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Estimating denitrification rates in estuarine sediments: A comparison of stoichiometric and acetylene based methods

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Abstract. We compared denitrification rates obtained using an adaptation of the acetylene block technique to rates estimated from benthic flux nutrient stoichiometry in the subtidal sediments of Tomales Bay, California (USA). By amending whole cores with acetylene and saturating nitrate concentrations, we obtained potential denitrification rates, which ranged between 4 and 30 mmol N m^{-2} d^{-1}. We determined the apparent Michaelis constant (K_{app}) and the maximum potential rate (V_{mp}) of the denitrifying community and used these constants in a rectangular hyperbola to estimate *in situ* denitrification rates. Both the K_{app} and V_{mp} of the denitrifying community exhibited significant variation over both depth in the sediment column and time of sampling.

Estimates of *in situ* denitrification obtained using our 'kinetic-fix' adaptation of the acetylene block ranged between 1.8 (March) and 9 (Sept.) mmol N m^{-2} d^{-1}. Denitrification rates obtained using benthic flux stoichiometry ranged between 0.7 and 4.1 mmol N m^{-2} d^{-1}. Average denitrification rates obtained using the 'kinetic-fix' acetylene block approach exceeded those obtained from net benthic flux stoichiometry; however, these differences were not significant. We conclude that our 'kinetic-fix' adaptation of the acetylene block technique provides realistic estimates of denitrification in sediments, even when pore water nitrate concentrations are low and nitrification and denitrification are closely coupled.

Introduction

In shallow coastal systems, a significant portion (25–50%) of organic matter mineralization occurs in sediments (Nixon et al. 1976; 1980; Nixon 1981). High rates of sediment metabolism, typical of bays and estuaries, can rapidly deplete the primary oxidant, oxygen (O_2), increasing the importance of alternative metabolic pathways. Of the suboxic and anaerobic oxidative pathways, denitrification provides the largest energy yield (Payne 1970; 1973). Denitrifying microorganisms transform combined nitrogen (NO_x) into gaseous

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(N_2 or N_2O) forms. Their activity balances the global nitrogen (N) cycle, compensating for new N input via microbial N_2 fixation (Tiedje 1988; Smith & Hollibaugh 1989). In systems impacted by anthropogenic combined N loading, denitrification removes excess combined N (Seitzinger 1988; Smith & Hollibaugh 1989). However, in many shallow coastal areas, phytoplankton production is N-limited (Dugdale 1967; Ryther & Dunstan 1971; Codispoti 1989). In these environments, sediment denitrification can exacerbate N limitation of water column phytoplankton (Koike & Sørensen 1988; Seitzinger 1988).

The ideal way to quantify denitrification rates is to measure dinitrogen (N_2) production directly (Seitzinger et al. 1980 & 1994; Devol 1991). Unfortunately, large background concentrations of N_2 make this measurement problematic and subject to large errors, even when slight contamination occurs (Devol 1991; Nowicki 1994). Measuring N_2 production directly may also require long (days) incubations or pre-incubations (Seitzinger et al. 1980 & 1994). Because of these problems, indirect approaches have been developed to estimate denitrification. These include: mass balance and stoichiometric modeling, measuring sediment-water interface NO_3^- fluxes, utilizing stable N isotope tracers, applying metabolic inhibitors and examining kinetic parameters of the denitrifying community (see Seitzinger 1988 for a review). In this paper, we compare denitrification rates obtained with a kinetic approach based on the acetylene (C_2H_2) block technique (referred to as the 'kinetic-fix' acetylene method) with rates estimated from benthic flux stoichiometry.

Acetylene inhibits the reduction of N_20 to N_2 (Balderston et al. 1976; Yoshinari & Knowles 1976). By amending samples with C_2H_2 and quantifying the change in N_2O concentration over time, the denitrification rate can be estimated (Sørensen 1978; Kaspar 1982; Knowles 1990). This technique yields detailed spatial (mm to cm) resolution of denitrification activity. However, C_2H_2 also inhibits nitrification (Hynes & Knowles 1978; Walter et al. 1979; Knowles 1990). When nitrification and denitrification are closely coupled, denitrification rates may be underestimated (Oremland & Capone 1987; Revsbech et al. 1988, Christensen et al. 1989 & 1990; Nielsen et al. 1990; Revsbech & Sørensen 1990). Underestimates of denitrification rates may also arise from sulfide alleviation of the C_2H_2 block (Tam & Knowles 1979; Sørensen et al. 1987) or from diffusion of N_2O into regions of low NO_3^- concentration where N_2O is consumed (Seitzinger et al. 1994).

Kinetic approaches have been used to estimate denitrification in sediments (Oren & Blackburn 1979; Kaspar 1982; Twilley & Kemp 1986; Raymond et al. 1992). Denitrification can be described by a Michaelis-Menten type equation (Payne 1973; Knowles 1982):

$$V_o = V_{max} * (S/(S + K_m)) \tag{1}$$

where V_o is the ambient rate, V_{max} is the maximum rate, S is the ambient NO_3^- concentration, and K_m is the Michaelis (half saturation) constant. Reported kinetic constants exhibit variability between and within systems as well as over depth in a single sediment environment (Kaspar 1983; Kaspar et al. 1985).

