

THE BENTHOS AND NUTRIENT CYCLING: INSIGHTS FROM MULTI SCALE ANALYSIS

By

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(Under the Direction of Christof Meile)

ABSTRACT

The goal of this dissertation is to investigate the impacts of benthic fauna across multiple scales, with particular emphasis on the importance of benthic fauna for two major marine interfaces: the sediment-water interface, and the interface between the terrestrial and marine environments. Combined model-laboratory studies are used to investigate benthic faunal impacts and variability at the scale of individual plots in order to document the importance of burrow patchiness. This work additionally expands on the scale-based nature of patchiness with explicit consideration of sediment reactivity and a discussion on the implications for sampling regimes in different environments.

Laboratory-modeling studies are used to describe the importance of individual organisms' variability, particularly in two important behaviors: burrowing depth and burrow irrigation intensity. Laboratory microcosms are used to parameterize a reaction network describing nitrogen cycling and early diagenesis in arenicolid-inhabited sediments. Burrowing depth, irrigation intensity, and environmental conditions are manipulated within the model to gain a clearer understanding of the importance of organism behavior for nitrogen cycling.

Large-scale analysis is used to consider the system-wide impacts of benthic fauna. Denitrification estimates for whole estuaries are obtained from the literature and combined with databases containing nutrient availability and benthic community status for those same estuaries. This dataset is used to investigate the relationships between benthic community status (diversity and abundance) and system scale denitrification. Literature data on the individual effects of benthic fauna are then combined with the large-scale database to create a model able to predict estuarine denitrification, using nutrient availability and benthic organism abundance as the fundamental controls of denitrification.

INDEX WORDS: Reactive transport, Benthic fauna, Nitrogen cycle, Oxygen flux, Denitrification, Bioturbation, Bioirrigation

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B.A, Hendrix College, 2009

A Dissertation Submitted to the Graduate Faculty of the University of Georgia in Partial
Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2015

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DEDICATION

To my Parents, my first and greatest source of encouragement.

ACKNOWLEDGEMENTS

My advisor, Christof Meile, without whom I have no doubt I would not have grown into the scholar I am today.

My committee, whose expertise and advice have been instrumental in shaping this dissertation into a body of work I am proud to call my own.

All of my friends and family, for the combination of supporting me when I needed it, and putting up with me when I probably didn't deserve it.

All of the faculty at Hendrix College, particularly my undergraduate advisor Dr. Jennifer Dearolf, for introducing me to the joys and wonders of doing and teaching science, and always having the time to support me even years after leaving their tutelage.

My longtime roommate and companion Josh, for his patience and understanding even when completing this dissertation made me a less than ideal roommate.

My dog and friend, Mocha, whose companionship I treasure even though he ate an entire Thanksgiving ham.

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CHAPTER 1

INTRODUCTION

Nitrogen

Nitrogen is a major constituent in a wide variety of organic molecules, but although nitrogen is the most abundant element in the atmosphere, its biological availability is one of the limiting factors in marine systems. As Galloway et al. (2004) point out, the vast majority of nitrogen is in the form of dinitrogen gas (N_2) and thus unavailable for biological use. Because the capacity to break the triple bond between nitrogen atoms in N_2 is possessed by a relatively small number of taxa, the level of bioavailable nitrogen controls ecosystem productivity (Paerl 1997). Nitrogen can also cause eutrophication in coastal ecosystems when present in excess, a phenomenon that has become increasingly widespread in the 20th and 21st centuries (Diaz & Rosenberg 2008). The onset of hypoxia can have dramatic and wide-ranging consequences, so effective management of these systems requires thorough knowledge of how limiting nutrients such as nitrogen enter, are transformed in, and eventually are removed from coastal ecosystems.

The Haber-Bosch process invented at the turn of the 20th century has resulted in a dramatic increase in total nitrogen fixation rates (Galloway et al. 2004); shortly before the end of the 20th century, roughly 100 Tg of nitrogen was created annually by human activity (Kramer 1999). This contrasts with the roughly 15 Tg of bioavailable nitrogen produced by humans in the middle of the 19th century, the majority of which was fixed as a result of the cultivation of legumes (Smil 1999) and was very small relative to the roughly 250 Tg

produced naturally at that time. As a consequence of this large increase in nitrogen production, N loading to coastal ecosystems has roughly doubled in the past century and a half, and it is projected to increase even further – to roughly 60 Tg yr⁻¹ – by the middle of this century (Galloway et al. 2004). This has had major impacts on the services coastal ecosystems provide (Cloern 2001, Levin et al. 2009). As just one example, eutrophication in the Chesapeake Bay has coincided with a near-total collapse of its oyster fisheries (Kemp et al. 2005). Given that global population – and thus the demand for nitrogenous fertilizers to support the human food supply – is increasing continuously, it is crucial to fully understand how nitrogen moves through these ecosystems (Canfield et al. 2010).

Nitrogen has a complex global biogeochemical cycle, (Figure 1.1) and bioavailable nitrogen enters the ecosystem via nitrogen fixation and uptake into biomass (particulate organic nitrogen, PON). The nitrogen contained within biomass is transformed through a variety of organic forms, including amines, nucleic acids, and urea, which are exchanged between and within trophic levels (Carpenter & Capone 1983, Zehr & Ward 2002). When biomass is degraded, reactive nitrogen is released in the form of ammonium, which in turn is oxidized by microbial activity through nitrification. The nitrate produced in this reaction can then be consumed as part of the redox cascade, producing dinitrogen gas and removing the nitrogen from the ecosystem (Seitzinger 1987). More recent additions to our knowledge of the nitrogen cycle include dissimilatory nitrate reduction to ammonium (DNRA, Sorensen 1978), which recycles nitrogen within a system, and anaerobic ammonium oxidation (anammox), which is an alternative removal pathway resulting in N₂ production (Mulder et al. 1995).

One notable aspect of the global nitrogen cycle is how closely it is linked to other biogeochemical cycles, including that of oxygen. Denitrification coupled to nitrification is dependent on the aerobic oxidation of ammonium produced during the breakdown of organic matter (Kristensen & Blackburn 1987), yet denitrification is an anaerobic process inhibited by the presence of oxygen. This represents the most direct link between the two elemental cycles (see Figure 1.1), but nitrogen cycling is also indirectly linked to oxygen dynamics via the differences in biogeochemical cycling under oxic or anoxic conditions. Low bottom water oxygen concentrations encourage alternate diagenetic pathways such as sulfate reduction, and the enhanced production of sulfide encourages nitrogen recycling via dissimilatory nitrate reduction to ammonium rather than removal by denitrification (Gardner & McCarthy 2009). The lack of oxygen combined with the inhibition of nitrification by sulfide additionally leads to an increase in ammonium efflux from the sediment in hypoxic conditions (Middelburg & Levin 2009), further contributing to retention of bioavailable nitrogen in the estuary as opposed to removal.

Because the consumption of nitrate is an anaerobic process and nitrification requires oxygen, efficient nitrogen removal is controlled by the close proximity of oxic and anoxic sediments. Nitrogen cycling is therefore closely associated with interfaces between oxic and anoxic niches. Two interfaces in particular are of prime importance and the unifying topic of this dissertation: the interface between sediment and water, where nitrogen cycling is most intense, and the interface between the terrestrial and marine environment, which heavily impacts the total nitrogen exported to the coastal environment (Nixon et al 1996).

The benthic environment

The degradation of organic material leads to steep concentration gradients in near-shore sediments that lead to a distinct zonation of redox conditions, and the close proximity of oxic and anoxic areas promotes rapid nitrogen cycling. Within these near-shore sediments, depth-integrated rates of nitrogen removal are typically controlled by the availability of substrate, specifically organic carbon and nitrate, which can either be supplied from the overlying water or *in situ* by nitrification of ammonium produced from organic matter degradation (Seitzinger 1988). In eutrophic environments with high levels of nitrate in the overlying water – typical of many estuaries and coastal environments – it is direct denitrification of overlying water nitrate that is responsible for the bulk of denitrification (Seitzinger et al. 2006). Thus, denitrification in many coastal systems is controlled in part by the extent of benthic-pelagic coupling, a process that is in turn heavily impacted by the benthic faunal community.

The recognition of bioturbation – the active mixing of particulates within soil or sediment – as an important process is not new, and it can be traced to Charles Darwin, who described the process in his final work (Darwin 1881). In marine sediments, the formation and irrigation of burrow structures leads to the introduction of oxidants and other reactive solutes into reduced, anoxic sediment (e.g. Aller 1988, Kristensen 2000, Meysman et al. 2006). This burrow formation has dramatic effects on both the ecology and biogeochemistry of marine sediments (Fenchel 1996, D’Andrea et al. 2002, Volkenborn et al. 2007, Volkenborn & Reise 2007). Indeed, the evolution of burrowing lifestyles was one of the formative moments in the Ediacaran to Cambrian transition, leading to major changes in the structure of the ocean floor and possibly even playing a major role in the

Cambrian Explosion (Thayer 1979). In the present day, the benthic community serves as one of the primary drivers of benthic-pelagic coupling between the overlying water and the sediment environment, so fauna can substantially impact multiple biogeochemical cycles, including that of nitrogen.

Benthic fauna stimulate nitrogen cycling through a variety of ways (Stief 2013), with the mechanism for this stimulation depending on the infauna functional traits (Mermillod-Blondin 2011). The close proximity of oxic zones to anoxic sediment promotes nitrogen removal via coupled nitrification-denitrification (e.g. Aller 1988, Aller & Aller 1998). Another pathway for enhanced nitrogen cycling is the stimulation of direct denitrification via increased supply of nitrate to the benthic environment (Kristensen et al. 1991, Dornhoffer et al. 2015); this pathway is especially important in eutrophic estuaries with high concentrations of nitrate in the overlying water (Seitzinger et al. 2006). There is therefore clear evidence of the ecological importance of benthic fauna in terms of their impacts on nitrogen cycling.

Anthropogenic impacts and the benthos

The use of fertilizer and increase in coastal populations has led to significant pressure on the coastal ocean ecosystem, as evidenced in hypoxic events (Diaz and Rosenberg 2008). Hypoxia occurs when rapid degradation of organic material results in depletion of oxygen, and it has a number of effects on the benthos, including losses of biomass and organism richness (Levin et al. 2009). This damage is further compounded by additional anthropogenic pressures such as trawling (Duplisea et al. 2001) and overfishing of the benthic community such as bivalves (Casey et al. 2014). Even when hypoxia does not

result in organism death, it can lead to individual behavioral changes such as increased respiration rates and changes in feeding strategies (Diaz & Rosenberg 1995), as well as changes in total community bioturbation (Belley et al. 2010).

The loss of benthic fauna leads to pronounced changes in sediment biogeochemistry. Specifically, critical elements such as nitrogen, phosphorus, and carbon are recycled or buried, leading to unpredictable recoveries or hysteresis when external nutrient loadings are reduced due to the release of stored highly labile organic matter during recovery (Middelburg & Levin 2009). This leads to a feedback cycle, wherein the loss of benthic fauna decreases nitrogen removal and therefore exacerbates hypoxia (Karlson et al. 2007), as shown in Figure 1.2. The changes in biogeochemical cycling that result from hypoxia not only slow system recovery by encouraging nutrient recycling and the burial of labile carbon, but they also enhance the geographical spread of eutrophication by extending the “reach” (i.e. the physical distance traveled) of anthropogenic nitrogen that would normally be consumed via nitrate reduction (Howarth et al. 2011).

The severity of the impacts caused by hypoxia is not evenly spread over the entire benthic community, because hypoxia tolerance varies widely among taxonomic groups: more complex invertebrates and echinoderms are more sensitive to low oxygen concentrations than, e.g., polychaetes (Levin et al. 2009). Over relatively short time scales (months to years), hypoxia leads to pronounced but short-lived changes in ecosystem function as a result of community changes during succession (e.g. Bartoli et al. 2000). Over longer time scales, anthropogenic forcing in the form of eutrophication leads to pronounced changes in community composition (Reise 1982, Conley et al. 2007), which

then can lead to permanent functional shifts depending on the functional traits of surviving or colonizing organisms.

Different functional traits among taxa, such as feeding strategy and burrowing behavior, can play a key role in determining overall ecosystem function (Norling et al. 2007, Waldbusser & Marinelli 2009). For instance, the colonization of disturbed Baltic Sea sediments by low-oxygen tolerant polychaetes leads to a permanent shift from dominance by the seagrass *Zostera marina* due to the burial of seagrass seeds by the polychaetes (Delefosse & Kristensen 2012). There is also evidence that shifts in community composition can make a system more resilient to hypoxia by the introduction of new functional traits or more tolerant species; for instance, the colonization of the Baltic Sea by the invasive Spionid *Marenzelleria* sp. was associated with the improvement of water quality in sediments inhabited by this polychaete (Norkko et al. 2012).

