



Denitrification in coastal ecosystems: methods, environmental controls, and ecosystem level controls, a review

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Abstract

In this review of sediment denitrification in estuaries and coastal ecosystems, we examine current denitrification measurement methodologies and the dominant biogeochemical controls on denitrification rates in coastal sediments. Integrated estimates of denitrification in coastal ecosystems are confounded by methodological difficulties, a lack of systematic understanding of the effects of changing environmental conditions, and inadequate attention to spatial and temporal variability to provide both seasonal and annual rates. Recent improvements in measurement techniques involving ^{15}N techniques and direct N_2 concentration changes appear to provide realistic rates of sediment denitrification. Controlling factors in coastal systems include concentrations of water column NO_3^- , overall rates of sediment carbon metabolism, overlying water oxygen concentrations, the depth of oxygen penetration, and the presence/absence of aquatic vegetation and macrofauna. In systems experiencing environmental change, either degradation or improvement, the importance of denitrification can change. With the eutrophication of the Chesapeake Bay, the overall rates of denitrification relative to N loading terms have decreased, with factors such as loss of benthic habitat via anoxia and loss of submerged aquatic vegetation driving such effects.

Introduction

Understanding the underlying causes of coastal eutrophication and using this information for management practices requires a sound knowledge of coastal and estuarine nutrient cycles. Nitrogen is often cited as the limiting nutrient in coastal ecosystems (Ryther & Dunstan, 1971; Boynton et al., 1982), though seasonal shifts in N and P availability in some systems may result in P limitation (D'Elia et al., 1986; Fisher et al., 1992). Denitrification, the reduction of NO_3^- or NO_2^- to N_2O or N_2 , represents an important N sink in estuarine (Seitzinger, 1988; Nixon et al., 1996) and oceanic ecosystems (Christensen et al., 1987; Falkowski, 1997). This process is mediated by anaerobic bacteria which can use NO_3^- (or NO_2^-) as a terminal electron acceptor in respiration. The physiology of denitrifying bacteria is well understood under controlled conditions, but their behavior is less well understood under natural conditions. To a great

extent our understanding of environmental denitrification has been hampered by inadequate measurement techniques. Despite these limitations, there is an increasing body of knowledge of the factors which control denitrification, particularly at physiological scales.

Sources of inorganic N in sediments include the remineralization of N in organic matter (algae, macrophytes, animals) and the flux of inorganic nitrogen (primarily nitrate) from the water column (Figure 1). Remineralization of N from organic compounds involves oxidative reactions, followed by a terminal electron acceptor step requiring terminal electron acceptors such as O_2 , NO_3^- , Mn(IV) , Fe(III) and SO_4^{2-} . Remineralization produces NH_4^+ as a primary end product. Ammonium in sediment can have several fates including (1) accumulation in pore water or adsorption on to sediment, (2) loss by diffusion or advection across the sediment-water interface, (3) assimilation by plants or bacteria, or (4) oxidation to

NO_3^- or NO_2^- (nitrification) which can exchange with overlying water or be reduced to N_2 (denitrification). The relative importance of these processes depends strongly on certain environmental conditions including oxygen concentration in overlying water, temperature, salinity, organic carbon loading, and the presence of macrophytes, microphytobenthos, or macrofauna.

Despite methodological uncertainties and limitations in spatial and temporal resolution of rate measurements, there have been numerous attempts to calculate contributions of denitrification to N budgets for whole coastal ecosystems. In a recent review of estuarine N mass balances (Nixon et al., 1996), the relative importance of denitrification to the N cycle varied considerably among systems (Table 1), with water residence time and bottom water oxygen concentrations cited as major factors contributing to these differences. Much of the denitrification data for coastal ecosystems are derived from a fundamentally flawed method (acetylene reduction, see methods comparison in Seitzinger et al., 1993) and from indirect mass balance calculations. This underscores the need for convenient direct techniques which can be broadly applied for diverse coastal ecosystems at a range of scales.

The goals of this review are: (1) to critically discuss the measurement of denitrification rates; (2) to identify the factors which control denitrification rates in coastal ecosystems; (3) to discuss the potential effects of environmental changes on denitrification rates; and (4) to consider temporal and spatial variability in the calculation of whole system denitrification rates. Other reviews of environmental denitrification in coastal (e.g. Seitzinger, 1988, 1990) and wetland ecosystems (Groffman, 1994) may be of interest to the reader. This analysis focuses on variability, regulation and extrapolation of measured rates and suggests integrated approaches for future research.

Denitrification methods

Direct techniques

For the purposes of this discussion, 'direct' techniques mainly use measurements of nitrogen fluxes to estimate the rate of denitrification. For example, both direct N_2 flux measurements and measurement of N_2O accumulation after inhibition of the $\text{N}_2\text{O} \rightarrow \text{N}_2$ transformation would be direct techniques. An example of an indirect technique would be estimating the rate of

denitrification by determining the difference between the rate of NH_4^+ flux and the rate of N remineralization estimated from the stoichiometric relationship between dissolved inorganic N (DIN) flux and either the flux of O_2 or dissolved inorganic carbon (DIC)

The direct measurement of denitrification is complicated by a high background concentration of N_2 dissolved in natural waters. For example, in 10 psu seawater at 20°C, the N_2 concentration is $\sim 498 \mu\text{mol L}^{-1}$. If during the course of incubation, all of the remineralized N is denitrified, and $100 \mu\text{mol L}^{-1}$ DIC is produced, then the N_2 change would be $7\text{--}8 \mu\text{mol L}^{-1}$, or a $< 2\%$ change in total N_2 . At lower rates of denitrification, direct N_2 flux measurements are generally difficult to make. It is because of the difficulty in detecting changes in N_2 against a large background that a number of indirect and direct measurement techniques have been developed (Table 2). There are important limitations to virtually every denitrification technique currently in use.