We amended cores with variable concentrations of NO_3^- and inhibited denitrification with C_2H_2; we then estimated apparent kinetic constants for the denitrifying community. Using this approach, we obtained estimates of potential and *in situ* denitrification rates. Kinetic constants determined in slurry experiments reflect community activity and should be considered "apparent" K_m and V_{max} (Williams 1973; Murray et al. 1989). Throughout this paper, we refer to the apparent Michaelis constant as "K_{app}" and to the maximum potential rate as "V_{mp}".

Estimating biological rates from system-scale or benthic flux nutrient stoichiometry is a classical geochemical approach that integrates spatial and temporal heterogeneity in microbial processes (Redfield 1958; Kjerfve 1981; Nixon & Pilson 1984). Elemental ratios of reacting organic matter and reaction stoichiometry of benthic dissolved inorganic nutrient fluxes [e.g., carbon to nitrogen (DIC:DIN) or phosphorus to nitrogen (DIP:DIN)] or oxygen to nitrogen (O_2:N) fluxes can be used to estimate denitrification by assuming that denitrification is responsible for the difference between 'predicted' and 'measured' DIN fluxes.

The stoichiometric approach is not without assumptions and considerations. For example, using O_2:N flux ratios to estimate denitrification can be problematic because 1) in sediments, O_2 is consumed by both biotic (organic matter degradation) and abiotic (oxidation of reduced S and metals) processes, which means that the benthic O_2 flux does not necessarily reflect organic oxidation coupled to O_2; and 2) sulfate reduction often accounts for a significant portion (often $\sim$50%) of net organic carbon oxidation, which means that DIC and DIN fluxes may be decoupled from the O_2 flux and instead coupled to sulfate reduction. An alternative to O_2:N flux ratios are DIC:DIN (hereafter C:N) or DIP:DIN (hereafter N:P) flux ratios.

The DIC flux is a relatively direct measurement of integrated sediment organic carbon metabolism. However, if benthic primary production is important, measured fluxes of DIC, as well as DIN and DIP, will be lower than the "actual" fluxes due to uptake by the photoautotrophic community. In some systems, DIC removal via $CaCO_3$ precipitation leads to underestimates of DIC production and thus of denitrification. Benthic DIP fluxes arise from complex geochemical sorption-desorption reactions as well as organic oxidation reactions. At times, the benthic DIP flux may reflect geochemical, more so than biological, reactions in this system (Vink 1994).

The present study was undertaken in March and September 1992 to compare denitrification rates estimated using C_2H_2-based methods to those obtained using benthic flux C:N and P:N stoichiometry. Temporal and spatial (depth) variability in kinetic constants of the denitrifying community was assessed. The comparison of different techniques within a single sediment environment allowed us to evaluate the consistency of these independent methods.

Methods

Site description

Tomales Bay (CA, USA) is a shallow (mean depth = 3m), semi-enclosed embayment with a 1m mean tidal range (see Smith et al. 1987 & 1991 for details). Phytoplankton production averages 300 g C m^{-2} yr^{-1} (Smith et al. 1989), a value typical of temperate coastal embayments (Nixon & Pilson 1983). This work was completed at a station 10 km from the mouth of the bay. Sediments are muddy silts with 2–3% organic carbon (OC), a high porosity ($\phi = 0.80$) and a bulk density of 1.3 g cm^{-3}. Sediments are mixed to depths of 30–40 cm (from ^{210}Pb; Snidvongs et al. submitted) by a variety of large and small polychaetes (macrofauna density ~60–150 g m^{-2}; A. Hatcher, pers. comm.). Pore water NO_3^- concentrations in the upper 30 cm vary between 2–25 μM while NH_4^+concentrations vary between 20–350 μM.

Acetylene based methods

Potential denitrification rates and kinetic constants of the denitrifying community were determined in whole core incubations. Fifteen to 25 replicate cores were collected in clear, polycarbonate tubes (5 cm wide, 45 cm long) by SCUBA divers and immediately returned to the laboratory. Cores were inspected visually and disturbed cores were discarded; experiments were begun immediately. The sediment height in each core tube was adjusted to 30 cm and the overlying water phase was removed and carefully replaced with 100 mL of filtered (GF/F) sea water.

Triplicate cores received each of the following treatments: (1) control – injected with N_2-saturated deionized water (DIW); (2) C_2H_2 – injected with C_2H_2-saturated DIW; and (3 through 8) + NO_3^- – injected with C_2H_2-saturated DIW amended with NO_3^-. Injection solutions were prepared as follows: 30 mL of N_2-flushed DIW was placed in an acid-washed, DIW-rinsed, serum bottle (78 mL). The headspace of each bottle was evacuated (2 min.), purged for 10 min. with ultra high purity (UHP) N_2, and re-evacuated. Ninety milliliters of either UHP-N_2 or CaC_2-generated C_2H_2 was then added. Nitrate amendment solutions were prepared by adding a volume of concentrated NO_3^- stock solution to O_2-free, C_2H_2-saturated DIW. Final concentrations were such that injection of 500 μl into sediment pore water increased the ambient concentration by 10, 50, 100, 500 or 1000 μM NO_3^-.