The problem of scale

The majority of studies documenting the impacts of benthic fauna have been designed and executed at the scale of single organisms (e.g. Stief 2013) up to plots of tens of square meters (e.g. Volkenborn & Reise 2007, Volkenborn et al. 2007). However, ecosystem services that are relevant to human society are usually considered at e.g. the scales of entire estuaries (Snelgrove et al. 2014). Bridging the gap between these scales is not entirely straightforward, because it requires understanding of 1) the heterogeneity present at progressively larger scales and its impact, 2) the importance of interactions at multiple scales, and 3) mechanisms or statistical relationships that explain observed patterns and can be used to predict patterns across multiple scales. In the context of the

benthic community, the need to bridge this scale gap has led to very active research into the ecosystem function at the scale of communities.

A number of key factors have emerged from investigations into the relationships between community aspects and ecosystem function (remineralization, benthic flux, nitrogen removal, etc.). Biodiversity, functional diversity, organism traits, and especially organism density all have impacts on ecosystem function (Emmerson et al. 2001, Marinelli & Williams 2003, Waldbusser et al. 2004, Norling et al. 2007, D'Andrea & DeWitt 2009, Michaud et al. 2009, Waldbusser & Marinelli 2009, Braeckman et al. 2010, Braeckman et al. 2014), but these relationships are not always consistent. The lack of clear relationships and especially mechanisms that can explain these relationships makes bridging the scale gap difficult. Modeling can be a powerful tool to address this problem, because numerical models can be used both to isolate variables that would be difficult to manipulate empirically (such as reaction rate constants to mimic different environmental conditions), and to provide key insight into underlying controls of ecosystem function. This dissertation is centered on the use of models to provide insight into the mechanisms underlying ecosystem function and apply those mechanisms across spatial scales.

Structure of dissertation

The goal of this dissertation is to examine the impacts of benthic fauna across multiple scales, with particular emphasis on the importance of benthic fauna for two major marine interfaces: the sediment-water interface, and the interface between the terrestrial and marine environments. Chapter 2 deals with benthic faunal impacts and variability at the scale of individual plots in order to document the importance of burrow patchiness.

Patchiness is a known pattern for marine benthic communities (e.g., Levin 1992, Underwood et al. 2000), so the importance of this patchiness must be understood in order to cross scales of interest. The second chapter uses a combined laboratory-modeling study to describe how benthic oxygen fluxes change as a result of variations in organism burrow distribution. The chapter additionally expands on the scale-based nature of patchiness (Morrisey et al. 1992) with explicit consideration of sediment reactivity and a discussion on the implications for sampling regimes in different environments.

The third chapter zooms in from the scale of plots to the scale of individual organisms. Where Chapter 2 is concerned with community-level variation (patchiness), the goal of chapter 3 is to describe the importance of individual organisms' variability, particularly in two important behaviors: burrowing depth and burrow irrigation intensity, which both can naturally change with organism age and size (Riisgard et al. 1996). Furthermore, where Chapter 2 is concerned with the effects of organisms that impact benthic-pelagic coupling by enhancing diffusive exchange between sediment and the overlying water, Chapter 3 deals with the importance of head-down deposit feeders, which actively create porewater advection through injection of water across a feeding pocket (Meysman et al. 2005). Laboratory microcosms are used to parameterize a reaction network describing nitrogen cycling and early diagenesis in arenicolid-inhabited sediments. Burrowing depth, irrigation intensity, and environmental conditions are manipulated within the model to gain a clearer understanding of the importance of organism behavior for nitrogen cycling.

Chapter 4 considers the system-wide impacts of benthic fauna. Denitrification estimates for whole estuaries are obtained from the literature and combined with

databases containing nutrient availability and benthic community status for those same estuaries. This dataset is used to investigate the relationships between benthic community status (diversity and abundance) and system scale denitrification. Literature data on the individual effects of benthic fauna are then combined with the large-scale database to create a model able to predict estuarine denitrification, using nutrient availability and benthic organism abundance as the fundamental controls of denitrification.

Finally, Chapter 5 summarizes the main findings and conclusions.

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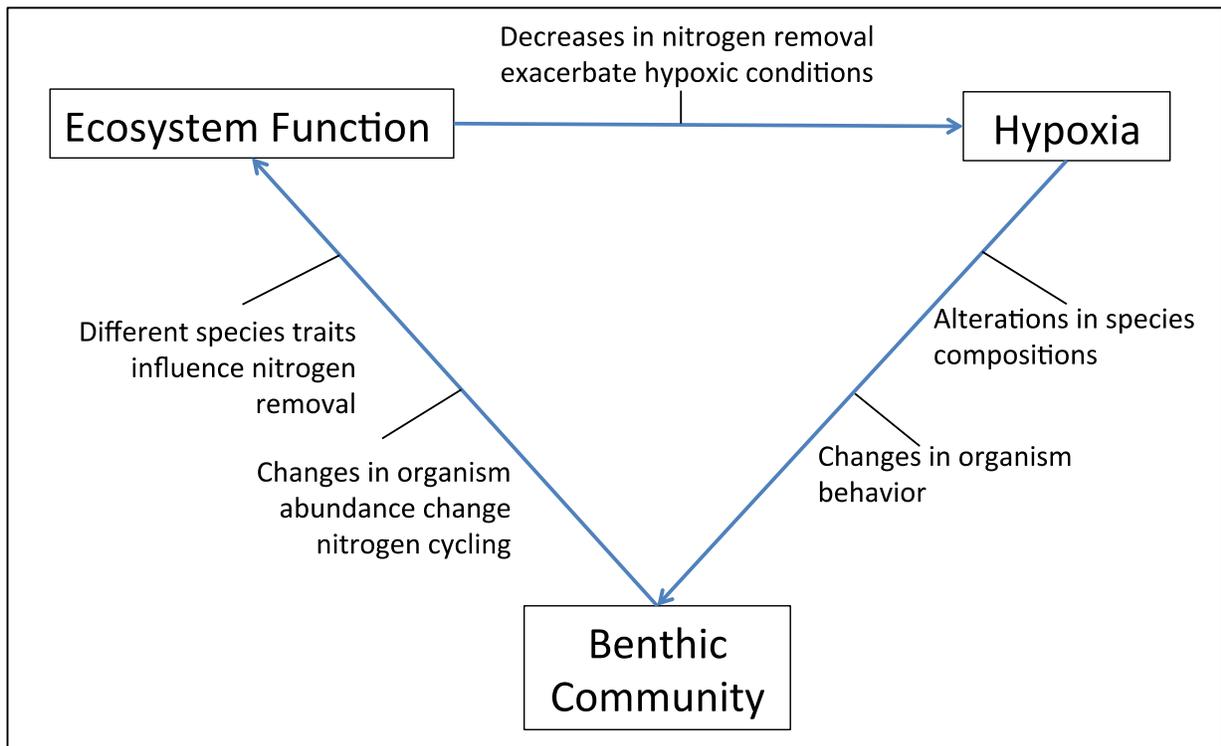


Figure 1.2: Conceptual diagram of feedbacks between hypoxia, benthic community state, and ecosystem function.

CHAPTER 2

BURROW PATCHINESS AND OXYGEN FLUXES IN BIOIRRIGATED SEDIMENTS¹

¹ Thomas Michael Dornhoffer, George Waldbusser, and Christof Meile. 2012. *Journal of Experimental Marine Biology and Ecology*. 412: 81 – 86. Reprinted here with permission of the publisher

Abstract

Bioturbation plays a crucial role in benthic nutrient cycling in many sedimentary environments. Burrowing animals affect benthic-pelagic coupling by mixing sediment and porewater and increasing the effective area of diffusive exchange between oxidizing and reducing environments. Here, we report on a coupled laboratory-modeling experiment that explores organism distribution patchiness and its implications on sedimentary oxygen fluxes. Microcosms were established with three different arrangements of artificial burrows. Data from the laboratory were used to parameterize a three-dimensional diffusion-reaction model, and the impact of burrow distribution on benthic O₂ fluxes at the plot (decimeter) scale was assessed for a range of sediment reactivities representing a variety of benthic habitats. At high O₂ consumption rates, as seen in the microcosms, burrow spacing had little to no effect on sedimentary O₂ uptake; at intermediate rates, the overlap of oxic halos surrounding burrows and benthic O₂ uptake depended significantly on the burrow distribution pattern. Using observed relationships between benthic O₂ flux and oxygen penetration depth in marine sediments, we predict that burrow patchiness has its greatest impact in settings with benthic oxygen fluxes on the order of 1-10 mmol m⁻² d⁻¹, typical for the continental shelf and slope. The biogeochemical heterogeneity caused by burrows also affects the interpretation of concentration measurements, and we present an estimate of the number of measurements needed to reliably estimate bulk O₂ concentrations in cohesive sediments as a function of organism density, measurement scale and sediment reactivity.

Introduction

Organisms that reside within sediments have significant impacts on the nature and properties of their benthic environment (Coleman and Williams 2002, Meysman et al. 2006). The effects of benthic faunal bioturbation manifest themselves at the scale of individuals (Aller 1980, Krantzberg 1985) and may propagate to the scale of ecosystems when organism densities are sufficiently high (Waldbusser et al. 2004). The cumulative effect of burrowing organisms is well illustrated by the dramatic alteration of oceanic sulfur cycling during the Phanerozoic, caused by the evolution of burrowing across the Ediacaran/Cambrian transition (Canfield and Farquhar 2009), or by the modern ecosystem engineering of bioturbating organisms (e.g. D'Andrea and DeWitt 2009). However, when investigating ecosystem-level implications of bioirrigation, inferences from spatially averaged plot-scale measurements are often used to describe effects over larger scales. Such extrapolations may be impacted by unresolved localized features and small-scale variability (Schneider et al. 1997).

At the infaunal organism scale (mm-cm), the biogeochemical effects of benthic infaunal burrows in diffusion-dominated environments stem in large part from the creation of additional surface area for diffusive solute exchange between reduced porewater and oxidized overlying water (Aller 1980). The balance of reaction and diffusion leads to sub-mm to cm-scale concentration and redox gradients across sediment-water interfaces, including burrow walls (Timmerman et al. 2006), which drive benthic solute exchange fluxes. A complex three-dimensional zonation of biogeochemical transformations results (Kristensen 2001), and numerous studies have shown burrow effects on chemolithotrophy and abiotic redox reactions (Kristensen and Kostka 2005) as well as

coupled nitrification/denitrification (Kristensen et al. 1988). The seminal work of Aller (1980) on the radial diffusion model to describe the local burrow environment is an elegant approach that has proven adequate in many cases for capturing effects of infauna on sediment biogeochemistry by describing a complex spatial domain as a collection of equally-spaced cylindrical vertical burrows. This micro-environment approach allows for very fine scale (sub-mm to cm) and computationally efficient descriptions of the biogeochemical dynamics around burrows. However, the application of a constant inter-burrow distance may limit the ability of this model to predict benthic exchange fluxes if patchy infaunal distributions result in overlap of geochemical zones of influence between burrows.

Patchiness has long been recognized as a structuring agent of sediment properties and benthic communities (e.g. Levin 1992, Underwood et al. 2000), and in a seminal work on spatial dynamics in the benthos, Morrisey et al. (1992) noted the variability of benthic infaunal populations across increasingly large (up to kilometers), nested spatial scales. However, the smallest sampling resolution considered by Morrisey et al. was that of core samples, capturing approximately 80 cm². In many settings, this may encompass numerous burrow structures in different spatial arrangements, exemplifying the disparity between the resolution at which benthic communities are sampled and the resolution at which animal-sediment interactions are studied or modeled. Thus, common coring or grab-type sampling does not address the question of how individual tube-building animals' spatial domains of biogeochemical influence interact (as they do ecologically, *sensu* Woodin 1978), and what the biogeochemical consequences of these interactions are. Indeed, fine-scale

patchiness as a factor shaping soft-sediment communities and as a potential driver of sediment biogeochemical processes has received only limited treatment.

Studies aimed at documenting the effects of density or burrow spacing on sediment chemistry have noted non-linear effects; for example, Marinelli (1994) and Marinelli and Williams (2003) found non-linear effects of burrow density on biogeochemical fluxes, and in sediment plug experiments mimicking evenly spaced burrows, Gilbert et al. (2003) report non-linear effects on denitrification with respect to burrow densities and inter-burrow distance. Such results represent important steps forward in more fully integrating the spatially explicit nature of life within marine sediments, and they emphasize the need to assess the implications of spatial variation in order to fully understand the intricacies of ecosystem interactions (e.g. Timmermann et al. 2006, Volkenborn et al. 2007).

Here, we report on a coupled laboratory-modeling approach towards gauging the importance of burrow spatial arrangement on sediment biogeochemical fluxes. A microcosm experiment using artificially irrigated burrow mimics was conducted to illustrate the effect of burrow patchiness on sediment oxygen fluxes. Finite-element reactive-transport modeling was used in tandem with the laboratory experiments to explore the effects of oxygen consumption rate and burrow arrangement on sediment biogeochemistry. This approach allows us to apply a mechanistic description to identify under what conditions burrow patchiness may be an important community level parameter in cohesive sediments.