The acetylene inhibition technique is based on the inhibition of the transformation of N_2O to N_2 (Sørensen, 1978); the accumulation of N_2O instead of N_2 is readily measured by gas chromatography using an electron capture detector and the total N_2O flux is equivalent to the denitrification rate. Several limitations have been identified, including the incomplete blockage of the N_2O transformation to N_2 (Seitzinger et al., 1993), particularly where hydrogen sulfide is present (Koike & Sørensen, 1988). The inhibition of nitrification by acetylene (Hynes & Knowles, 1978) may result in the depletion of NO_3^- during the course of incubation, thus limiting denitrification rates. This is an important problem where the overlying water is not the main NO_3^- source. In some cases, NO_3^- additions may partially mitigate this effect (Joye et al., 1996). Overall, this approach should work best when denitrification is supported by high NO_3^- concentrations in the water column and where hydrogen sulfide concentrations are low.

Experiments using ^{15}N as a tracer for denitrification are made difficult by incomplete labeling of sediment $^{15}\text{NO}_3^-$ and $^{15}\text{NH}_4^+$ pools. Nishio et al. (1983) and Jenkins & Kemp (1984) used ^{15}N to identify the importance of sediment nitrification to the production of NO_3^- to support denitrification in coastal sediments. The ^{15}N isotope pairing technique was introduced recently by Nielsen (1992) as a way of determining fluxes attributable to substrate derived from the water

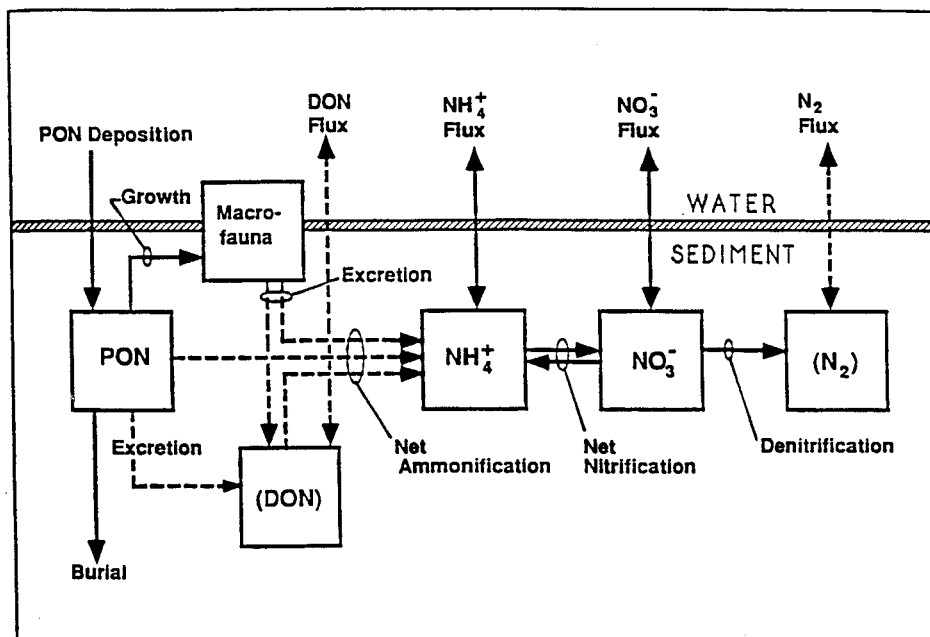


Figure 1. Idealized nitrogen cycle (Kemp et al., 1990).