A 500 μl volume (3% of pore water volume) of either N_2 or C_2H_2-saturated solution was injected at 1 cm intervals through silicone-filled injection ports down the length of the core. After injection, cores were sealed with rubber bungs fitted with gas-tight, 3-way stopcocks. Either UHP-N_2 or C_2H_2 was added to aqueous and gaseous phases (20% vol:vol) overlying the core. Cores were incubated in darkness for $\leq$45 min. at *in situ* temperature with constant stirring of the water phase via a motorized magnetic stirring device (Joye 1993). After incubation, overlying gas and water phase samples were collected. Subcores (2.5 cc) were collected every 1.5 cm as the sediment was extruded. Two subcores (3 cm interval) were placed in a tared 38 mL serum bottle containing 10 mL of 2 M NaCl. Gas samples (15 mL) were collected after headspace equilibration and transferred to a 15 mL evacuated serum bottle for storage.

Nitrous oxide was analyzed using a Shimadzu Model 14 gas chromatograph equipped with a ^{63}Ni electron capture detector. Quantification of N_2O was achieved by comparison to a standard curve generated using purified N_2O (N_2O in He, Scott Specialty Gases, Plumsteadville, Pa.). Variation between triplicate standards was <3%. Denitrification rates were calculated on a gram wet sediment (gws) basis and were converted to volume (cm^{-3}) or area-integrated (m^{-2}) rates using sediment wet density (g/cm^3) and the depth interval. After calculating denitrification rates, kinetic constants were estimated using the following adaptation of the Michaelis-Menten equation (Henderson 1993):

$$S/V_o = (1/V_{mp} * S) + (K_{app}/V_{mp}), \qquad (2)$$

where S is the NO_3^- concentration, V_o is the measured denitrification rate, V_{mp} is the maximum rate, and K_{app} is the half saturation constant.

Benthic flux stoichiometry

Benthic fluxes were measured using dome incubation chambers (Dollar et al. 1991). Briefly, triplicate clear plexiglass chambers were placed on the sediment by SCUBA divers. The enclosed water volume (33 L) was mixed continually during incubation. Water samples were collected soon after deployment (<1 hr) and then ca. 24 h later. Time series experiments have shown that benthic fluxes are linear for $\leq$36 hours (Joye et al. in prep.).

Samples were immediately analyzed for dissolved O_2 and temperature using a YSI dissolved O_2 meter. Sample pH was measured (within 3 hr) using a combination electrode and high impedance electrometer. Alkalinity was determined within 1–2 days using a single point titration with 0.01 N HCl (Smith et al. 1987). DIC concentrations were calculated from nutrient-corrected total alkalinity and pH (Smith et al. 1991).

Nutrient samples were collected and analyzed according to Smith et al. (1991). Benthic fluxes were calculated according to Dollar et al. (1991). Denitrification (DNF) rates were calculated using the suspended particulate organic matter (SPM) C:N:P ratio and the following equations:

$$\text{Denitrification Rate} = ((\text{measured DIC flux} / \text{SPM C:N ratio}) - \text{measured DIN flux}) \tag{3}$$

$$\text{Denitrification Rate} = ((\text{measured DIP flux} / \text{SPM P:N ratio}) - \text{measured DIN flux}) \tag{4}$$

The general format of these equations is [Denitrification = Expected N flux - Observed N flux]; the rate is in mmol N m^{-2} d^{-1}. The average C:N and P:N ratios of SPM are 7.4 and 0.07, respectively (Smith et al. 1991). Previous work on nutrient cycling in Tomales Bay has shown that benthic C:P fluxes are well correlated with the organic C to organic P ratio in suspended particulate organic matter (see Smith et al. 1989 & 1991 for further discussion). Most reactions appear to be occurring near the sediment-water interface, where the C:N:P ratio approximates that of suspended more so than bulk sediment (SPM C:N:P = 105:15:1, 1 cm sediment C:N:P = 198:15:1; Vink 1994). Thus, we use the SPM C:N and N:P ratios to estimate denitrification rates. Since mineralized NH_4^+ must be nitrified before it is denitrified, this calculation estimates "complete denitrification", i.e. that directly coupled to nitrification (Smith & Hollibaugh 1989).

Results

'Kinetic-fix' acetylene block estimates of denitrification

Pore water NO_3^- concentrations were significantly higher in surface (1.5 & 7.5 cm, 14 and 6 μM, respectively) compared to deep sediments (1 μM) in September; pore water NO_3^- concentrations (14 μM) were 10 times higher than bottom water NO_3^- concentrations (<1 μM). In March, concentrations were high throughout the upper 14 cm (4 to 11 μM) and decreased gradually below that depth (2.5 μM); water column NO_3^- concentrations (25 μM) were ca. 2 times greater than pore water concentrations (11 μM). Despite detectable pore water NO_3^- concentrations (>1 μM), denitrification rates were similar in N_2 and C_2H_2-inhibited cores that did not receive NO_3^- amendments. Rates in C_2H_2-inhibited cores were often near the detection limit of this method (Figure 1). Potential denitrification rates in C_2H_2-inhibited, NO_3^--amended cores increased in proportion with NO_3^- concentration and were generally higher in September (Figure 1).