Methods

Laboratory Methods

Laboratory microcosms were established in four 10 cm long by 10 cm wide by 20 cm deep rectangular containers. These aquaria were filled with homogenized surface sediment collected from a muddy-sand intertidal flat, Little Tom's Cove, VA, USA (lat = 37.886, lon = 75.345). The sediment was poorly sorted, with the following average properties across all microcosms as determined by methods of Folk and Ward (1957): porosity 0.43 ± 0.01 , mean grain size $281.8 \mu\text{m} \pm 6.7$ and 0.50 ± 0.25 % fines (passing a 75 μm sieve). Sediment was added to a depth of about 15 cm, leaving 5 cm of overlying water. Sediment was allowed to settle for one day before burrow structures were added by core replacement.

Artificial burrows with an inner radius of 0.4 cm and length of 10 cm were constructed of Magna nylon filter paper (0.45 μm filter) surrounded by 125 μm nominal sieve opening Nitex to provide structure, and were cinched at the bottom. Four burrows were placed in each microcosm, resulting in a density ρ of 400 per m^2 (e.g. Miron and Kristensen 1993, Volkenborn et al. 2007), in three different arrangements (Fig. 2.2, top): even, grouped, and cornered. These arrangements represent an approximately uniform burrow distribution, many small and evenly-spaced burrow clusters, and widely-spaced large clusters of burrows, respectively. In the even arrangement, burrows were aligned in a square with roughly 2.5 cm between burrows located roughly 3 cm from the edge of the aquaria. In the grouped treatment burrows were approximately 0.5 cm apart and 4 cm from the edge of the aquaria. The cornered treatment had the same inter-burrow distances, but all burrows were within 1.5 cm of the corner of the microcosm. The Clark-Evans

Indices (Clark and Evans 1954), defined as $R = 2\bar{r}\sqrt{\rho}$, where \bar{r} is the average distance to the nearest neighbor within the plot, of these plots are 1.6, 0.8 and 0.8, respectively (where $R = 1$ in a uniform random distribution and $R = 2$ in a completely uniform arrangement).

After artificial burrows were added to microcosms, the microcosms were placed in a seawater bath that was fed from a filtered, recirculating seawater system with a temperature of 22°C and salinity of 33. Artificial burrows were flushed with a 12 channel peristaltic pump (Masterflex Computerized Drive, Model 75550-60), one channel per burrow, at a rate of 1 ml min⁻¹ per burrow. The irrigation tubes were run through the acrylic lids and sealed with silicon, with one return line that fed each of the four pump channels per microcosm, so that microcosms could be sealed to measure fluxes of oxygen while water was being irrigated into burrows without contact to atmosphere. Except for the peristaltic tubing, all tubing used externally of the microcosms was low gas permeability, silver-embedded tubing (Tygon Silver Antimicrobial Tubing) to prevent atmospheric contamination and limit microbial biofilms inside tubing. When fluxes were not being measured, the tops of microcosms were propped open by 3-5 cm and vigorous mixing of the seawater bath ensured exchange between sediment and water bath, while allowing for irrigation of burrows. Flux measurements were performed in August 2009 and run over the course of 2 hours. The experiments were repeated 4 times, and oxygen levels in the overlying water were monitored using a Thermo-Orion O₂ probe. Oxygen flux was determined by fitting a slope to the measured oxygen levels in the microcosm water.

To determine O₂ consumption rates and kinetic rate constants, sediment flask incubations were run separately. Sediment was removed from the microcosms at the end of the experiment and sealed in a flask. O₂ concentrations were measured every half-hour

for six hours, and again at 26 and 30 hours using a Thermo-Orion 4-Star oxygen meter and probe. Winkler titrations and spectrophotometric analyses of oxygen were also used to verify accuracy of the oxygen electrode.

Modeling Approach

Reactive transport simulations were set up to mimic the laboratory microcosms, with 4 cylindrical burrows distributed according to the even, grouped, or clustered settings. For simulations with a lower organism density, the domain was extended to 20 cm x 20 cm x 20 cm, with inter-burrow distances increased proportionally. The O₂ concentration field was computed taking into account diffusion and reaction, and run to steady state:

$$0 = \nabla \cdot (D\nabla C) - k \frac{C}{C+K_m} \quad (1)$$

where C is the O₂ concentration. The effective diffusion coefficient D is estimated as $D = D_{mol}\phi^2$ (Ullman and Aller 1982), with a molecular diffusion coefficient D_{mol} of $1.73 \cdot 10^{-9}$ m² s⁻¹. O₂ consumption was modeled using Monod kinetics with K_m set to 20 μM (Furukawa 2001). The maximum sediment oxygen consumption rate k was determined from flask incubations, and subsequently varied to assess the impact of both sediment reactivity and burrow distribution patterns on O₂ distribution and exchange fluxes. At the sides and bottom of the model domain, fluxes were set to zero, while an O₂ concentration of 220 μM was imposed at the sediment surface and burrow walls as the artificial burrows were continuously flushed.

Model-generated concentration fields were used to assess the variability of measurements expected in a bioturbated field setting. A random sampling algorithm was used to determine the number of sampling points necessary to obtain a value within 5% of the actual average concentration at a given depth (N_s). In order to obtain a value for N_s , the average oxygen concentration in a depth horizon was determined analytically, and then the value of the sampled concentration was averaged over n sampling points to provide a running average of the sampled oxygen levels: $C(n)$. This value was tracked from a value of $n \gg N_s$ down to a value of n such that $C(n)$ is no longer within 5% of the true average. In order to account for variations in the random sample locations, the determination of N_s was repeated multiple times within the plot and averaged to give a plot value.

Results

Microcosm and Model Fluxes

The laboratory microcosms showed relatively large oxygen uptake fluxes, on the order of 80 ± 5.6 mmol m⁻² d⁻¹. The fluxes tended to be highest in the even arrangement and lowest when highly clustered (Fig. 2.1, Experimental), but these differences were not statistically significant ($F = 1.34$, $p = 0.32$). The maximum sediment O₂ consumption rate (k) was calculated to be $1.4 \cdot 10^{-5}$ mol m⁻³ s⁻¹ using least-square fitting.

Modeled sediment O₂ uptake ratios for the cornered and grouped arrangements relative to the even setting are shown in Figure 2.1, for reactivities ranging from $1.4 \cdot 10^{-5}$ mol m⁻³ s⁻¹ (in line with values reported for coastal sediments, Hall et al. 1989) to $1.4 \cdot 10^{-8}$ mol m⁻³ s⁻¹ (corresponding to deep ocean sediments, Wenzhoefer et al. 2001). Consistent with the experimental data, simulated fluxes are virtually identical in the three burrow

arrangements at the value of k derived from the flask incubations. At lower reactivities, there are pronounced burrow arrangement effects, while at very high and very low values for k , there is no apparent effect of burrow distribution. At their most pronounced ($k = 1.38 \cdot 10^{-7} \text{ mol m}^{-3} \text{ s}^{-1}$), the effects due to arrangement can account for up to a 30% decrease in benthic flux when compared to an even burrow arrangement. While a reduction of the burrow radius and hence the burrow surface area leads to a smaller absolute O_2 flux, the same relative effect of burrow clustering on benthic O_2 uptake is also observed for smaller burrow radii ($r = 2 \text{ mm}$; not shown). Similarly, variations in porosity affect the magnitude of benthic fluxes, but the same flux ratios are obtained using the lab-measured porosity of 0.43 or a value of 0.6, representative of fine sand or muddy sediments (cf. Volkenborn et al. 2010).

Modeled concentration fields for the different arrangements (Fig. 2.2) show the role of burrows on O_2 spatial distribution patterns. Model cross-sections illustrate that different burrow clustering patterns can result in different degrees of overlap between each burrow's oxygenated zone; note in particular the lack of anoxic zones between burrows in the clustered model cross-sections (Figs. 2.2b and c). The grouped and cornered distributions - reflecting many small groups versus one large cluster respectively - show noted overlap in these cross-sections, a feature not seen at the high reactivity and low oxygen penetration depth observed in the laboratory microcosms.

Patchiness and Sampling Reliability

Model-obtained oxygen concentration fields were also used to assess sampling reliability as a function of organism density, measurement scale, and sediment reactivity

(Fig. 2.3). Although the average sedimentary oxygen concentration was similar for all model settings, more sampling points were needed in the cornered arrangement than in the grouped or even setting (Fig. 2.3 inset). Increasing reactivity (k) necessitated a larger number of samples be taken to accurately measure the average concentration in a given sediment horizon, as did lower organism density, expressed by a length scale $L_d = \frac{1}{\sqrt{\rho}}$, where ρ is the areal burrow density. For a given measurement device of radius L_M , the number of sampling events (N_s) necessary to reliably reproduce the average concentration in a select sediment depth interval peaks at high values for k and a low measurement to density scale ratio ($P=L_M/L_D$). It can be approximated by (Fig. 2.3):

$$N_s = 62.84e^{(1.94 \times 10^5 k - 6.3P)} \quad (2)$$

where k is in $\text{mol m}^{-3} \text{s}^{-1}$. As the rate of O_2 consumption decreases, the number of burrows increases or a larger measurement device is used, the calculated value for N_s drops quickly to only a few measurements.

For about 100 burrow openings per m^2 and assuming a sediment oxygen demand on the order of $1 \times 10^{-5} \text{ mol m}^{-3} \text{ s}^{-1}$, sediment cores present a reliable average concentration-measuring tool, though missing the underlying variability within the sediment.

Microelectrodes, on the other hand, may require repeated deployment to obtain a measure of the average porewater concentration (e.g. Jørgensen et al. 2005). Moreover, N_s increases with decreasing oxygen penetration depth, because a larger number of points is needed to ensure the probe samples within a burrow's oxic zone. Decreasing reactivities lead to more uniform O_2 concentration fields, decreasing the number of necessary sampling points.

Finally, burrow arrangement also has an effect on N_s as can be seen in the different values

for the even, grouped, and cornered arrangements (Fig. 2.3 inset); the distribution effect is more pronounced at higher reactivities, in contrast to the trends seen for flux values.

Discussion

Benthic Flux

Burrowing infauna affect the biogeochemical structure of their sedimentary habitat and consequently elemental cycling, and can govern benthic exchange fluxes (see e.g. Kristensen 2000, 2001 for reviews). In the diffusion-dominated settings studied here, burrow formation increases the benthic flux simply by means of the increased surface area for exchange between porewater and overlying water, making burrow density the master variable affecting benthic exchange for a given type of macrofaunal community. The flux accentuation, however, is dependent on the concentration gradient at the burrow walls; treating burrows as simple extensions of the sediment-water interface ignores the impact of the potential overlap of the oxic halos of neighboring burrows.

The degree of overlap of the oxygenated zone around burrows also depends largely on the sediment reactivity. At the reactivity seen in the microcosm sediment, the oxygenated halos do not overlap, because the inter-burrow distance is large relative to the oxygen penetration depth. However, when the sediment is less reactive, O₂ penetrates deeper into the sediment, and burrows' oxic zones can begin to interact with each other. Different burrow arrangements show different degrees of overlap at moderate reactivity (Fig. 2.2). At these values for k (on the order of 10^{-6} mol m⁻³ s⁻¹), there is a clear trend of decreasing fluxes with increasing overlap (Fig. 2.1), so that sediment oxygen uptake in the even setting is higher than in the grouped arrangement, which is in turn higher than in the

cornered setting. Finally, as the reactivity decreases even more, the oxygen penetration depth is always large relative to the burrow spacing used here; the diffusive flux approaches zero and there is no detectable pattern due to arrangement. Trends in settings with lower burrow densities are similar to those discussed above, except that as the average burrow spacing is increased, a larger oxygen penetration depth (i.e. a lower reactivity) is necessary for oxic zones to overlap.

In addition to differences due to burrow wall properties (Aller 1983) and burrow geometry (Koretsky et al. 2002), overlap of oxygenated regions surrounding burrows and the variation of such overlap in a patchy burrow distribution explains why benthic flux may not scale linearly with organism density or surface area (e.g. Marinelli and Williams 2003). However, the scale of this variation – centimeters – is rarely considered in ecological studies but can have significant effects on benthic oxygen uptake, which is often used as a proxy for whole sediment metabolism. Our model simulations suggest that ignoring the patchiness of burrow distributions can alter such estimates up to approximately 30%.

Concentration and Variability

The presence of irrigated burrows generates fine-scale heterogeneity that can have a strong influence on the structuring of sediment microbial communities (Marinelli et al. 2002, Fenchel and Finlay 2008). In bioirrigated settings, a large number of single point measurements with microelectrodes may be necessary to capture the sediment's spatial variability, e.g. due to oxic microniches that form within bioturbated zones. Burrowing organisms impact not only large-scale benthic exchange fluxes but also finer-scale solute distribution in the sediment, and therefore affect sampling requirements: in a highly

heterogeneous environment, it takes significantly more measurements to obtain an accurate value of the “average” concentration. This effect is also directly linked to the scale in question, emphasizing the need for a scale-based measure of patchiness.