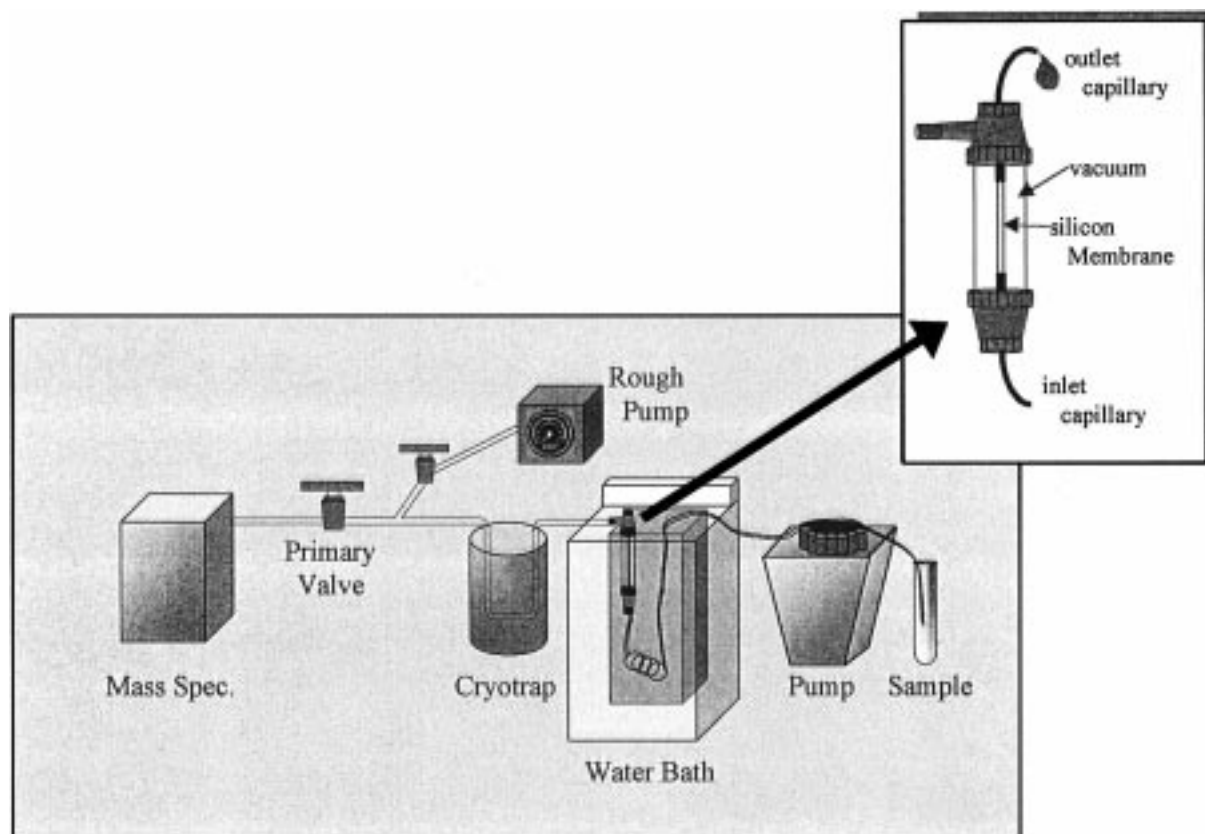


Figure 2. Dissolved Gas Analyzer (DGA) for the high precision analysis of $N_2:Ar$ gas ratios in water samples (Kana et al., 1994).

Table 1. Approaches to estimating denitrification budgets in selected estuarine, coastal and shelf ecosystems. Most of the data is from Nixon et al. (1996), with Norsminde Fjord data from Nielsen et al. (1995), Boston Harbor from Giblin et al. (1997) and the North Atlantic continental shelf information from Seitzinger and Giblin (1996)

System	Denitrification (% of total N loading)	Denitrification Technique
Baltic Sea	74	Whole system mass balance
Boston Harbor	17	Stoichiometry
Chesapeake Bay	26	Mass balances, acetylene inhibition
Delaware Bay	43–54	Direct N ₂ flux after gas purging
Narragansett Bay	13–27	Direct N ₂ flux after gas purging
Ochlockonee Bay	11	Direct N ₂ flux after gas purging
Norsminde Fjord (DK)	2	¹⁵ N isotope pairing
Potomac River	16	Mass balances, acetylene inhibition
Scheldt Estuary (NL)	40	Whole system mass balance
North Atlantic continental shelf	> the sum of estuarine, river & atmospheric inputs	Regression of sediment oxygen uptake and denitrification; regression of sediment oxygen uptake and primary production

column versus that derived from coupled nitrification-denitrification. This approach uses ¹⁵NO₃⁻ labeling of the overlying water of incubated cores to partition the denitrification attributable to overlying water NO₃⁻ and NO₃⁻ supplied by nitrification (Nielsen, 1992). The measurement of ¹⁴N¹⁴N, ¹⁴N¹⁵N and ¹⁵N¹⁵N dinitrogen by mass spectrometry is required. Despite concerns about the complete mixing of ¹⁵N and ¹⁴N isotope pools (Middelburg et al., 1996a), this technique generally appears to give reliable estimates of denitrification.

Seitzinger (1987) has used a long-term incubation technique to measure direct N₂ fluxes. In this method, overlying water is changed daily with N₂-free water for 10 days to remove most of the N₂ the system; incubations of about 24 h are then sufficient to measure N₂ fluxes using gas chromatography. The assumption that sediment conditions are unchanged over the long preincubation period may also be problematic, with experimental incubations of intact estuarine sediments revealing rapid depletion of labile organic matter within weeks (Kelly et al., 1985; Boynton et al., 1995). In general, this technique appears to give higher rates of denitrification than the acetylene or ¹⁵N techniques (Seitzinger et al., 1993; van Luijn et al., 1996a). Nowicki (1994) modified this approach by using anoxic control cores to estimate the continuing flux of pore water N₂ to overlying water; the absence of

nitrification during such incubations results in a better estimate of fluxes attributable to residual pore water N₂.

As described earlier, direct N₂ fluxes under ambient N₂ concentrations are difficult to determine, but are desirable because of the minimal perturbation to the sediment system. Precise gas chromatographic techniques (< 0.5% coefficient of variation) have been used in some environments to determine N₂ concentration in relatively brief core incubations (Devol, 1991; Devol & Christensen, 1993; LaMontagne & Valiela, 1995). The duration of denitrification experiments may be limited because of oxygen depletion. Such depletion of O₂ presents a practical limit to direct N₂ flux measurements because changes in nitrification rates and other redox pathways may be caused by low O₂ concentrations. When either NH₄⁺ or NO₃⁻ efflux are the predominant N fluxes from sediments, direct measurement of denitrification with direct chromatographic techniques is difficult.

Recently, Kana et al. (1994) described a mass spectrometer that is able to rapidly measure N₂ in water at a precision of < 0.05% for the N₂/Ar ratio. This makes it possible to measure denitrification (specifically net N₂ flux) in sediment core samples using incubations of <12 h, or by measuring the difference in the N₂ concentration between inlet and outlet water of continuous flow incubations. The mass spectrometer utilizes

Table 2. Some different approaches to measuring denitrification

Technique	Approach	Limitations	Example references
Acetylene inhibition	Blocks $N_2O \rightarrow N_2$, measure N_2O accumulation	Incomplete block; blocks nitrification	Sørensen, 1978; Lohse et al., 1993
^{15}N	Label NO_3^- or NH_4^+ , follow incorporation in N_2	Potential enhancement by NO_3^- additions	Nielsen, 1992; Lohse et al., 1996
Direct N_2	Degas system of N_2 , measure N_2 increase	Degassing step, long-term incubation, controls	Seitzinger, 1987; Nowicki, 1994
Direct N_2	Time course change in N_2 measured by gas chromatography	High background, gas disequilibria from changing temperatures or from CH_4 ebullition	Devol, 1991; LaMontagne and Valiela, 1995
N_2/Ar ratio change	Measure time-course changes in gas ratio via mass spectrometry, continuous flow gas stripping	Gas disequilibria from changing temperatures or from CH_4 ebullition	Kana et al., 1994, 1998
Stoichiometry	Difference between C or O_2 -based N remineralization and net DIN flux	Not direct, imprecision, problem with 'chemical' oxygen demand or storage of reducing species (S(II), Fe(II)) when O_2 is used	Nixon, 1981; Joye et al., 1996; Giblin et al., 1997
Nitrification-N balance	Measure all DIN fluxes and nitrification, make a mass balance	Indirect	Kemp et al., 1990
Pore water modeling	Model rates from pore water chemistry	In shallow environments, need mm scale profiles and transport coefficients	Jahnke et al., 1982
Sediment diagenetic modeling	Using labile organic matter inputs and bottom water O_2 , the nitrogen cycle is modeled.	Need information on organic loading, water column chemistry. May be difficult to validate.	Soetaert et al., 1996; Middelburg et al., 1996a
Whole-system mass balance	Measure all N fluxes, calculate denitrification by difference	Imprecise, major error associated with measurement of other terms in the budget	Nielsen et al., 1995; Nixon et al., 1996