The community K_{app} and V_{mp} were calculated for each depth interval throughout the upper 30 cm. Coefficients of determination were usually $\geq$0.95; only two regressions were not significant. An example of this transformation is presented in Figure 2. The K_{app} and V_{mp} (Equation 2) of the denitrifying community varied over depth and time. Half saturation constants were significantly higher in March compared to September (Figure 3). In September, we were unable to determine the K_{app} for the 25.5 cm depth interval and the V_{mp} for the 1.5 cm depth interval, due to poor regression statistics. In March, K_{app} values exhibited considerable depth variation while September K_{app} values increased slightly with depth (Figure 3).

V_{mp} exhibited a surface (1.5 & 4.5 cm) maximum in March; however, values were similar, and generally higher, throughout the upper 19.5 cm in September (Figure 4). Average integrated potential denitrification rates (i.e., Σ V_{mp} @ 500 μM NO_3^-) were higher in September than in March (Figure 5). By substituting the apparent kinetic constants and pore water NO_3^- concentrations into Equation 1, we obtained estimates of *in situ* denitrification rates (Figure 6). In March, highest rates were detected in the near-surface interval (1.5 cm). Secondary peaks were noted at 10.5 and 19.5 cm. In September, similar rates were obtained within the upper 13.5 cm. Rates were generally higher than those estimated in March at comparable depths. Total integrated denitrification was estimated to be 1.8 ($\pm$0.9) mmol N m^{-2} d^{-1} in March and 9.3 ($\pm$4.6) mmol N m^{-2} d^{-1} in September. Estimated *in situ* rates amounted to 8 and 24% of the maximum integrated potential denitrification rates, in March and Sept. respectively.

Stoichiometric estimates of denitrification

Fluxes of DIN, DIP and DIC were higher in Sept. than in March. We used benthic flux stoichiometry and the SPM C:N:P ratios to calculate denitrification rates (Table 1). Mean estimates based on C:N and P:N stoichiometry were similar during March; however, mean C:N rates were higher in Sept.. High variability among fluxes and thus in estimated denitrification rates was not surprising (Dollar et al. 1991). Mean estimates of denitrification derived from stoichiometric methods were lower than those derived from the 'kinetic-fix'

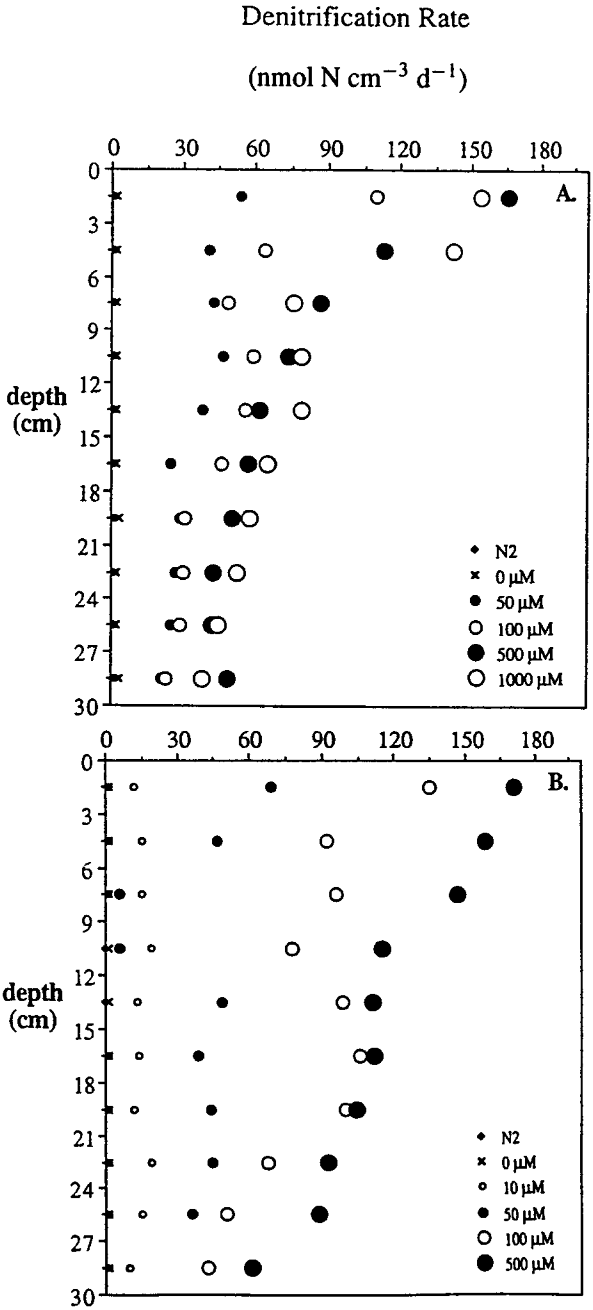

Figure 1. Variation in the depth distribution of denitrification as a function of NO_x concentration. Panel A presents results from March 1992 and panel B presents results from Sept. 1992.

