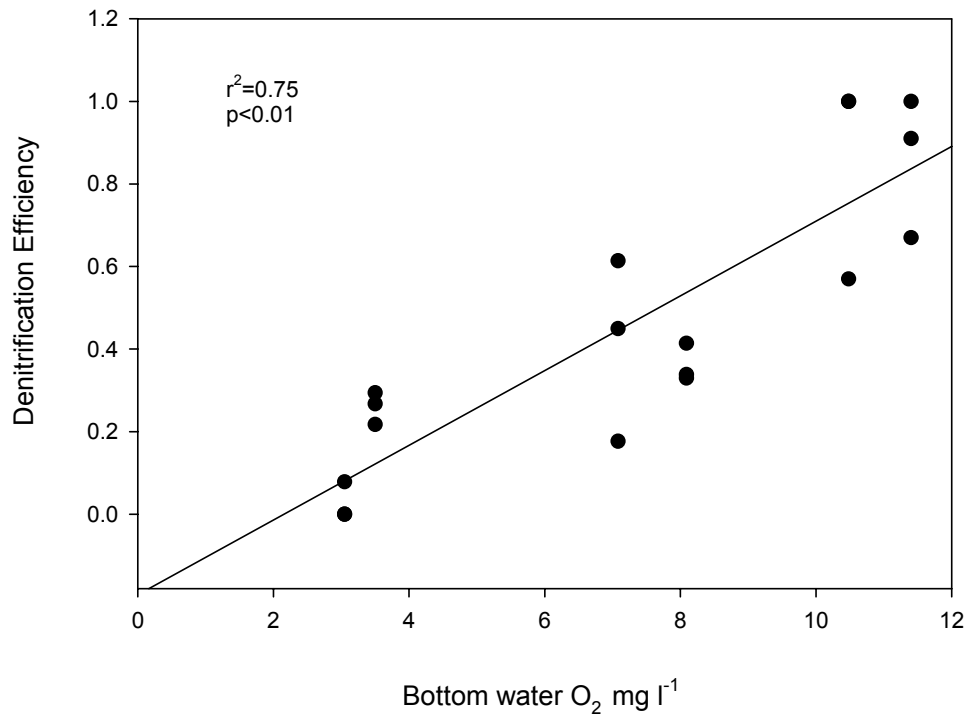


Figure 2.13 Denitrification efficiency plotted against bottom water O₂ in mg l⁻¹. Bottom water O₂ is a strong indicator of efficiency explaining 75% of the variability in DE.

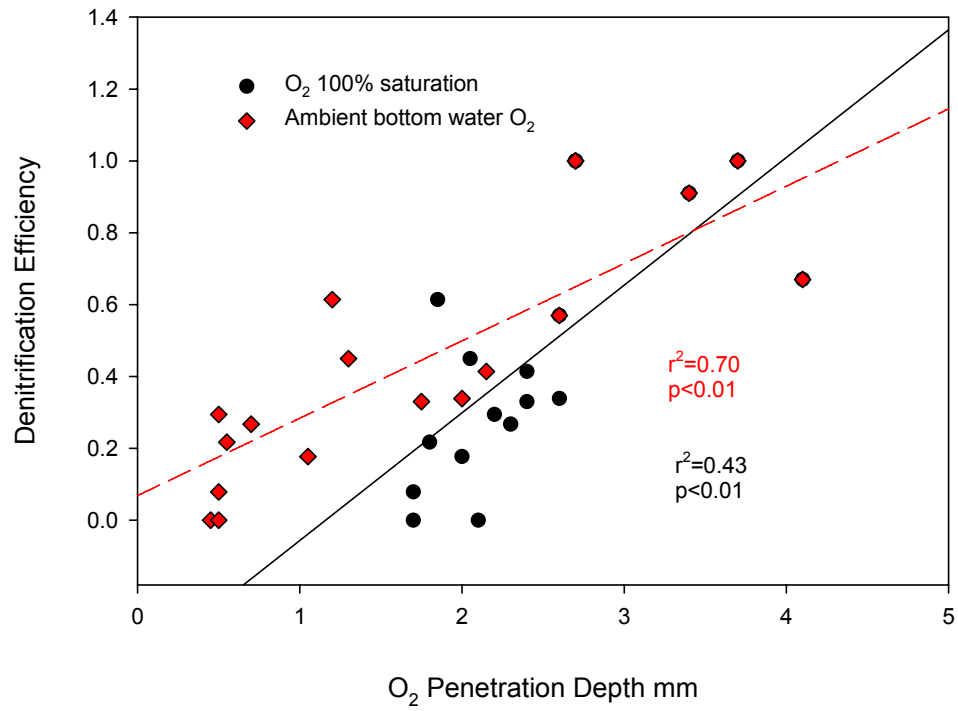


Figure 2.14 Denitrification efficiency plotted against the estimated *in situ* O₂ sediment depth penetration (L) in mm (diamonds) and the measured L under saturated O₂ conditions (closed circles).

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