a membrane tube interface that allows dissolved gases to permeate into the vacuum inlet (Figure 2). The instrument, which we refer to as a Dissolved Gas Analyzer (DGA) because of its ability to measure multiple gases with high precision, is capable of measuring 30 samples per hour on <5 ml of water per sample. Practical advantages of the DGA over either gas chromatography or conventional mass spectrometers for measurements of N_2 flux include the lack of a gas stripping step, rapid throughput, small sample size, high precision, and simultaneous measurement of O_2 concentration.

We have begun to exploit this instrument in studies of denitrification associated with estuarine sediments. The DGA can be used with both batch and continuous flow experiments, the choice depending on the type of data desired. The determination of denitrification

in batch cores requires a time-series measurement of N_2 (or N_2/Ar). After thermal equilibration, cores are closed and subsequently subsampled for 4–24 h depending on the sediment oxygen demand. Generally, it is possible to establish a linear increase in N_2 prior to the O_2 concentration dropping below 50% of saturation, which is a desirable condition if the ambient water is oxic (Figure 3). This technique is suitable for estimates of ambient denitrification rates, given its relatively rapid set-up and incubation time and speed of measurement.

We have also incubated sediments with continuously flowing source water to produce steady-state conditions. Such cores have exhibited constant oxygen demand and N_2 efflux for a period exceeding one week. Fluxes are determined from the difference between the gas concentrations entering and exiting

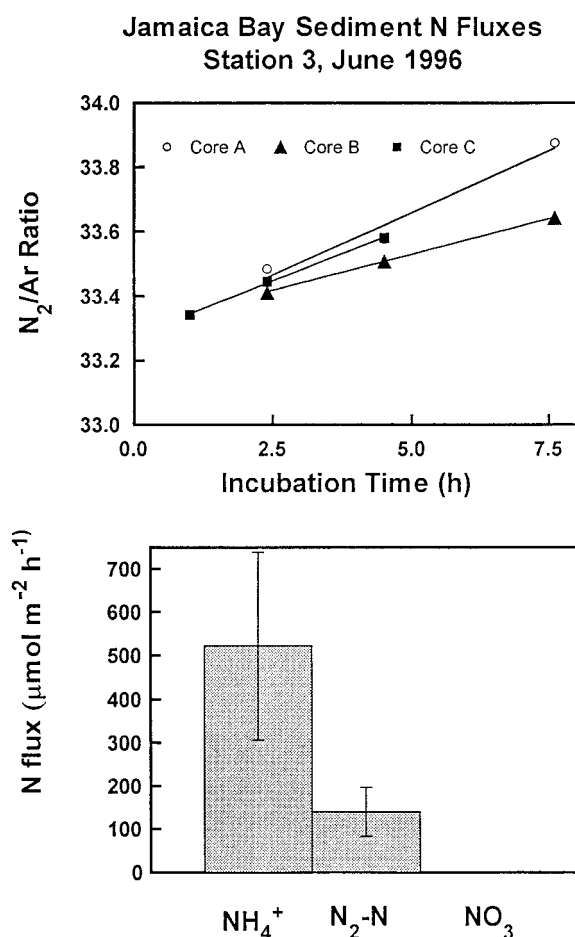


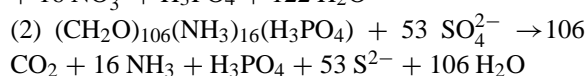
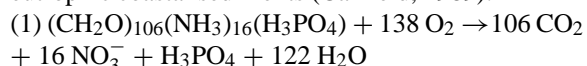
Figure 3. Time course of N₂:Ar ratio changes in a sediment incubation from eutrophic Jamaica Bay, New York (upper graph). The flux rates of N₂-N, NH₄⁺ and NO₃⁻ are the averages of 3 cores and the error bars are 1 SD (lower graph). (data from Cornwell and Owens, unpubl.). There were no significant NO₃⁻ fluxes.

the reaction chamber. We set the flow conditions to yield approximately a 1% change in the N₂/Ar ratio, a difference that is readily measured. In practice, this results in a 50% drop in the concentration of O₂ overlying the core, thus maintaining the sediment surface in an oxic state. The continuous flow method is particularly suitable for studying factors that influence denitrification, such as water column nitrate concentration, or process kinetics (Kana et al., 1994, 1998).

Indirect techniques

The application of stoichiometric assumptions to measurements of sediment-water exchange provides an indirect approach to estimating denitrification. This

calculation requires constant elements ratios (for algae, C:N = 6.6; N:P = 16) associated with the dominant terminal electron accepting processes in eutrophic coastal sediments (Canfield, 1989):



Other terminal electron acceptors not represented here (NO₃⁻ and metal oxides) have been recognized as being important in carbon metabolism in some coastal sediments (Middelburg et al., 1996b; Slomp et al., 1997). The ratios of C:N and N:P shown here are for remineralization processes, and are often at variance with the bulk ratios observed in sediments (Cornwell et al., 1996). The process of nitrification can change the ratio of C:O₂ because of the utilization of both N and C by nitrifying bacteria. The formation/dissolution of carbonate or phosphate minerals, as well as the adsorption of P on to Fe oxides, will necessarily complicate the use of C:N and N:P stoichiometry. The use of O₂:N flux ratios is complicated by seasonal production and reoxidation of reduced sulfur in iron sulfide minerals (Cornwell & Sampou, 1995; Giblin et al., 1997), with low ratios during mineral formation and high ratios during dissolution/reoxidation.