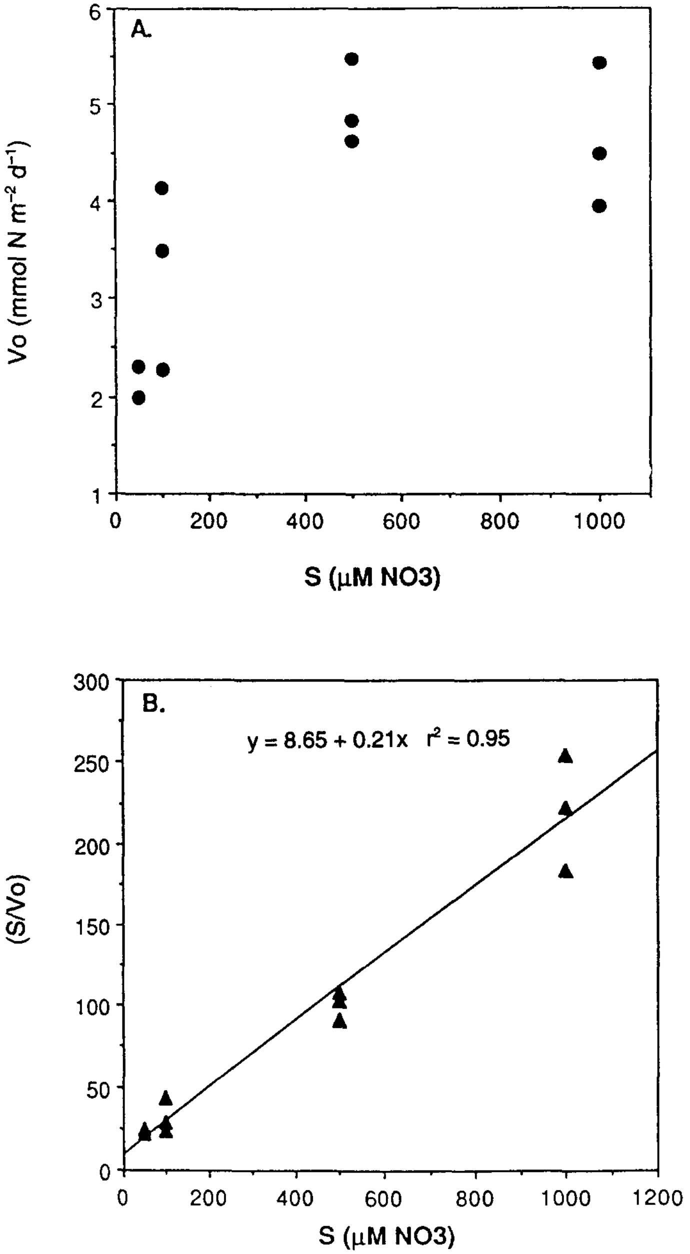

Figure 2. Example of the effect of substrate concentration on denitrification rate at a single depth interval. The denitrification rate (V_o) versus substrate concentration (S) is shown in Panel A and the linearization of these data are presented in Panel B. Each symbol marks the mean rate determined in one core (*n* = 2 per core; *n* = 3 cores).

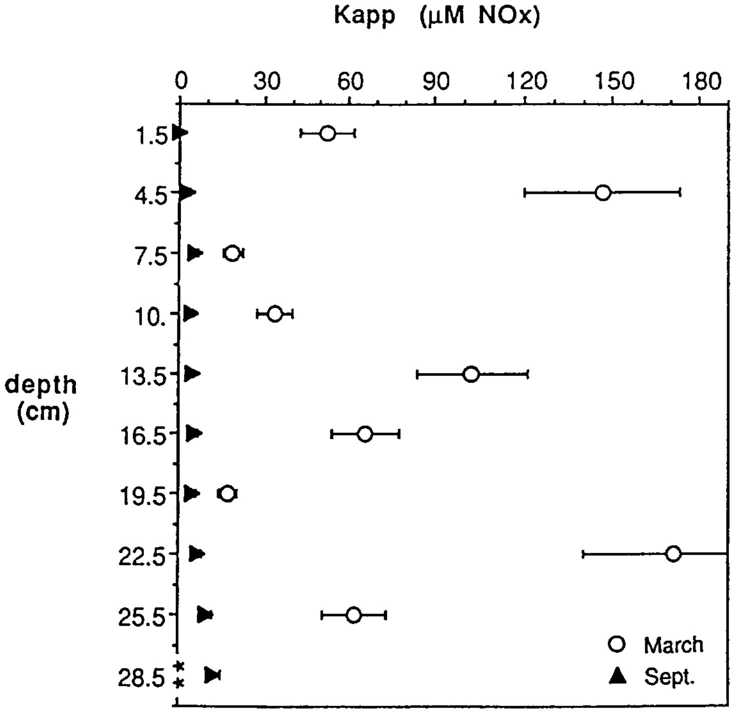

Figure 3. Depth variation in K_{app} (μmol NO_x) in March and Sept. 1992. Symbols represent the mean K_{app}; error bars represent the standard deviation of the mean ($n = 3$). ** denotes no data for that depth.

Table 1. Benthic fluxes of DIC, DIN and DIP and estimated denitrification rates for Station 10 sediments. Denitrification rates were calculated using DIC:DIN (**C-DNF**) and DIP:DIN (**P-DNF**) flux ratios (Equations 3 and 4). Mean rate or flux ($n = 3$ domes) and standard deviation of the mean (parentheses) are presented. All fluxes and rates in μmol m^{-2} d^{-1}.

Date	DIN flux	DIP flux	DIC flux	C - DNF	P - DNF
March	932 (162)	114 (15)	12700 (2700)	784 (710)	778 (421)
Sept.	1517 (726)	233 (149)	41500 (1500)	4100 (2800)	1900 (1700)

approach; however, the CV's for both estimates were high and the means were statistically indistinguishable.