The variability within a sedimentary O₂ concentration distribution comes from two primary sources: the consumption of O₂ in the sediment and the presence of burrows as a source of lateral heterogeneity. Model-obtained information can be used to assess this variability and predict the number of measurement events necessary to capture an accurate picture of O₂ in the benthos (N_s ; Fig. 2.3). However, while determination of the measurement- to density-scale ratio (P) is straightforward, sediment reactivity (k) may not be as easily obtained in the field directly. It is thus useful to relate the reactivity to the oxygen penetration depth (L_{O_2}), which may either be measured directly, constrained from measurements in comparable environments, or visually assessed based on observed color changes in sediment cores (Diaz and Trefry 2006). Assuming a rate of O₂ consumption (k) constant with depth, as is approximately the case if reoxidation of reduced metabolites is significant near the O₂ penetration depth (Glud et al. 1994), it can be approximated as (Cai et al. 1996):

$$L_{O_2} \approx \sqrt{2\phi DC/k} \quad (3)$$

Thus, Equation 2 can be approximated by:

$$N_s = 62.84e^{(3.88 \times 10^5 \phi DC L_{O_2}^{-2} - 6.3P)} \quad (4)$$

C is the bottom-water concentration of oxygen (in mol m⁻³), D is the effective diffusion coefficient (m² s⁻¹), and L_{O_2} is the oxygen penetration depth in meters. Because the probability of encountering a burrow is related to not only abundance but also burrow size, the estimation of N_s is impacted by changes in burrow radii: smaller burrows are less likely

to be encountered, and thus the calculation of N_s will slightly underestimate the true extent of variability in the sediment oxygen distribution when used with very small burrows.

Scaling up and ecological context

Understanding the small-scale effects of patchy distributions on oxygen dynamics is important where scaling up from an ‘average’ burrow is not sufficient. Our data show that burrow arrangement has negligible effect on sediment O_2 uptake in settings with small O_2 penetration depths, which are characteristic for benthic environments with a high sediment load and rapid remineralization of organic matter. Similarly, in entirely oxic sediments such as in the deep sea, burrow grouping is inconsequential for benthic O_2 uptake. The areas most likely to be impacted by burrow patchiness are those where burrow spacing is on the same order of magnitude as the scale over which biogeochemical gradients are evident. For organism densities similar to those investigated here, patchiness has most impact in settings with oxygen penetration depths on the order of centimeters, indicative of intermediate reactivities. For O_2 penetration depths of 1 cm, a typical flux is on the order $2 \text{ mmol m}^{-2} \text{ d}^{-1}$ (estimated as $F_{O_2} = 2\phi D \frac{[O_2]}{L_{O_2}}$; Cai et al. 1996). This is well within the range where bioturbation is a significant contributor to total O_2 uptake (Meile and Van Cappellen 2003) and corresponds to water depths typical for the continental shelf and slope (Wijsman et al. 2002).

If patchiness is to be considered as an important community variable when scaling up, it is crucial to have measures that relate directly to the scale of observation, and account for clustering. The ratio of measurement scale and characteristic burrow spacing (P) yields insight into the relative probability of encountering burrow structures and is

easily estimated in the field; however, it does not reflect the actual organism *arrangement*. Some arrangement information is reflected in the Clark-Evans Index, but because it considers only the nearest neighbor for each organism, the cornered and grouped arrangements in this particular study are not distinguished despite the differences in sediment biogeochemistry evident in the benthic O₂ fluxes (Fig. 2.1). As a more concise measure of patchiness we propose a normalized probability (Q) of encountering one or more burrow openings with a measurement device of a given sampling radius. Normalization by the probability for a uniform random distribution of the same density removes the impact of burrow density, similar to the Clark-Evans index. This measure emphasizes oxygenated burrow microenvironments as a critical factor for sediment biogeochemistry in predominantly anoxic coastal sediments, and it offers the improvement of explicit consideration of both observation scale and increased sensitivity to subtle changes in burrow arrangement. For instance, for a measurement scale of 2 cm, the grouped and cornered distribution, which are characterized by the same Clark-Evans index, have Q -values of 0.8 and 0.6, respectively. However, for measurements encompassing several burrow clusters, Q does not distinguish between arrangements, similar to R .

Given the importance of burrow arrangement for the diffusive oxygen flux, patchiness likely also plays a role in other elemental cycles, particularly of redox sensitive species. For example, Gilbert et al. (2003) showed a pronounced stimulation of coupled denitrification related to inter-burrow spacing. Although the extent of oxygen penetration is a primary driver in the various redox reactions within sediments, other elements will be sensitive to spatial scales dependent on their respective reaction rates. For example,

ventilation of burrows stimulates opal dissolution by removing silicic acid from porewaters, and the response of burrowing organisms to the presence of burrows in their vicinity alters these silica dynamics (Marinelli 1994). Differences in organism distributions may also explain the lack of strong relationships between species density and nitrogen and phosphorous concentrations (e.g. Ieno et al. 2006). Extrapolating these impacts to the field scale – and taking into account the presence and impact of patchiness documented here – suggests that patchiness can be an important factor for biogeochemical fluxes and benthic-pelagic coupling.

The finding of unexpected non-additive density effects on benthic nitrogen, phosphorous and oxygen flux (Michaud et al. 2009) highlights the potential importance of arrangement and the need for measures that capture small-scale (centimeter) variations in burrow grouping patterns. This recent finding indicates that organism distribution is a factor leading to net community effects diverging from simple “sum-of-parts” aggregations, and suggests that effective study requires consideration of both organism ecology and distribution in terms of biogeochemical functioning (Waldbusser et al. 2004, Norling et al. 2007, Michaud et al. 2009). The distribution of larger burrow structures has been shown to have a controlling influence on the distribution of smaller burrowing organisms (Woodin 1978), potentially via the oxygenation of otherwise anoxic sediments (Marinelli 1994), and the findings of this study provide mechanistic evidence for such biogeochemical connectivity.

Conclusions

One way in which infauna ecosystem engineers structure their habitat is through alterations of the physical or spatial structure, e.g. by establishing (semi-)permanent burrows which affect biogeochemical zonation and benthic exchange fluxes. The spatial distribution pattern, or patchiness, can be a significant factor in the creation of habitats and hence the benthic community structure.

Combining microcosm experiment with permanently flushed artificial burrow structures and reactive transport modeling, we showed that in diffusion dominated settings, it is possible to quantitatively describe the relationship between burrow distribution, organism density, sediment reactivity, and sediment oxygen demand. At high reactivity, burrow spacing has no impact on benthic O₂ demand because all oxygen is consumed in close vicinity of the burrow walls. However, as reactivity decreases and oxygen penetration into the sediment increases, such as the case on the continental shelf, oxic zones surrounding burrow overlap and significant effects on dissolved oxygen fluxes can be observed.

Our findings highlight the importance of spatial arrangement in some sediment habitats, suggesting that fully understanding the role of the benthic community in elemental cycling requires consideration of not only organisms' effects on their environments, but also the inextricable feedback between these organisms and the ecological context in which they live.

Acknowledgments

This publication was supported by the National Science Foundation (OCE 0751882 to CM and OCE 1014226 to GGW) and the Office of Naval Research (ONR # N000140610316 to GGW). We thank Heather Bergschneider and Steven Fenske for their assistance with the laboratory experiments.

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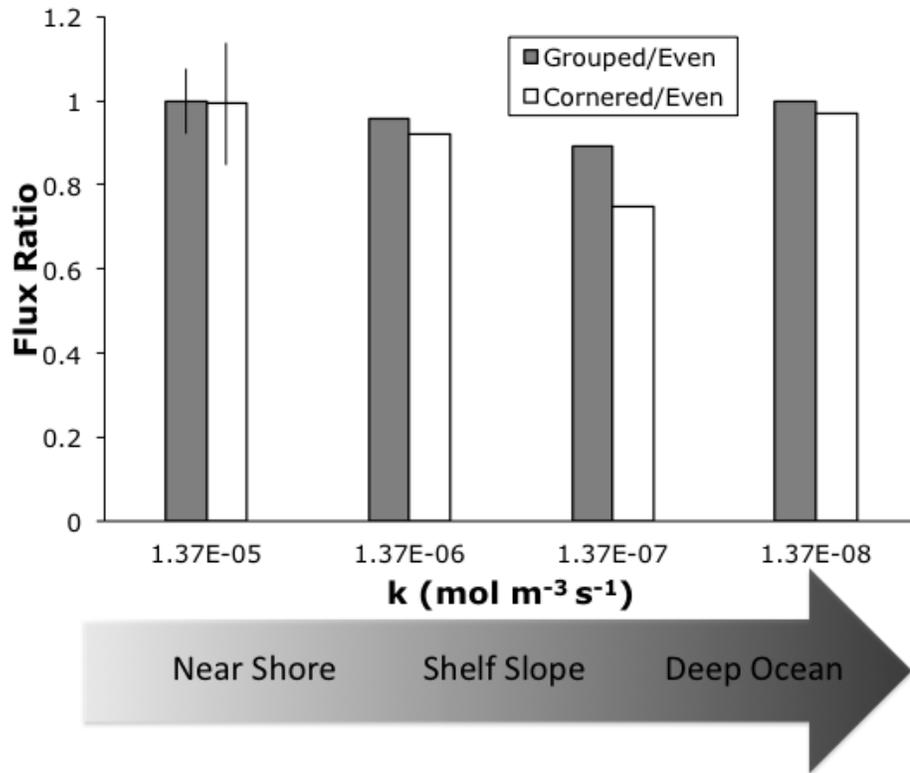


Figure 2.1: Modeled and experimental flux in the grouped and cornered burrow distributions relative to the fluxes from the setting with an even burrow distribution ($F^{O_2} / F_{Even}^{O_2}$) for a range of benthic habitats. Error bars represent the standard deviation in laboratory measured fluxes, sediment from which was used to obtain the base value for the organic matter degradation constant of $1.37 \times 10^{-5} \text{ mol m}^{-3}\text{s}^{-1}$.

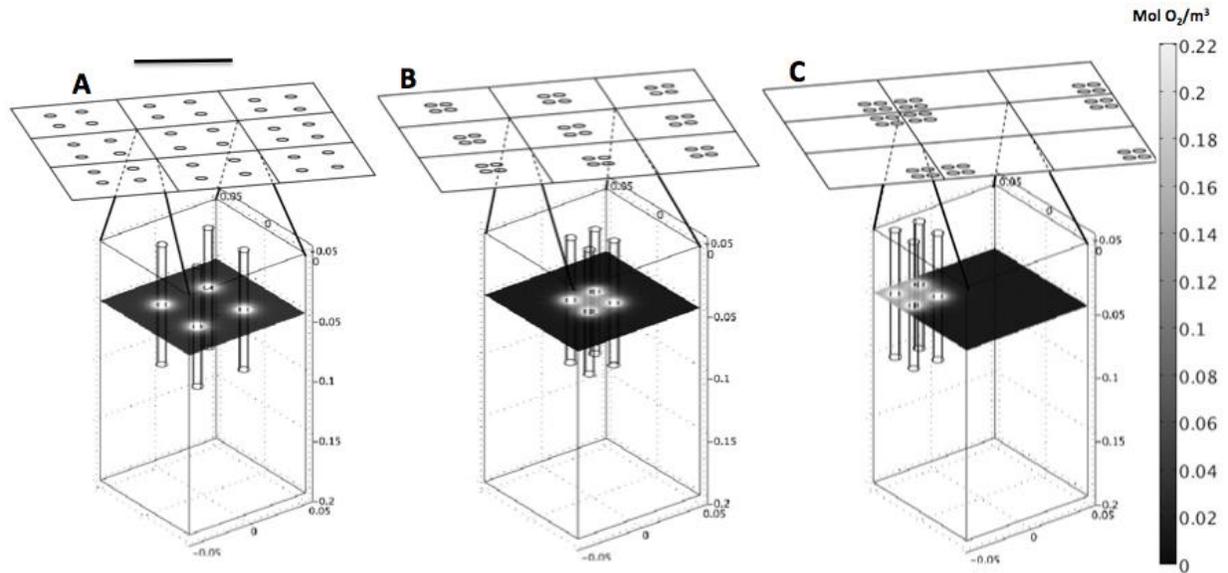


Figure 2.2: Model results showing O₂ concentration at 5 cm depth for a medium reactivity ($k = 1.37 * 10^{-6} \text{ mol m}^{-3} \text{ s}^{-1}$) for the **(A)** even **(B)** grouped and **(C)** cornered arrangement. Sketches at the top represent the three burrow arrangements, while the bottom half shows the sediment columns with the cross section of the O₂ concentration fields. The scale bar equals 10 cm.