If the flux of dissolved inorganic N (DIN = NH₄⁺ + NO₂⁻ + NO₃⁻) from sediments is less than the NH₄⁺ remineralization flux predicted from the measured dissolved inorganic carbon or P fluxes, the difference is assumed to be due to denitrification. In Giblin et al.'s (1997) study of benthic fluxes in Boston Harbor, annual DIC:DIN flux ratios ranged from 9.6 to 15.5, considerably higher than those observed for organic matter. This suggests that 31–57% of remineralized N was denitrified. Implicit in this analysis is the assumption that the C:N ratio of sedimenting organic matter has Redfield composition. Modest imprecision in DIC fluxes or DIN fluxes will propagate uncertainty in the estimation of denitrification, a major problem when denitrification is a low proportion of total N remineralization.

Other indirect approaches have included the measurement of DIN fluxes and nitrification rates, with denitrification assumed to be equivalent to the nitrification rate (e.g. Kemp et al., 1990). Pore water modeling can be a useful approach in deep-sea sediments (e.g. Jahnke et al., 1982), and a more holistic approach to understanding nitrogen cycling in the larger milieu

of sediment diagenetic processes can be helpful in the absence of direct measurements (Soetaert et al., 1996; Middelburg et al., 1996a, 1996b).

Environmental controls on denitrification rates

In the last decade, significant progress has been made in identifying principal factors that control denitrification in the coastal environment (Table 3). A combination of field observation and laboratory experimentation has been used, and despite methodological difficulties, there has been considerable growth in our understanding of processes regulating denitrification. The influence of environmental factors on denitrification often is different for coupled nitrification/denitrification than denitrification supported by overlying water NO_3^- . Thus, any discussion of the proximate controls on denitrification needs to include this distinction.

Organic matter loading may be one of the most important variables controlling denitrification in aquatic ecosystems. The increased loading of organic matter will increase rates of sediment metabolism, oxygen demand and N remineralization. Increased sediment oxygen demand will decrease the oxygen penetration depth (Cai & Sayles, 1996) and minimize nitrification rates (Caffrey et al., 1993) by limiting the depth interval in which nitrification can occur. In nature, it may be hard to distinguish between low water column oxygen and high organic matter loading because these two conditions often coincide in eutrophic coastal systems. Caffrey et al. (1993) mixed organic matter into homogenized coastal sediments and observed a decrease in nitrification rates but an overall increase in denitrification. The increased denitrification occurred because of increased utilization of high concentrations of water column NO_3^- . In contrast, Smith et al. (1989) have argued that in Tomales Bay, carbon limits denitrification at the whole ecosystem scale. For a better understanding of the effect of eutrophication on denitrification, further studies of the effects of organic matter loading are required to provide a better description of eutrophication-related changes in coupled nitrification/denitrification rates in coastal sediments.

Nitrate in the overlying water directly affects denitrification in estuarine sediments (Lohse et al., 1993; Pelegri et al., 1994; Nielsen et al., 1995). A direct proportionality between sediment denitrification rate and water column nitrate concentration (Figure 4a) has been reported in a number of studies recently

(Seitzinger, 1993; Pelegri et al., 1994; Nielsen et al., 1995; Kana et al., 1998), indicating that it is controlled by the rate of diffusion across the layer separating the mixed water column from the anoxic sites of denitrification (Kana et al., 1998). Moreover, the *in situ* denitrifying bacteria respond rapidly to increases in the nitrate concentration in the overlying water (Kana et al., 1998). Together, these findings suggest that the sediment microbial population remains poised to utilize available nitrate and that the denitrification rate is modulated by short-term temporal changes in the nitrate concentration in the overlying water. More work needs to be done to determine the factors that affect the slope of the nitrate vs. denitrification relationship (i.e. the diffusive properties of the sediment). This will be important for developing ecosystem denitrification models.

The importance of the overlying water oxygen concentration on nitrification and denitrification has been examined both experimentally (Figure 4b; Rysgaard et al., 1994) and at the whole ecosystem level (Kemp et al., 1990). The concentration of oxygen at the sediment-water interface and the sediment oxygen demand control the oxygen depth distribution near the sediment-water interface. While increased oxygen penetration depths may enhance coupled nitrification/denitrification (Rysgaard et al., 1994), denitrification supported from the flux of NO_3^- from overlying water will be hindered by an increased diffusive path length between the sediment surface and anoxic sediment horizons. At the ecosystem level, hypoxia/anoxia reduces denitrification via reduction of nitrification, and in eutrophic systems such as the Chesapeake Bay, hypoxia/anoxia decreases the importance of denitrification as a N sink (Kemp et al., 1990; Kemp & Boynton, 1992).