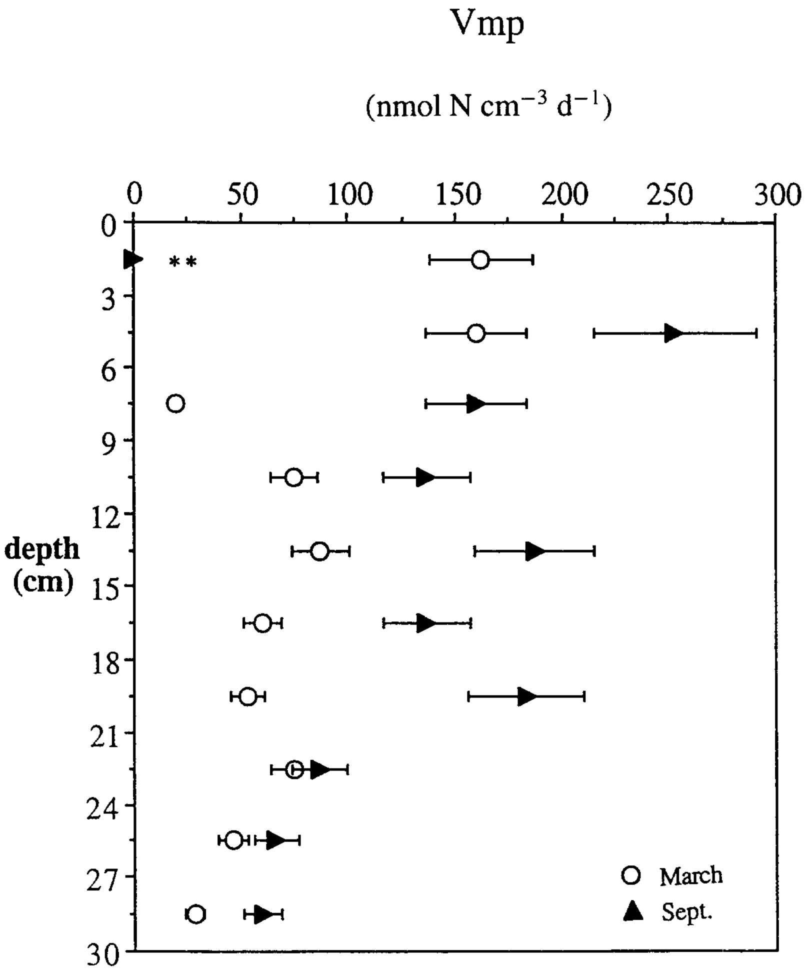

Figure 4. Depth variation in V_{mp} (nmol N cm^{-3} d^{-1}) in March and Sept. 1992. Symbols represent the mean V_{mp}; error bars represent the standard deviation of the mean ($n = 3$). ** denotes no data for that depth.

Discussion

At present, there is no widely-accepted method for quantifying denitrification in sediments. In all likelihood, the applicability of an individual method depends on the biogeochemical characteristics of the system under study. In Tomales Bay, sediment pore waters lack free sulfide (HS^- is typically below detection; Chambers et al. 1994), bulk sediment is relatively oxidized throughout the upper 100 cm of the sediment column (Vink 1994), and pore waters have measurable NO_x (≥ 1 μM) within the upper 30 cm (Joye 1993).

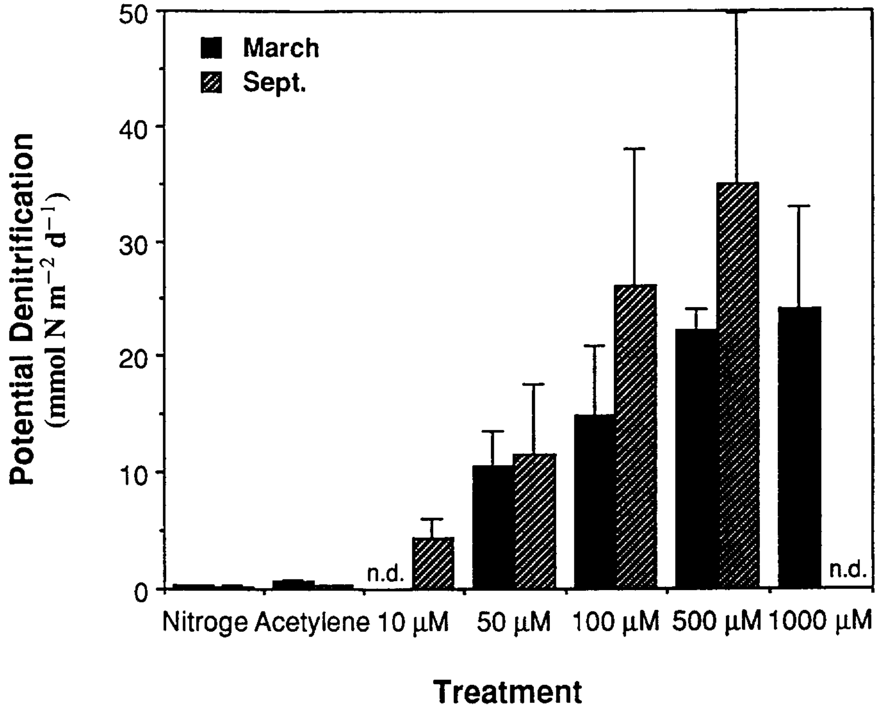

Figure 5. Integrated rates of potential denitrification in March and Sept. 1992. The treatment (N addition level) is shown on the X-axis. Bars represent the mean ($n = 3$) potential denitrification rate in mmol N m^{-2} d^{-1} and error bars represent the standard deviation of the mean. n.d. = no data.