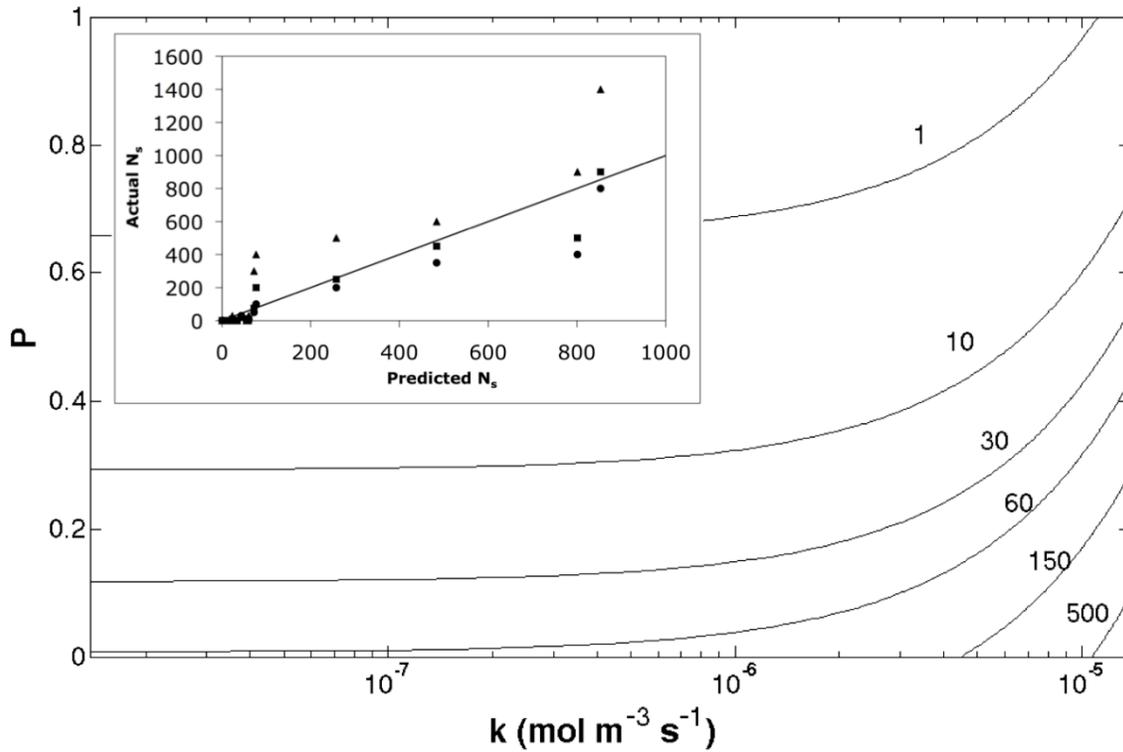


Figure 2.3: Contour plot of N_s as a function of reactivity (k) and P , defined as the ratio of the scale of measurement (L_M) and the typical distance between burrows (L_D) based on sampling a 5 cm below the sediment surface. **Inset:** Actual values of N_s (Circles, squares, and triangles denote the microcosm values for the even, grouped, and cornered distributions, respectively) versus values predicted by equation 6.

CHAPTER 3

**MODELING LUGWORM IRRIGATION BEHAVIOR EFFECTS ON SEDIMENT NITROGEN
CYCLING²**

² Thomas Michael Dornhoffer, George Waldbusser, and Christof Meile. 2015. *Marine Ecology Progress Series*. 534: 121 – 134. Reprinted here with permission of the publisher

Abstract

Benthic infauna in marine sediments have well-documented effects on biogeochemical cycling, from individual to ecosystem scales, including stimulation of nitrification and nitrogen removal via denitrification. However, the effects of burrowing depth and irrigation patterns on nitrogen cycling have not been as well described. Here we examine the effects of lugworm behavior on sediment nitrogen cycling using a reaction-transport model parameterized with literature and laboratory data. Feeding pocket depth and pumping characteristics (flow rate and pattern) were varied, and rates of nitrification, denitrification, and benthic exchange fluxes were computed. As expected, more intense burrow irrigation stimulates denitrification and coupled nitrification-denitrification. At high pumping rates and low sediment oxygen consumption rates ($\sim 10^{-6} \text{ mol m}^{-3} \text{ s}^{-1}$), simulation results show a decrease in rates of nitrification and denitrification with decreasing burrow depth due to incomplete consumption of injected oxidants. Model results also suggest that discontinuous irrigation leads to temporal variability in sediment nitrogen cycling, but that the time-averaged rates do not depend on the irrigation pattern. We identify 1) the poorly constrained chemical composition of lumen fluid injected into sediments and 2) the response of microbial activity/distribution to oscillating redox conditions as critical knowledge gaps affecting estimates of sediment N removal.

Introduction

Nitrogen is an essential nutrient in marine systems that can control productivity and – in excess – lead to coastal eutrophication and hypoxia (Diaz & Rosenberg 2008, Pearl & Piehler 2008, Canfield et al. 2010). Eutrophication and the subsequent advent of coastal ocean hypoxia can have severe negative effects on the marine community, with pronounced impacts on benthic macrofaunal diversity and composition (Levin et al. 2009, Zhang et al. 2010) as well as individual behavior (Riedel et al. 2014). These impacts in turn compromise the ecosystem services that the benthos provides, including organic matter recycling and nitrogen removal (Cloern 2001, Diaz & Rosenberg 2008). The loss of these essential ecosystem functions can have wide-ranging ecological consequences, even exacerbating the hypoxia problem by enhancing nitrogen recycling rather than removal (Kemp et al. 2005).

One of the most significant nitrogen sinks in coastal environments is denitrification, whereby nitrate is reduced to molecular nitrogen gas and thus becomes biologically unavailable. Denitrification is inhibited by oxygen, and is typically confined to sediments where the aerobic degradation of organic material exhausts dissolved oxygen. Nitrogen is supplied to the sediment both via transport from the overlying water, and from organic matter degradation, which supplies ammonium that undergoes nitrification to nitrate and subsequently can be consumed through denitrification by coupled nitrification-denitrification (Seitzinger 1988, Galloway et al. 2004). Efficiently coupled nitrification-denitrification is reliant on the close proximity of oxic and anoxic zones in sediments, and so the fate of nitrogen is affected heavily by sediment oxygen distribution (Kristensen et al.

1987), which in many coastal sediments is substantially influenced by macrofauna (e.g. Volkenborn et al. 2012).

Benthic infauna can significantly enhance rates of elemental cycling (Henriksen et al. 1983, Huettel 1990, Aller & Aller 1998, Banta et al. 1999) and alter the distribution and cycling of nitrogen through a variety of means. At the most basic level, the formation of burrow structures enhances benthic-pelagic coupling by creating a larger surface area for diffusive exchange (Aller 2001). Burrowing also accelerates the dispersion of solid particles - including organic matter - in the sediment (Fornes et al. 1999, Berg et al. 2001), and in large-scale *Arenicola* exclusion experiments, Volkenborn et al. (2007a) observed significant changes to both sediment structure and composition as a result of the presence or absence of adult arenicolid polychaetes. These changes directly affect the permeability of sediments, enhancing the transport of solutes caused by advective flow. At the burrow scale, irrigation in permeable sediments results in the injection of oxic seawater into otherwise reducing sediment (D'Andrea et al. 2002, Waldbusser & Marinelli 2006, Volkenborn et al. 2010), which powers a cascade of redox reactions. However, although the individual time-averaged effects of bioirrigation have been extensively studied and modeled, ecosystem function of complex communities (as measured by e.g. remineralization rates) is often poorly estimated by summation of the known individual effects (Waldbusser et al. 2004).

One of the key challenges in predicting the function of complex ecosystems is the delineation of relationships between community characteristics and ecosystem function. Several studies have documented statistically significant relationships between ecosystem function and biogeochemical settings, macrofaunal diversity or density (Emmerson et al. 2001, Marinelli et al. 2003, Waldbusser et al. 2004, Norling et al. 2007, D'Andrea et al. 2009,

Michaud et al. 2009, Waldbusser & Marinelli 2009), but these correlations tend to be unable to entirely predict ecosystem function (Waldbusser et al. 2004, Norling et al. 2007). This may be due in part to variations in organism behavior affecting burrow spacing - which can alter benthic oxygen fluxes (Dornhoffer et al. 2012) and rates of nitrification and denitrification (Gilbert et al. 2003) - or burrowing depth, which could potentially impact oxygen distribution (Michaud et al. 2009) and fluid residence times (e.g. Santos et al. 2012).

Our limited knowledge of the importance of variations in organism behavior is related at least in part to the scarcity of measured reaction rates at the spatial and temporal resolution necessary to capture redox oscillations in the vicinity of burrows (Marinelli & Boudreau 1996, Volkenborn et al. 2010, Volkenborn et al. 2012). Numerical reaction transport models of multiple chemical species can help fill this gap by providing high-resolution calculated concentration fields and reaction rates. In this paper we present a modeling study parameterized with laboratory and literature data to determine the effects of a common lugworm, *Abarenicola pacifica*, on sediment nitrogen cycling. Lugworms (Family Arenicolidae) are a group of polychaete annelid commonly found in sandy coastal areas across a worldwide distribution. These head-down deposit feeders are considered ecosystem engineers because their presence and actions have a formative effect on their ecosystems, influencing the physical, chemical, and ecological makeup of their communities (Volkenborn et al. 2007a, Volkenborn 2007b). Specifically, we investigate 1) to what extent increasing burrow irrigation rates increase rates of nitrogen cycling, 2) the extent to which changes in burrow depth alter the rates of nitrification and denitrification, and 3) the implications of different irrigation patterns, particularly the importance of continuous vs.

discontinuous irrigation. We use the model results to yield insight into the major controls of benthic nitrogen cycling and highlight important areas for future investigation.

Methods

Microcosm Methods

In order to determine the impacts *Abarenicola pacifica* has on solute exchange, five microcosms (15 cm radius by 45 cm deep) were established and filled with homogenized sediments to a depth of 30 cm, which was allowed to settle for 2 weeks. Sediment and organisms were collected from tide-flats in Yaquina Bay, OR, USA, and microcosms were maintained in a flow-through seawater tank at the Hatfield Marine Science Center, Newport, OR. Salinity and temperature of incubation tanks were subjected to variations in source water from Yaquina Bay and were 28-30 and 8-12°C, respectively. After 2 months acclimation, fluxes of oxygen, ammonium and nitrate were measured in closed incubations with magnetic stirrers attached to a fishing wire inside the microcosm to agitate overlying water without creating radial pressure gradients. Incubations were run on December 20 and 30, 2009, and January 6 and 20, 2010. During each incubation, overlying water samples (~5 ml) were taken every 1-1.5 hours until either oxygen levels dropped to 50% of the starting value or 4 samples were taken. In all cases, at least 3 data points were collected, and the change in overlying water concentration was used to compute benthic fluxes. Oxygen samples were measured with an oxygen optode (PSt1, PreSens), and nutrient samples were 0.2 µm filtered then frozen in sterile sample vials. Nutrient samples were analyzed colorimetrically following the protocols of Waldbusser & Marinelli (2006).

Sediment reaction rates were determined at the termination of the microcosm study (Feb 1, 2010). Approximately 2 ml sediment samples were collected at depth intervals of 0-2, 2-5, 5-10, 10-15, 15-20, 20-25 cm and incubated as a slurry with 2 ml of filtered seawater on a shaker table. Oxygen concentrations were measured every 2-3 hours and oxygen consumption rates were computed from the linear decrease in dissolved oxygen over time. O_2 consumption rates were then used to estimate the rate constant for organic matter mineralization (k_{DOM} , see below).

Model Description

A cylindrical model domain with a radius of 10 cm was established, containing a single lugworm injection pocket located on the central symmetry axis. These dimensions were chosen to represent typical organism densities (Volkenborn et al. 2007b). The domain encompasses 2 cm of water overlying 20 cm sediment with a constant porosity of 0.6, and a constant permeability ($k = 1 \times 10^{-12} \text{ m}^2$, after Volkenborn et al. 2010) except for a feeding column of radius 0.025 m located above the spherical injection pocket (Huettel 1990, Retraubun et al. 1996), with a permeability an order of magnitude greater than the surrounding sediment ($k = 1 \times 10^{-11} \text{ m}^2$) as a first-order approximation. The porosity used is slightly higher than the laboratory-measured porosity, which has negligible impacts on volume-integrated reaction rates. The tailshaft was not explicitly modeled because its presence has been shown to have negligible effects on sediment flow fields (Meysman et al. 2006).

In conjunction with an incompressibility condition (Eq. 1c), fluid flow was simulated using the Navier-Stokes equation in the overlying water (Eq. 1a). Flow in the sediment was

modeled using the Stokes-Brinkman equation (Eq. 1b), which neglects the inertial term in the porous medium but accounts for the exchange of stress between the fluid and the sediment matrix (Bars & Worster 2006):

$$\rho \frac{\partial u}{\partial t} + \rho(u \cdot \nabla)u = \nabla \cdot [-pI + \mu_{eff}(\nabla u + (\nabla u)^T)] \quad (1a)$$

$$\frac{\rho}{\phi} \frac{\partial u}{\partial t} = \nabla \cdot \left[-pI + \frac{\mu}{\phi}(\nabla u + (\nabla u)^T) - \frac{2\mu}{3\phi}(\nabla \cdot u)I \right] - \left(\frac{\mu}{k} \right) u \quad (1b)$$

$$\rho \nabla \cdot u = 0 \quad (1c)$$

where ρ is the fluid density, u is the flow velocity (in the sediment, eq. 1b, this is the Darcy velocity), p is the pressure, I is the identity tensor, k is the permeability, ϕ is the porosity, μ is the dynamic viscosity of 0.001 Pa-s, and μ_{eff} is the depth-dependent effective dynamic viscosity term in the overlying water composed of the dynamic viscosity plus the eddy viscosity $E(z)$, as determined by the Reichardt equation (Eq. 2), times the fluid density:

$$E(z) = \kappa z u_* \left[1 - \left(11 / \left(\frac{\rho z u_*}{\mu} \right) \right) \tanh \left(\frac{\rho z u_*}{11 \mu} \right) \right] \quad (2)$$

where κ is the Karman constant of 0.4, z is the height above the sediment-water interface in meters and u_* is the shear velocity, set to 0.1 cm s⁻¹ (Boudreau 2001). The bottom and side of the cylindrical domain were set as no flow boundaries, while at the upper boundary, atmospheric pressure was imposed, allowing for the flow of water. The boundaries of the feeding pocket served as the porewater injection site, with a pumping rate imposed (as an input velocity for a given feeding pocket size). In simulations with discontinuous irrigation, a 5-minute period of pumping at a constant rate followed by 5 minutes of resting was imposed, following the general pumping pattern reported by Volkenborn et al. (2010). In the case of continuous irrigation, the input velocity was halved relative to that in the

corresponding discontinuously irrigated model, so that the time-integrated flow rate was the same.