Macrofauna can stimulate overall denitrification rates in coastal or estuarine sediments. Pelegri et al. (1994) observed a 3-fold increase in coupled nitrification/denitrification in sediment with high densities of the amphipod *Corophium volutator* (Figure 4c). Increased nitrification is observed for many species of macrofauna in coastal sediments (Henriksen et al., 1983) and arises because of increased surface area and enhanced areal rates in burrow walls (Mayer et al., 1995). Modeling efforts (Aller, 1988) have identified the nitrification zone thickness, burrow abundance and burrow radius as important nitrification controls. Bio-irrigation from macrofauna can increase fluxes of water column NO_3^- into the sediments, increasing denitrification rates (Pelegri et al., 1994). In the Ches-

Table 3. Factors which influence denitrification rates in estuarine and coastal ecosystems

Factor	Influence	Selected references
Primary factors		
Organic matter loading	With high overlying water NO_3^- , enhanced denitrification; coupled nitrification/denitrification likely hindered	Caffrey et al., 1993
Overlying water NO_3^-	Increased NO_3^- enhances denitrification	Nielsen et al., 1995; Kana et al., 1998
Bottom water oxygenation	Decreased O_2 results in low couple nitrification/denitrification and increased denitrification of water column NO_3^-	Kemp et al., 1990; Rysgaard et al., 1994
Secondary factors		
Macrofauna	Increased rates of coupled nitrification/denitrification	Pelegri et al., 1994
Submersed macrophytes	Increased denitrification rates	Caffrey and Kemp, 1990, 1992
Benthic microalgae	Increased rates of coupled nitrification/denitrification; decreased rates of denitrification from water column NO_3^-	Risgaard-Petersen, 1994
MnO_2 ; Mn^{2+}	Mn cycling in sediments results in direct oxidation of NH_4^+ to N_2 and in reduction of NO_3^- to N_2	Luther et al., 1997
H_2S	Results in NO_3^- reduction to NH_4^+ , decreases denitrification	Koike and Hattori, 1978
FeS	Enhances denitrification	Garcia-Gil and Golterman, 1993

peake Bay, historically high populations of benthic filter feeders such as oysters (Newell, 1988) may have had both direct and indirect effects on denitrification by increasing particulate carbon and nitrogen fluxes to oxygenated shallow water sediments rather than to hypoxic or anoxic deep water sediments.

Submersed plants can have a strong influence on denitrification rates (Iizumi et al., 1980; Kristensen et al., 1988), directly through enhancement of nitrification by increased O_2 inputs into sediments (Henriksen & Kemp, 1988; Caffrey & Kemp, 1990, 1992) and indirectly through increased metabolic rates and the 'filtration' and focusing of water column particulates into plant beds. Caffrey & Kemp (1992) observed that sediments vegetated with *Potamogeton perfoliatus* had higher rates of coupled nitrification/denitrification than unvegetated sediments (Figure 4d). Conversely, high rates of NH_4^+ uptake by macrophytes may result in decreased rates of nitrification.

Microphytobenthos can have a major effect on the chemistry of the sediment-water interface, including assimilatory and dissimilatory cycling of nitrogen (Revsbech et al., 1988). Field observations and modeling of the effects of microphytobenthos in a coastal ecosystem in Delaware, U.S.A., indicated that benthic algae had a large impact on overall benthic nutrient fluxes, but little impact on denitrification budgets (Cercio & Seitzinger, 1997). Using the ^{15}N isotope pairing technique, Risgaard-Petersen et al. (1994) showed that photosynthetic enhancement

of oxygen penetration enhanced coupled nitrification-denitrification, but decreased the rate of denitrification from overlying water NO_3^- by increasing the NO_3^- diffusive path length. Overall their study showed higher rates of denitrification in the dark than in the light. Van Luijn et al.'s (1995) study of lake sediments showed that increased oxygen penetration resulting from benthic algal photosynthesis resulted in increased rates of coupled nitrification/denitrification. Differing results between these systems may be expected because of (1) competition for NH_4^+ by both nitrifiers and primary producers and (2) competition for NO_3^- by primary producers and denitrifiers.

There has been an increasing awareness that the interaction of the sedimentary N cycle with other sedimentary redox cycles (Mn, Fe, S) may be an important influence on denitrification in coastal sediments (Sørensen, 1987). Luther et al. (1997) provide evidence that denitrification in Mn-rich sediments may be mediated by a non-biological cycling of sedimentary Mn redox species. In the presence of O_2 , the interaction of MnO_2 and NH_4^+ can yield N_2 ; in the absence of oxygen, reduction of NO_3^- by Mn^{2+} occurs. Garcia-Gil & Golterman (1993) have shown that the oxidation of added FeS in sediments can enhance non-biological denitrification. It is likely that other chemical processes that lead to denitrification will be identified.

Another control on the rate of denitrification in coastal ecosystems is the dissimilatory reduction of