Given these characteristics, the C_2H_2 block method should work well in these sediments if problems associated with the decoupling of nitrification and denitrification are overcome.

'Kinetic fix' acetylene block method

Denitrification rates determined using the C_2H_2-block technique (Sørensen 1978, no NO_3^- addition) were consistently low (Joye 1993), suggesting that residual pore water NO_3^- pools were quickly exhausted and that N_2O was consumed in either NO_3^--depleted or HS^--enriched microzones during these short (30–45 minute) incubations. Seitzinger et al. (1994) reported that C_2H_2-inhibition of N_2O reductase ('NOR') was 50% effective in detecting denitrification fueled by water column NO_3^- in freshwater sediments and that it did not detect any denitrification coupled to nitrification. Rapid NO_3^- consumption resulting in NO_3^- limitation and subsequent N_2O consumption could result in low efficacy of C_2H_2-inhibition in these sediments.

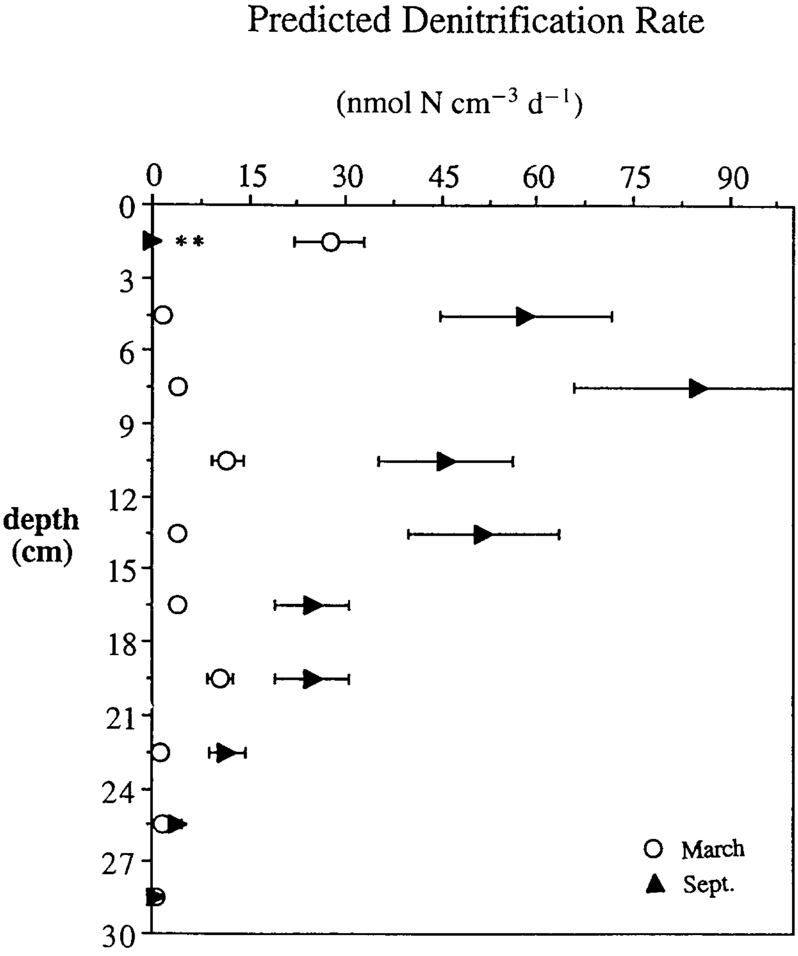

Figure 6. Kinetic estimates of *in situ* denitrification (nmol N cm^{-3} d^{-1}). Symbols (defined on figure) represent the average estimated rate; error bars reflect the estimated standard deviation of the mean rate.

Performing joint C_2H_2-NO_3^--additions mitigates the NO_3^--limitation problem to some extent; however, ambient rates may be overestimated at high [NO_3^-]. Joye (1993) found that concentrations as low as 5 μM NO_3^- resulted in measurable rates of denitrification. We used several (3 to 7) NO_3^- concentration additions in this work. An easier approach would be to choose a single NO_3^- concentration such that pore water concentrations are not dramatically altered. This requires that NO_x concentrations be determined prior to performing experiments. However, a constant NO_3^--enrichment used at different times may yield poor estimates of *in situ* rates if there is significant variation in the kinetic parameters of the denitrifying community or in pore water NO_3^- concentrations.