The distributions of nine dissolved chemicals were computed as

$$\phi \frac{\partial C}{\partial t} = \nabla \cdot (D\phi\nabla C) - \nabla \cdot (uC) + R \quad (3)$$

where D is the effective diffusion coefficient, C is the concentration, and R is the net reaction rate, reflecting the reactions listed in Table 3.1 within the sediment and set to 0 in the overlying water. In the sediment, the effective diffusion coefficient for solutes reflected molecular diffusion corrected for tortuosity ($D = D_{mol}/(1 - \ln(\phi^2))$), while in the overlying water, the effective diffusion coefficient also accounted for eddy diffusion, $D = D_{mol} + E(z)$, using the Reichardt equation (Eq. 2) in order to simulate well-mixed overlying water above the diffusive boundary layer. This approach was necessary because advective flow induced by infauna can influence solute concentrations at the SWI (Volkenborn et al. 2010), and therefore the concentrations must be imposed sufficiently high above the SWI to avoid interference with this effect. A reaction network describing the breakdown of organic matter was implemented, consisting of 9 reactions (Table 3.1), following the general approach of Van Cappellen & Wang (1996) and Porubsky et al. (2011). Nitrate is removed via denitrification; not included are dissimilatory nitrate reduction to ammonium (DNRA) and anaerobic ammonium oxidation (anammox). DNRA has been shown to be responsible for a large portion of nitrate reduction (Christensen et al. 2000, An et al. 2002, Gardner et al. 2006, Lam et al. 2011), but typically is most prevalent under sulfidic conditions (Koop-Jakobsen et al. 2010) that are not present in the irrigated scenarios considered here. Anammox tends to be important under anoxic conditions with relatively low sediment organic material loading (Engstrom et al. 2005), but in shallow water coastal

environments with plentiful organic matter such as those modeled here, it generally accounts for a small percentage of total N_2 production (Thamdrup & Dalsgaard 2002, Dalsgaard et al. 2005, Engstrom et al. 2005, Thamdrup 2012). Finally, assimilation of nitrogen into new biomass could account for a significant portion of remineralized nitrogen sequestration (Sundbaeck et al. 2004). However, this amount is poorly constrained, and so was not included in the model formulation.

Rate constants for sediment reactions and their sources are presented in Table 3.2. Most were obtained from the literature, though the rate constant of the organic matter degradation k_{DOM} was based on incubation experiments in which the consumption of O_2 was observed over time. Using an assumed reactive dissolved organic carbon (DOM) porewater concentration of $115 \mu M$, in line with measured total porewater DOC (Alperin et al. 1999), the average oxygen consumption rate (R) measured in slurries was used to approximate k_{DOM} , such that $k_{DOM} = R/DOC$ and $k_{POM} = R/POM$ (POM determined from loss on ignition of sediment). This assumes that DOM consumption and production from POM are balanced during the slurry incubation. Although we cannot entirely rule out oxygen consumption by reoxidation of metabolites, we assume that oxygen consumption directly reflects OM degradation because lugworm irrigation leads to depletion of reduced metabolites (e.g. Volkenborn et al. 2007a).

For solutes, domain sides and bottom were impermeable, and a fixed concentration was imposed at the top and at the injection pocket based on laboratory data; reduced substances were assumed to have a concentration of 0 at the upper boundary and in the burrow lumen. The model was implemented in COMSOL Multiphysics 4.4. The 2D axisymmetric domain was discretized into approximately 50,000 finite elements. Models

were run to steady state using generalized-alpha time-stepping (Chung et al. 1993); in the case of discontinuous models a maximum time step of 50 s was imposed in order to properly capture temporal variability over a pumping cycle.

To document the effects of burrow depth, feeding pockets were established in the models at 5, 10 or 15 cm depth. Additionally, in order to examine the importance of environmental context, the rate constants of organic matter mineralization and nitrification, and the concentrations of NO_3 and DOM in the injected porewater were varied.

Results

Microcosm

Ventilation of burrows by *A. pacifica* led to the formation of deep oxic pockets in otherwise anoxic sediments. The typical O_2 penetration depth (as indicated by the redox color discontinuity) was 1 – 2 mm below the sediment-water interface, except around the feeding pocket, where sediments were oxidized more uniformly in the microcosm around the feeding pocket. O_2 consumption rates measured in slurry incubations of microcosm sediment were 1.17 ± 0.49 (1 SD) $\mu\text{mol m}^{-3} \text{s}^{-1}$, giving values of $1.017 \times 10^{-5} \text{s}^{-1}$ and $7.9 \times 10^{-9} \text{s}^{-1}$ for k_{DOM} and k_{POM} , respectively (using a POM concentration of 150 mol m^{-3} based on loss on ignition). Nitrate and oxygen fluxes measured in the microcosm experiments were on the order of 1 and $50 \text{ mmol m}^{-2} \text{d}^{-1}$, respectively (Table 3.3).

Model simulations

Pressure imposed by lugworm irrigation leads to significant fluid flow in the sediment, with majority of the velocity being in the vertical (z) direction, particularly in the

feeding column. There is also significant downward and horizontal velocity in the immediate vicinity of the feeding pocket, but these components of the velocity decrease quickly with increasing distance from the feeding pocket (for a visualization of such flow fields see Meysman et al. 2005). The injection of oxic overlying water into otherwise anoxic sediment porewater results in the formation of a volume of oxygenated sediment around the feeding pocket. Due to the higher permeability of the feeding column, the simulations show sediments directly above the feeding pocket that are more oxidized than the surrounding sediment (Fig. 3.1A). The upward advection caused oxygen penetration depths at the sediment-water interface (SWI) to be very small (less than 1 mm) and when high advection velocities are imposed, the oxic porewater from the burrow is ejected from the sediment to the overlying water. When oxygen is consumed rapidly relative to advection, lugworm-induced advective flow leads to the ejection of anoxic porewater (cf. results in Volkenborn et al. 2010). The input of nitrate at the injection pocket also stimulates denitrification, which extends from areas with low oxygen levels out into the anoxic zone (Fig. 3.1B).

Effects of burrowing and irrigation behavior

The model simulations show a clear impact of the amount of fluid pumped on depth-integrated rates of denitrification (Fig. 3.2). Higher flow rates, typically corresponding to larger organisms, lead to increased areal rates of denitrification; additionally, a decrease in the flow rate through the sediment diminishes the effect of feeding pocket depth on denitrification, especially in the case of slow rates of organic matter oxidation ($k_{DOM} = 1 \times 10^{-5} \text{ s}^{-1}$). In the case of environments with faster rates of organic matter oxidation, all

nitrate injected into the sediment is consumed, so that denitrification increases linearly above rates of 1 mL min^{-1} up to the maximum irrigation rates reported for arenicolid polychaetes. Irrigation intensity also tends to increase rates of nitrification (Fig. 3.3), but because this coincides with a large increase in nitrate supply through the injection of burrow water, an increase in irrigation intensity lowers the proportion of denitrification coupled to nitrification (Fig. 3.4). When ammonium produced by actively respiring and excreting organisms is present in the burrow water (Kristensen et al. 1991), the initial dip in nitrification with increased pumping (Fig. 3.3) is not seen, as the oxidation of injected ammonium offsets the decrease in nitrification due to flushing of surficial sediments. Irrigation also alters the partitioning of oxygen consumption: increasing irrigation rates lead to a decrease in the overall proportion of oxygen consumed in oxidation of reduced metabolites as opposed to aerobic respiration, though there is a stimulation of the absolute rates of secondary metabolite oxidation. In the absence of irrigation ($Q = 0 \text{ mL min}^{-1}$) oxidation of secondary metabolites can account for more than 50% of oxygen consumption. This percentage drops very quickly with increasing irrigation to $\sim 20\%$ at $Q = 0.6 \text{ mL min}^{-1}$ and $\sim 11\%$ at $Q = 1.8 \text{ mL min}^{-1}$ in our simulations. This is because irrigation introduces reactive DOM in addition to oxygen, and the precise partitioning of oxygen consumption is dependent on the ratio of oxygen to carbon in the burrow lumen fluid, even though the overall pattern of partitioning remains the same.

In addition to showing strong effects of irrigation intensity, reactive transport modeling reveals a substantial impact of burrow depth on sediment nitrogen cycling (Fig. 3.2). The effect of burrow depth depends on sediment organic matter reactivity, with faster organic matter breakdown diminishing the impact of feeding pocket depth on sediment

denitrification. At low to intermediate oxygen consumption, indicative of sediments with relatively low organic matter content, deeper burrows lead to a higher predicted nitrification (and by extension, coupled denitrification) when compared to shallow burrows. However, faster rates of OM degradation lead to complete consumption of injected solutes regardless of burrow location, negating the effects of burrow depth seen in less reactive sediments (Figs. 3.2 and 3.3).

Importance of continuous versus discontinuous burrow irrigation

In simulations with slow organic matter mineralization, $k_{DOM} = 10^{-5} \text{ s}^{-1}$, the volume of oxygenated sediment - here defined as $[\text{O}_2] > 10 \text{ } \mu\text{M}$ - is near-constant regardless of irrigation type (ranging from 244 cm^3 to 152 cm^3 , depending on burrow depth), as the drawdown of oxygen is very slow relative to the time scale of pumping. However, faster rates of organic matter decomposition lead to pronounced oscillations of the oxygenated sediment volume. When the DOM oxidation rate constant is increased to 10^{-3} s^{-1} , the volume of sediment subject to oxidic oscillation (defined as sediment that experiences $[\text{O}_2]$ greater and smaller than $10 \text{ } \mu\text{M}$ within a flushing cycle) is roughly 30 cm^3 , which represents approximately 30% of the maximum oxidic volume observed over a pumping cycle for the higher reactivity setting. This oxidic oscillation due to intermittent pumping leads to temporally variable rates of nitrogen cycling and transport; however, the modeled time-integrated rates of nitrogen cycling for constant and intermittent pumping are indistinguishable under the conditions simulated, due to the complete consumption of injected solutes over the period of a pumping cycle.

Uncertainties in model formulation

Increasing the nitrification rate constant from $k_{NH_4} \sim 5 \mu\text{M}^{-1} \text{yr}^{-1}$ to $\sim 500 \mu\text{M}^{-1} \text{yr}^{-1}$ (after the sensitivity analysis in Na et al. 2008) results in a roughly three-fold intensification in the areal rates of nitrification (from 0.2 to 0.62 mmol N m⁻² d⁻¹) in models with high organic matter loading, and up to a five-fold increase in models with low OM (from 0.31 to 1.52 mmol m⁻² d⁻¹). The enhanced nitrification in turn stimulates denitrification, from 1.6 to 2.0 mmol N m⁻² d⁻¹ in models with high organic matter reactivity.

The depth distribution of particulate organic matter (POM) has a small effect on sediment nitrogen cycling, on the order of a 5% reduction in sediment denitrification when POM is uniform across depth as opposed to an exponential decay with increasing depth in the sediment: 1.65 mmol m⁻² d⁻¹ denitrification vs. 1.75 mmol m⁻² d⁻¹ for an irrigation rate of 1.5 mL min⁻¹. Uniform distribution of reactive POM, representative of heavily bioturbated sediments, increases the availability of POM at depth. However, this decreases the depth-integrated rates of both nitrification and denitrification because more O₂ is used for aerobic respiration rather than nitrification that fuels coupled denitrification.

Discussion

Model Validation

Model results show that pumping by a lugworm leads to distinct spatial structuring of concentration distributions, with injected solutes concentrated around a central feeding pocket (Fig. 3.1A). Calculated oxygen penetration depths at both the sediment-water interface and feeding pocket agree well with penetration depths measured using planar optodes in laboratory aquaria containing *Arenicola marina* – a species with similar

behavior and irrigation patterns as *A. pacifica* (Woodin & Wethey, 2009) - and homogenized organic matter-rich (organic content = 1.5%) sediment (Timmermann et al. 2006, Volkenborn et al. 2010). Additionally, the computed horizontal pressure decay of $p(r) \sim r^{-1.12}$ for a single organism in an infinite domain is similar to the peak pressure decay observed in the field following a defecation event (Wethey et al. 2008), indicating that the modeled physics are consistent with observations.