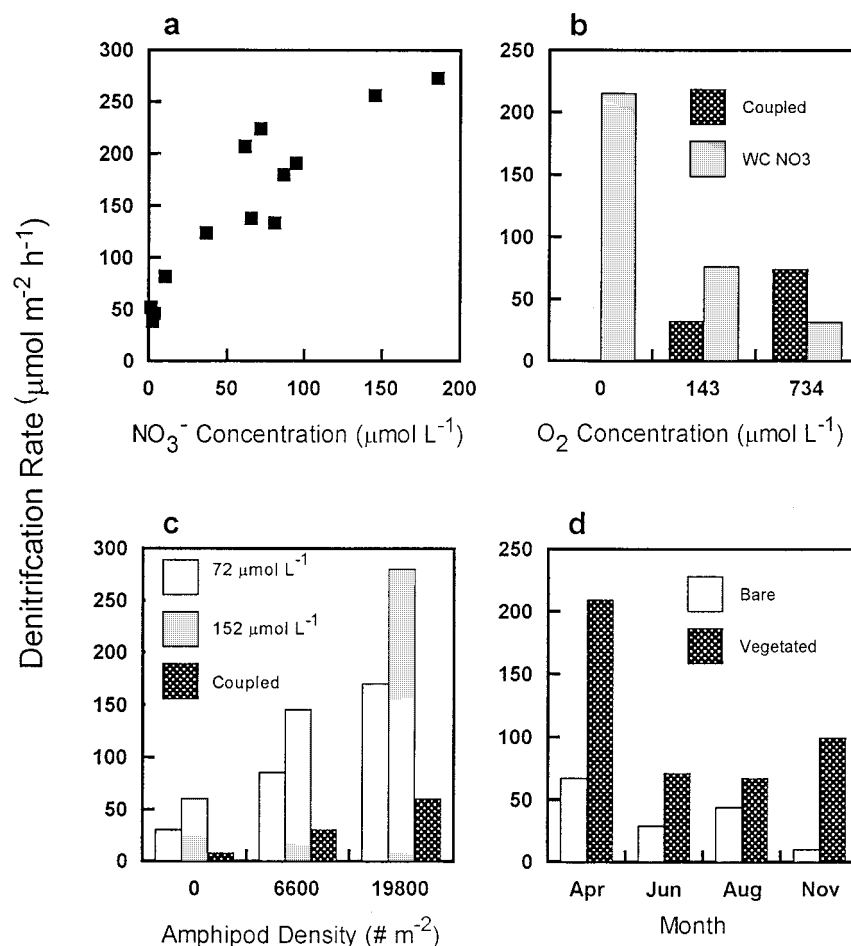


Figure 4. Denitrification response to changing environmental conditions. (a) Linear relationship between the concentration of NO_3^- in of overlying water and the rate of denitrification (Kana et al., 1998). (b) The effect of overlying water oxygen concentration on denitrification rates, both that coupled to nitrification and that supported by water column NO_3^- (Rysgaard et al., 1994). (c) The effect of amphipod density on denitrification rate, with rates split into those utilizing 72 and 152 $\mu\text{mol L}^{-1}$ NO_3^- concentrations and those derived for coupled nitrification/denitrification (Pegleri et al., (1994). (d) The effect of submersed macrophytes on denitrification rates (Caffrey and Kemp, 1992).

NO_3^- to NH_4^+ , also called dissimilatory NH_4^+ production. In this case, anaerobic nitrate respiration leads to enhanced NH_4^+ recycling rather than N removal by reduction to N_2 gas (Koike & Hattori, 1978). Recent studies have indicated that it's importance in coastal sediments varies considerably (Enoksson & Samuelson, 1987; Jorgensen, 1989). Under high organic loading conditions, dissimilatory NO_3^- reduction can be considerably more important than denitrification (Gilbert et al., 1997). More information on the conditions that favor this process over denitrification are needed.

Ecosystem level controls

At the ecosystem level, the residence time of the water may have an important effect on the proportion of N loading that is denitrified (Figure 5). The likelihood that recycled NH_4^+ will be transformed to N_2 and shunted from biological cycling depends on how quickly water and associated water column dissolved and particulate N exit the ecosystem boundary. In rapidly flushed systems, rates of particulate organic matter deposition may tend to be retarded so that minimal N is remineralized in sediments (Nielsen et al., 1995).

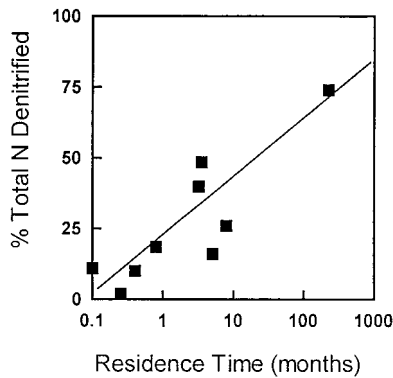


Figure 5. The residence time of water as a controlling variable on the proportion of N loading that is denitrified (Nixon et al., 1996).

Estimation of ecosystem denitrification budgets

Biogeochemical mass balances are a fundamental part of understanding nutrient cycles in coastal and estuarine environments, and are essential for the management of these ecosystems. The calculation of whole ecosystem mass balances is difficult for virtually all major budget terms, with sediment nutrient burial and denitrification budgets being no exception.

The extrapolation of limited sets of sedimentation and biogeochemical flux rate data to annual N and P budgets is usually difficult; the large uncertainties in important budget terms are well illustrated in the Chesapeake Bay (Boynton et al., 1995) and in ecosystem comparisons (Nixon et al., 1996). The spatial and temporal variability inherent in sediment biogeochemical rates make the determination of ecosystem budgets of nutrient cycling processes in estuaries and coastal ecosystems difficult at best. Denitrification has a large seasonal variability in most coastal ecosystems (Kemp et al., 1990), but often little is known about spatial variability due to the difficulty in making measurements.

The denitrification budgets summarized for North Atlantic coastal ecosystems (mostly from Nixon et al., 1996; Table 1) generally have a large uncertainty because of (1) calculation by difference in mass balances (e.g. the Baltic Sea, Scheldt Estuary) or by using less than optimal techniques (Chesapeake Bay), (2) minimal temporal sampling (Delaware Bay), or (3) minimal spatial sampling. The definition of system boundary may also have an influence on the calculation of total ecosystem denitrification. Because the boundary can include the land-water interface, it is necessary to include substantial losses associated with riparian systems and tidal marshes (Howarth et

al., 1996). In Chesapeake Bay subestuaries, our observations of denitrification via direct N_2 flux and ^{210}Pb -based estimates of N burial in tidal freshwater wetlands indicate that N removal by these systems is important to downstream water quality (Zelenke & Cornwell, unpublished).

There are several approaches for estimating annual mean rates of denitrification over an entire coastal ecosystem: (1) developing an extensive field research program, in which dependable rates of denitrification are measured across key scales of temporal and spatial variability, (2) using empirical models to relate denitrification rates to major forcing functions (e.g. DIN loading or ecological processes (such as primary production) or (3) developing numerical models calibrated with field and laboratory data and used to calculate denitrification rates in relation to spatial and temporal variations in water column and sediment properties. The limit to using the first approach is often financial or logistical, with interannual differences in ecosystem properties or fundamental changes in nutrient loading (increase or decrease) which may make the budget for a single year inapplicable for future years.