Seasonal variations in denitrification occur in most systems. This variability stems from fluctuations in substrate supplies, sediment redox conditions, and the physiology or abundance of denitrifying microorganisms. The kinetic parameters of the denitrifying community may vary because the factors influencing them fluctuate spatially and temporally. We observed that K_{app} and V_{mp} varied over depth in the sediment column and time, a finding similar to that of Raymond et al. (1992). Among the most important variables affecting denitrification are sediment O_2 concentration, NO_3^- concentration, and organic carbon availability. Oxygen concentration affects denitrification directly since many denitrifiers are facultative and increase NO_3^- respiration rates when O_2 concentrations fall below 10 μM (Tiedje et al. 1982; Tiedje 1988; Dalsgaard & Revsbech 1992). Changes in NO_3^- availability may alter either the cell-specific enzyme activity of denitrifiers (i.e., increased activity per cell) or stimulate growth (i.e., increased number of cells) (King & Nedwell 1987; Twilley & Kemp 1986). Either scenario could affect the kinetic constants of denitrifying microorganisms. Properly addressing these factors requires quantification of both the biomass of denitrifiers and enzyme expression. These measurements cannot be made in a practical manner using molecular or biochemical techniques currently available (Ward et al. 1993).

The spatial and temporal variability in the kinetic parameters of the denitrifying community correlates well with other changes in sediment geochemistry noted between March and September. The mixed layer depth deepens as temperatures increase due to increased bioturbation by macro- and meiofauna. This change is apparent in the depth distribution of dissolved nutrients and trace metals (Vink 1994) as well as in the physiology, and possibly the abundance, of denitrifying microorganisms (this work). In March, the majority of denitrification occurred in the upper 10 cm of sediment, implying that the activity of denitrifiers was limited at depth, most likely by substrate supply. Low temperatures may also have contributed to this pattern. During September, V_{mp} values were comparable throughout the upper 22 cm of sediment. We speculate that increased denitrification at depth reflects stimulation of nitrification rates by enhanced O_2 supply due to the activity of macrofauna (Joye 1993), which would directly affect the NO_3^- supply. A marked color change (from brown to black) occurred below this region (25 cm) and the majority of potential denitrification occurred above this zone.

Significant reductions in K_{app} were documented at all depths in Sept. (Figure 5), suggesting that the denitrifying community had increased their affinity for NO_3^-, possibly in response to low NO_3^- supplies. This pattern could also reflect changes in composition of the denitrifying community (i.e., selection for bacteria with a higher affinity for NO_3^-). In March, bottom water NO_3^- concentration was 2× greater than pore water concentration, so mixing of bottom and pore waters may have increased the NO_3^- available to denitrifiers. Conversely, in Sept., pore water NO_3^- concentration was 10× bottom water NO_3^- concentration and mixing of bottom and pore waters may have decreased NO_3^- availability. Given the obvious complexity of these interactions, it may be impossible to correlate kinetic constants with one specific variable because there is obviously a complex feedback between nitrification, denitrification, bioturbation, substrate and O_2 supplies, microbial community composition and physiology, and possibly other parameters. The use of diagenetic models is one way to unravel interactions between these factors (Joye et al. in prep.).

Denitrification rates have been estimated using Michaelis-Menten kinetic parameters in several estuarine and marine environments, including Tokyo Bay (K_{app} = 24 μM; Koike et al. 1978), Kysing Fjord, Denmark (K_{app} = 344 μM; Oren & Blackburn 1979), Chesapeake Bay (K_{app} = 2–92 μM; Twilley & Kemp 1986), and most recently in the Western Mediterranean (K_{app} = 200–1700 μM; Raymond et al. 1992). The range of reported K_{app} values lies between 2 to 170 μM and the range of V_{mp} lies between 2 and 18 mmol N m^{-2} d^{-1} (Raymond et al. 1992). The sediments of nearby San Francisco Bay exhibited a K_{app} of 50 μM NO_3^-, similar to the values obtained during this study; however, values of V_{mp} (< than 1 mmol N m^{-2} d^{-1}) were lower than those measured in Tomales Bay sediments (Oremland et al. 1984). While K_{app} of the Tomales Bay denitrifying community lie well within the reported range, the V_{mp} values are higher than any previously reported.

Comparison of stoichiometric and kinetic estimates of denitrification

Are the estimates obtained by our "kinetic-fix" variation of the C_2H_2-block method reasonable? Average rates determined using the "kinetic-fix" were 1.8 (±0.9) mmol N m^{-2} d^{-1} in March and 9.3 (±4.6) mmol N m^{-2} d^{-1} in September. Compare those values to the ones obtained using benthic C:N flux stoichiometry: 0.9 (±0.7) mmol N m^{-2} d^{-1} and 4.1 (±2.8) mmol N m^{-2} d^{-1} in March and September, respectively. The estimates derived from the two approaches are not significantly different, but 'kinetic-fix' estimates were consistently higher. Given the drawbacks associated with each method and the inherent variability of metabolic rates in sediments, this agreement is encouraging.

We conclude that our 'kinetic-fix' adaptation of the C_2H_2-block method provides reasonable estimates of denitrification in Tomales Bay sediments. The combination of kinetic and stoichiometric approaches allowed us to: 1) evaluate where denitrification was occurring within the sediment column; 2) evaluate how the physiology of the denitrifying community changed over time; 3) evaluate the potential for denitrification in these sediments; and 4) place reasonable constraints on the range of actual denitrification rates. Studies such as those reported here will help us to better fine-tune independent methods of estimating denitrification in aquatic sediments.

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