In the simulations using the laboratory-derived organic matter degradation constant ($k_{DOM} = 10^{-5} \text{ s}^{-1}$, based on sediment slurry incubations), advective transport is fast relative to the rate of oxygen and nitrate consumption, which leads to ejection of O_2 -containing porewater from the sediment into the overlying water. However, faster rates of organic matter oxidation result in the ejection of net reduced porewater; the modeled ejection of porewater at the SWI compares favorably to oxygen optode data from Volkenborn et al. (2010) that also show ejection of anoxic porewater into the overlying water during phases of burrow irrigation (average irrigation rate = $0.2 - 2.6 \text{ mL min}^{-1}$). This flushing of porewater could serve as an important source of reduced substances - especially ammonium - for the overlying water and autotrophic communities at the sediment surface (Marinelli 1992).

Modeled nitrate fluxes are in good agreement with the microcosm data, though the model consistently underestimates total oxygen consumption and ammonium efflux compared to the microcosms (Table 3.3). Modeled fluxes also compare well with a study by Na et al. (2008), who reported ammonium fluxes out of the sediment of 1.1 ± 3.2 to $4.7 \pm 7.9 \text{ mmol m}^{-2} \text{ d}^{-1}$ for acclimated *Arenicola marina* and mechanical mimics, respectively. Our modeled nitrate uptake also falls within their measured range (3.9 ± 4.6 and -3.2 ± 4.0

mmol m⁻² d⁻¹ for live worms and mimics, respectively). The mismatch between our measured and modeled oxygen and ammonium fluxes likely reflects processes at the sediment-water interface that are not fully reflected in the model. CT scans of the laboratory microcosms revealed a significant population of small meiofauna in the upper layers of the cores, which was not replicated in the model simulations. Consumption of oxygen and secretion of ammonium by meiofauna, as well as the bioirrigation caused by their shallow burrowing in the topmost sediment layer, can increase both the benthic oxygen uptake and efflux of ammonium. When these potential faunal effects are included as an enhanced diffusion coefficient ($D_{enhanced} = D \times 10$) and elevated aerobic respiration in the surface 1 cm layer (a ten-fold increase reflecting meiofauna respiration that was not captured in our slurry incubation), models predict higher oxygen consumption, ~30 mmol m⁻² d⁻¹. Notably, because nitrate penetration depths exceed the depth of the zone inhabited by the meiofauna in the microcosms, the enhanced transport in the upper layer of sediment has relatively small impacts on the modeled nitrate supply, leading to a closer match between modeled and measured nitrate fluxes (Table 3.3).

Energetics of pumping

Although models are able to accurately recreate observed pressure signals with single organisms, pressure fields computed in multi-organism settings at a burrow density of 10 ind. m⁻² exhibit a significantly smaller horizontal decay of the pressure signal ($p(r) \sim r^{-0.4}$) than burrows without nearby neighbors ($p(r) \sim r^{-1.12}$), due to the influence of nearby pumping organisms. Despite this impact on pressure fields, the pumping power equation from Riisgard et al. (1996) – $P_p(Q) = \rho * g * \Delta H_p(Q) * Q$, where P_p is the energetic cost of

pumping in Watts, Q is the volumetric flow rate, and $\Delta H_p(Q)$ is the sum of the pressure over the pump system and the imposed back pressure – shows that these variations result in minimal energetic costs to arenicolids (less than 1% of the total metabolism of a typical arenicolid; Riisgard et al. (1996)). The minimal metabolic effect of interacting pressure fields suggests that interactions between neighboring arenicolids are mediated by factors other than the energetic costs directly associated with burrow irrigation, such as increased food availability from increased downward surface deposit movement or localized depletion of food deposits due to the competitive feeding of multiple arenicolids (Longbottom 1970, Boldina & Beninger 2014).

The impacts of burrowing and irrigation behavior on nitrogen cycling

Burrowing depth and volumetric irrigation rate had major impacts on calculated nitrogen cycling (Figs. 3.2 and 3.3). Increases in the irrigation rate Q enhance denitrification (Fig. 3.2), especially in the case of deeper burrows; shallow burrows can offset the effects of increasing flow rate due to the ejection of reactive solutes. The impacts of irrigation intensity are also dependent on environmental context: at higher rates of organic matter breakdown (Fig. 3.2, diamonds, representing all simulated burrow depths between 5 and 15 cm), the increase in denitrification is linear above moderate flushing rates (1 mL min^{-1}) regardless of burrow depth. This linear relationship between denitrification and Q is because in highly reactive sediments with excess labile organic matter, denitrification is limited by the supply of nitrate from the overlying water (see e.g. Seitzinger et al. 2006). Furthermore, in highly reactive sediments, our model shows that nitrification tends to decrease with irrigation (Fig. 3.3), as oxygen is preferentially

consumed via aerobic respiration of the injected DOM, rather than being used in nitrification, which is kinetically slower than aerobic respiration.

Burrow irrigation leads to a shift from SWI-dominated to deep feeding pocket-dominated denitrification. When Q is low, diffusive transport across the SWI is the dominant process supplying nitrate compared to advective supply from the feeding pocket, so the steeper concentration gradient in highly reactive sediments supports higher rates of denitrification relative to sediments with lower organic matter loading. Thus in poorly-irrigated, SWI-dominated sediments, organic matter availability and reactivity is a primary control on nitrogen cycling (Fig. 3.2), which agrees with previous empirical and modeling studies of denitrification (Berg et al. 2003, Lee et al. 2006). After an initial decline in nitrification related to flushing of surficial ammonium, both nitrification and denitrification increase with irrigation rates (Figs. 3.2 and 3.3). This represents a switch from a diffusion-dominated environment, where the SWI accounts for 75% of total denitrification, to one dominated by advective supply and nitrogen cycling associated with the feeding pocket. In the latter case, consumption associated with the feeding pocket accounts for more than 90% of denitrification at high irrigation rates. This is because irrigation introduces not only nitrate, but also oxygen and dissolved organic matter (Gardner et al. 1993), which is broken down into ammonium that supplies nitrification. Our modeled effects of irrigation intensity agree well with findings by Na et al. (2008) that similarly show an increase in both N_2 production as a function of Q , and a higher rate of nitrification in intensely irrigated microcosms relative to controls.

The model results suggest that system responses to changes in burrow depth are governed by changes in the time scales of competing processes within the domain,

especially the residence time of the injected fluid (Fig. 3.5). In cases with rapid consumption of organic matter and corresponding complete consumption of oxygen and nitrate, feeding pocket depth has minimal effect on nitrogen cycling due to the complete consumption of nitrate before ejection across the SWI is possible. However, cases with incomplete consumption of nitrogen due to lower sediment reactivity lead to pronounced decreases in sediment denitrification for shallow relative to deep burrows (compare 5, 10, and 15cm depths in Fig. 2; dashed lines in Fig. 3.5). When nitrate produced through nitrification - as opposed to injected nitrate - is the dominant source for denitrification, feeding pocket depth is unimportant (Figs. 3.3 and 3.4 at low values of Q), because nitrogen produced *in situ* is rapidly consumed in denitrification regardless of burrow location.

Our results, particularly uncertainties in the model parameterization, highlight important knowledge gaps. The oxygen to DOM ratio in injected burrow water plays a critical part in determining the redox oscillation of the sediment and in turn the potential for nitrification and denitrification. When levels of oxygen exceed the available reactive reducing equivalent, the sediment around a feeding pocket remains oxidized, and denitrification rates are low due to a lack of reactive organic matter for use in denitrification. Alternatively, injected porewater with a DOM to O_2 ratio greater than 1 (an excess of DOM) creates oxygen distribution patterns that are more in line with observations that show areas of oxidized sediment closely associated with the feeding pocket but extending no more than 2-3 cm into the surrounding sediment (e.g., Volkenborn et al. 2010). Although oxygen levels in the burrow lumen and injected water are fairly well constrained (Volkenborn et al. 2010), there is scant information on the levels of labile organic material or nitrogen compounds in the burrow lumen. The assumed value of 115

$\mu\text{M C}$ in the burrow water leads to modeled oxygen concentrations that closely match measured optode data, but the concentration of DOM nonetheless represents an important area of uncertainty. Our model results show that system responses to irrigation intensity are highly dependent on burrow water composition, so knowledge on the chemical makeup of burrow lumen water, which may differ from the overlying water due to microbial processes associated with the burrow lining, is critical to accurately predict infaunal effects on nitrogen cycling.

Benthic Context and Controls on Nitrogen Cycling

Extrapolating our predicted denitrification rates for individual organisms to the field scale using an organism density of 32 ind. m^{-2} , which is typical for a sand flat dominated by *A. marina* (Volkenborn et al. 2007b), leads to a maximum integrated denitrification potential of $25 \text{ mmol N m}^{-2} \text{ d}^{-1}$. This is very high compared to commonly reported rates of $<1 - 6 \text{ mmol N m}^{-2} \text{ d}^{-1}$ (Seitzinger 1988), suggesting that limits on nitrate supply to the anoxic sediment may prevent this maximum from being reached. This discrepancy may alternatively be due to the fact that some nitrogen flux measurements may not capture the effects of larger infauna due to the use of undisturbed cores for measurements (Cowan & Boynton 1996, Eyre & Ferguson 2002), even though the irrigation rates that are modeled here have pronounced effects on nitrogen cycling. Additionally, if larger infauna such as those modeled here produce relatively low-density burrow openings, their effect may not be captured if the size of the benthic flux chambers is small relative to inter-burrow distance; in this case, chambers with a diameter less than 10 cm would potentially miss burrow openings, especially if burrows are not distributed evenly

(Dornhoffer et al. 2012). Individual variability in irrigation rate and – to a lesser extent – burrow depth may also explain at least in part the large variabilities in maximum denitrification rates that have been documented empirically (Stief 2013), further solidifying the importance of considering infauna when measuring and predicting system-wide rates of nitrogen reduction.

In most coastal environments with high organic matter loading, our results suggest that denitrification is controlled by irrigation intensity, which determines the availability of nitrate. On an areal basis, spatially integrated volumetric rates of biologically driven fluid exchange between sediment and the overlying water reflect both organisms' intrinsic irrigation rate and organism density. Our results suggest that increasing organism density will enhance sediment denitrification rates by increasing the areal irrigation rate. This is a possible mechanism leading both to observed density effects (Marinelli et al. 2003, Waldbusser & Marinelli 2009) and to species-specific effects (Norling et al. 2007), as irrigation behaviors are largely characteristic for given arenicolid species (Riisgard et al. 1996). Furthermore, changes in areal irrigation rate due to a species interaction response can be a potential mechanism underlying the effects of functional diversity on ecosystem function (Waldbusser & Marinelli 2006, Norling et al. 2007, Michaud et al. 2009).

Irrigation intensity and burrowing depth are commonly related to organism size and thus age (Riisgard et al. 1996) suggesting that aging and subsequent changes in irrigation behavior can increase sediment denitrification substantially, up to 4 – 5 fold over non-irrigated sediments (Fig. 3.2). Our findings on the importance of burrow irrigation suggest that an environment being initially recolonized by infauna after a hypoxic event – represented in our models by an increase in the overall irrigation rate – may initially

experience enhanced ammonium effluxes, followed by increased rates of nitrification (and thus a decreased efflux of ammonium). This change in nitrogen cycling is because opportunistic pioneer organisms both increase in number and increase in age, creating deeper, more intensely irrigated burrows. The enhanced supply of nitrate from the overlying water via irrigation will rapidly increase denitrification, decreasing the proportion of coupled nitrification-denitrification (Fig. 3.4). This is precisely the pattern seen by Bartoli et al. (2000) in a microcosm simulation of the initial stages of such a recolonization event.

The importance of discontinuous irrigation

When sediment reactivity is low, the slow consumption of oxygen relative to the frequency of irrigation periods leads to minimal oscillation in oxic volume. However, as the reactivity of organic matter is increased, model simulations exhibit pronounced redox oscillation in sediment surrounding the feeding pocket (not shown), consistent with the analysis of Volkenborn et al. (2012) and the oxygen dynamics caused by the pumping of *Arenicola marina* documented in Volkenborn et al. (2010). Our results expand on these findings and show that the redox oscillation unsurprisingly leads to oscillations in nitrogen fluxes and instantaneous nitrification and denitrification rates, reflecting the redox-sensitive nature of these reactions and movement of the oxic-anoxic interfaces.

Despite the observed temporal variability, the time-averaged areal nitrogen cycling rates appear to be largely independent of irrigation pattern. In the model, these rates are independent of irrigation pattern because in the models that produce significant fluctuations in redox conditions ($k = 10^{-3} \text{ s}^{-1}$), all injected nitrate and oxygen is consumed.

This contrasts with findings documenting a distinct change in porewater ammonium concentrations and organic matter degradation rates due to redox oscillation (Aller et al. 1994, Sun et al. 2002), but that effect has been observed as a result of days-scale oscillations, rather than the minute-scale considered here. In our model, the microbial community is assumed to be at a steady state in terms of size and composition, and rate constants used to parameterize the reaction network reflect a (uniform) reaction potential, wherein process rates are modulated only by the distribution of substrates. However, redox oscillation likely causes shifts in microbial community composition and activity that are not considered in our approach. It is also possible that environmental conditions at the minute scale are too fast to elicit a strong microbial response, such that high frequency redox oscillations cease to be an important consideration. We are not aware of published data that addresses this potentially important issue impacting benthic nitrogen fluxes.