The identification of empirical relationships between sediment variables has been an important tool for the study of coastal sediments. Examples include (1) estimating the depth of sediment oxygen penetration from sediment oxygen demand and overlying water oxygen concentrations (Cai & Sayles, 1996) and (2) determining relationships between sediment chlorophyll a and NH_4^+ fluxes (Cowan & Boynton, 1996). Seitzinger & Giblin's (1996) denitrification estimate for the North Atlantic continental shelf illustrates the use of such empirical relationships. Their denitrification budget was developed based on (1) a strong correlation between sediment oxygen consumption and denitrification rate and (2) a considerably weaker relationship between sediment oxygen demand and phytoplankton production. While it may be argued that this indirect approach has little precision, it does provide reasonable estimates of denitrification in such a large continental shelf region. Such correlative approaches may also be useful in shallow water environments.

Process-based numerical model computations can also be used to estimate denitrification rates for whole ecosystems. Such models can, in principle, deal with the significant interannual variability in freshwater and nutrient inputs which lead to changes in the sediment area suited to macrofauna and submersed plant habitats typical of estuaries. An approach based on un-

Understanding the response of denitrification to changing temperature, salinity, overlying water NO_3^- , organic loading, macrofaunal densities and other limiting factors would have more predictive power than a denitrification measurement program alone. Spatially explicit water quality/physical models now often include terms for such sediment processes (Cercio & Cole, 1993). The understanding of temporal and spatial controls of denitrification may allow a better calculation of both current whole ecosystem denitrification rates and potential changes with improved or deteriorated water quality. Middelburg et al.'s (1996b) application of a diagenetic model to calculate denitrification at basin to whole ocean spatial scales further illustrates the potential application of models for system-wide denitrification estimates.

Environmental change and denitrification

Potential changes in denitrification rates associated with eutrophication may be illustrated using the Chesapeake Bay as an example (Table 4). The Chesapeake Bay, a highly productive coastal plain estuary, has experienced eutrophication associated with elevated point source and diffuse inputs of N and P (Malone, 1992; Kemp & Boynton, 1992). The mainstem of the Chesapeake Bay has three regions with distinct physical, chemical and biological characteristics: the oligohaline region, the mesohaline region, and the polyhaline region. The oligohaline region is characterized by high turbidity, high water column NO_3^- concentrations in the spring, dissolved oxygen in the water column in all seasons, and low annual primary production. The major losses of aquatic grasses that occurred in the 1960s (Orth & Moore, 1984) may have led to lowered relative denitrification rates as a fraction of N inputs, but increasing NO_3^- concentrations most likely have increased the absolute rate of denitrification. Marsh losses in upper estuarine systems may also have a negative impact on total system denitrification (Howarth et al., 1996). In the polyhaline region of the Chesapeake, increased rates of phytoplankton production and carbon deposition to the sediments may have stimulated denitrification. Increased primary production could support the relatively higher densities of macrofauna which could effectively contribute to enhance nitrification of recycled sediment NH_4^+ production (Mayer et al., 1995) and tighter coupling to denitrification (Jenkins & Kemp, 1984).

Eutrophication effects are most pronounced in the mesohaline region of the Chesapeake Bay, with (1) increased rates of primary production and organic matter deposition; (2) increased respiration rates below the pycnocline (Officer et al., 1984); (3) reduced benthic filtration of phytoplankton associated with overharvested populations the American oyster, *Crassostrea virginica* (Newell, 1988); and (4) reduced contributions of benthic primary production including benthic microalgae (Cooper & Brush, 1993) and aquatic grasses (Orth & Moore, 1984; Kemp et al., 1983), resulting from decreased light penetration. Because of anoxia, a total loss of the coupled nitrification-denitrification N cycling pathway occurs in the summer. In addition, the loss of benthic macrofauna in summer results in less efficient nitrification-denitrification during all seasons. Denitrification associated with aquatic grasses has also declined with the reduction of grass cover. While the effects of anoxia and the loss of aquatic grasses and benthic macrofauna have been demonstrated experimentally, the overall quantitative consequences of increased carbon loading on Chesapeake Bay's denitrification rates are less well documented. An examination of the consequences of environmental change on a hypothetical east-west transect of the mesohaline Chesapeake Bay suggests that current denitrification efficiencies (denitrification rate/N remineralization rate) are lower than those expected prior to eutrophication. Kemp et al. (1990) have identified a decrease in denitrification as an important eutrophication feedback that may have 'amplified' the effect of N loading. Decreases in N loading which increase denitrification efficiencies may improve water quality beyond that based on simple loading response scenarios.

Conclusions

The measurement of denitrification in coastal and estuarine sediments remains a difficult undertaking. Recent advances in ^{15}N techniques (isotope pairing) and in the direct measurement of N_2 have made the measurements more reliable than previous techniques. Considerable understanding about the factors which control denitrification has been developed, with organic matter loading, macrofauna, aquatic grasses, microphytobenthos, anoxia and NO_3^- identified as important environmental controls.

Two areas that need further development in studies of coastal denitrification are (1) more detailed and

Table 4. Chesapeake Bay environmental changes with eutrophication: possible effects on relative denitrification rates

Parameter	Change	Effect on denitrification
Carbon Loading	Increase	Decrease in coupled nitrification-denitrification; possible increase in denitrification from water column NO_3^-
Nitrate Concentration (Direct Effects)	Increased loading, higher concentrations in spring	Increase
Hypoxia/anoxia	Increase	Decrease
Macrofauna	Decrease, loss of habitat via anoxia	Decrease
Benthic Grazers (Oysters)	Large decrease via habitat loss and harvesting	Unknown
Submerged Aquatic Vegetation	Large decrease	Decrease
Benthic algae	Large decrease	Unknown
Water Residence Time	?	?
Marsh area	Decrease	Decrease?

thoughtful approaches to develop seasonal and annual denitrification budgets in heterogeneous sedimentary environments and (2) a better understanding of how eutrophication of coastal ecosystems has changed the relative and absolute rates of denitrification. The estimation of ecosystem denitrification budgets is difficult and more consideration of the relevant scales of temporal and spatial variability in sedimentary processes is required. The effects that environmental changes, including both increases and decreases in nutrient loading, have upon denitrification and other sediment N cycling processes need to be identified for better management of coastal resources.

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