Conclusions

Variations in the feeding pocket depth and burrow irrigation rate of lugworms can have substantial effects on nitrogen cycling in the vicinity of individual burrows. Increasing the amount of nitrate provided through irrigation activity stimulates denitrification, yet shallow feeding pockets can lead to ejection of burrow lumen fluid out of the sediment before consumption of reactive solutes is complete. Although discontinuous irrigation leads to distinct temporal variability within the sediment, the time-averaged rates of nitrogen cycling differ minimally from a case of continuous irrigation. However, this model finding does not take into consideration the response of the microbial community to redox oscillations, a feedback which could account for changes in nitrogen cycling rates that have

been observed in empirical studies at the individual scale. Finally, our results suggest that variations in organism behavior such as burrow irrigation are an additional aspect of the benthic community that must be taken into account to predict ecosystem function, as well as possibly being one mechanism to explain the observed relationships between the infaunal community and system function.

Acknowledgements

This publication was supported by the National Science Foundation (OCE 0751882 to C.M. and OCE 1014226 to G.G.W.). Additional support was received from the Gulf of Mexico Research Initiative Ecological Impacts of Oil and Gas Inputs to the Gulf to C.M. This is ECOGIG contribution no. 358 and the benthic flux data reflect GRIIDC accession number R4.x268.183:0001.

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Table 3.1: Reactions and rate laws; subscripts x and y describe the composition of the organic matter and are set to 106 and 16, respectively.

Reaction	Equation	Expression
Aerobic DOM degradation (R1)	$(\text{CH}_2\text{O})_x(\text{NH}_3)_y + x\text{O}_2 + y\text{H}^+ \rightarrow x\text{CO}_2 + y\text{NH}_4^+ + x\text{H}_2\text{O}$	$k_{\text{DOM}} * \text{DOM} * \text{O}_2 / (\text{O}_2 + K_{\text{mO}_2})$
Denitrification (R2)	$(\text{CH}_2\text{O})_x(\text{NH}_3)_y + \frac{4x}{5}(\text{NO}_3^-) + (\frac{4x}{5} + y)\text{H}^+ \rightarrow x\text{CO}_2 + y\text{NH}_4^+ + \frac{2x}{5}\text{N}_2 + \frac{7x}{5}\text{H}_2\text{O}$	$(k_{\text{DOM}} * \text{DOM} - \text{R1}) * \text{NO}_3^- / (\text{NO}_3^- + K_{\text{mNO}_3})$
Iron Reduction (R3)	$(\text{CH}_2\text{O})_x(\text{NH}_3)_y + 4x\text{Fe}(\text{OH})_3 + (8x+y)\text{H}^+ \rightarrow x\text{CO}_2 + y\text{NH}_4^+ + 4x\text{Fe}^{+2} + 11x\text{H}_2\text{O}$	$((k_{\text{DOM}} * \text{DOM} - \text{R1} - \text{R2}) * \text{Fe}(\text{OH})_3) / (\text{Fe}(\text{OH})_3 + K_{\text{mFe}(\text{OH})_3})$
Sulfate Reduction	$(\text{CH}_2\text{O})_x(\text{NH}_3)_y + \frac{x}{2}\text{SO}_4^{2-} + (\frac{x}{2} + y)\text{H}^+ \rightarrow x\text{CO}_2 + y\text{NH}_4^+ + \frac{x}{2}\text{HS}^- + x\text{H}_2\text{O}$	$(k_{\text{DOM}} * \text{DOM} - \text{R1} - \text{R2} - \text{R3}) * \text{SO}_4^{2-} / (\text{SO}_4^{2-} + K_{\text{mSO}_4})$
Nitrification	$\text{NH}_4^+ + 2\text{O}_2 \rightarrow \text{NO}_3^- + 2\text{H}^+ + \text{H}_2\text{O}$	$k_{\text{NH}_4} * \text{NH}_4^+ * \text{O}_2$
Sulfide Oxidation	$\text{H}_2\text{S} + 2\text{O}_2 \rightarrow \text{SO}_4^{2-} + 2\text{H}^+$	$k_{\text{HS}} * \text{HS}^- * \text{O}_2$
Iron Oxidation	$\text{Fe}^{2+} + \frac{1}{4}\text{O}_2 + \frac{5}{2}\text{H}_2\text{O} \rightarrow \text{Fe}(\text{OH})_3 + 2\text{H}^+$	$k_{\text{Fe}} * \text{Fe}^{2+} * \text{O}_2$
Iron Precipitation	$\text{Fe}^{2+} + \text{HS}^- \rightarrow \text{FeS} + \text{H}^+$	$k_{\text{precip}} * (\text{Fe}^{2+} * \text{HS}^- / (K_{\text{FeS}} * \text{H}^+) - 1)$
POM degradation	$\text{POM} \rightarrow \text{DOM}$	$k_{\text{POM}} * \text{POM}$

Table 3.2: Parameters used in the reactive transport model and their respective sources.

Parameter	Description	Value	Units	Source
O_{2o}	Oxygen in the overlying water	0.22	mol m ⁻³	This Study
O_{2inj}	Oxygen injected across the feeding pocket	0.088	mol m ⁻³	Volkenborn et al. 2010
NO_{3o}	Nitrate in the overlying water	0 - 0.02	mol m ⁻³	This Study
DOM_{inj}	DOM injected across feeding pocket	0 - 0.115	mol m ⁻³	Alperin et al. 1999
k_{DOM}	Rate constant for DOM degradation	1 x 10 ⁻⁵ - 1 x 10 ⁻³	s ⁻¹	This Study
k_{POM}	Rate constant for POM degradation to DOM	1 x 10 ⁻⁸	s ⁻¹	This Study
k_{NH4}	Rate constant for nitrification	1.59 x 10 ⁻⁴ - 1.59 x 10 ⁻²	(mol m ⁻³) ⁻¹ s ⁻¹	Na et al. 2008
k_{HS}	Rate constant for sulfide oxidation	5.1 x 10 ⁻⁵	(mol m ⁻³) ⁻¹ s ⁻¹	Van Cappellen and Wang 1996
k_{Fe}	Rate constant for iron oxidation	3.17 x 10 ⁻⁴	(mol m ⁻³) ⁻¹ s ⁻¹	Van Cappellen and Wang 1996
K_{mO₂}	Half-saturation for oxygen	0.02	mol m ⁻³	Van Cappellen and Wang 1996
K_{mNO₃}	Half-saturation for nitrate	0.005	mol m ⁻³	Van Cappellen and Wang 1996
K_{mFe(OH)₃}	Half-saturation for iron oxides*	8.75x 10 ⁻²	mol m ⁻³	Van Cappellen and Wang, 1996
K_{mSO₄²⁻}	Half-saturation for sulfate	0.03	mol m ⁻³	Kuhl and Jorgensen 1992
K_{FeS}	Solubility constant for FeS	10 ^{-2.95}	mol m ⁻³	Van Cappellen and Wang 1996
k_{precip}	Precipitation constant for FeS	1.9 x 10 ⁻⁹	s ⁻¹	Van Cappellen and Wang, 1996

* This half-saturation constant is more than two orders of magnitude lower than the iron oxide concentration estimated for our site, resulting in 0 order kinetics for dissimilatory iron reduction.

Table 3.3: Laboratory and model fluxes of nitrate, ammonium, and oxygen (mean and standard deviation). Positive fluxes are into the sediment.

	Oxygen (mmol m ⁻² d ⁻¹)	Nitrate (mmol m ⁻² d ⁻¹)	Ammonium (mmol m ⁻² d ⁻¹)
Measured*	52.62 ± 3.29	1.46 ± 0.15	-3.40 ± 0.51
Modeled			
<i>Q</i> = 1.6 mL min ⁻¹	16.11	1.45	-1.57
<i>Q</i> = 1.6 mL min ⁻¹ , with meiofauna**	31.26	1.62	-2.60
<i>Q</i> = 1.0 mL min ⁻¹	10.67	0.90	-1.26
<i>Q</i> = 2.0 mL min ⁻¹	19.66	1.77	-1.73

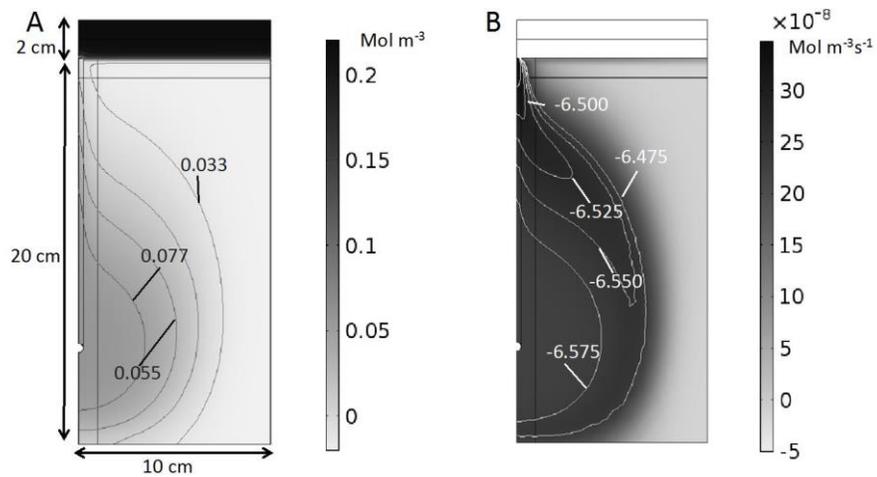


Figure 3.1: Radial cross section of modeled distribution of oxygen concentrations (**A**) and rate of denitrification (**B**) within the sediment around a 15-cm deep feeding pocket in low reactivity sediment ($k_{DOM} = 10^{-5} \text{ s}^{-1}$) and under continuous irrigation. The top 2 cm is the water overlying the sediment, and contour lines are the oxygen concentration (in mol m^{-3}) and the \log_{10} of denitrification rates, respectively.

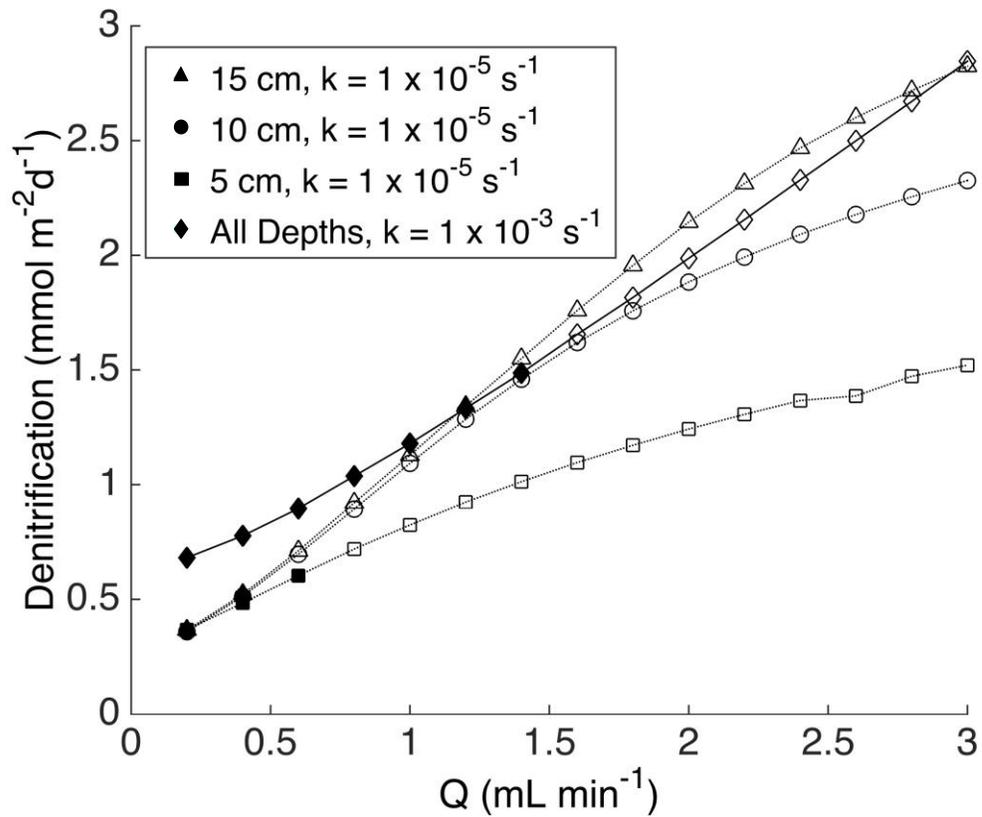


Figure 3.2: Depth-integrated denitrification rates as a function of irrigation rate, Q (continuous irrigation). In models with high organic matter lability ($k_{DOM} = 1 \times 10^{-3} \text{ s}^{-1}$), denitrification is independent of burrow depth, so diamonds with solid lines indicate all burrow depths; Triangles, circles and squares with dashed lines represent feeding pockets at 15, 10 and 5 cm depth, respectively, for an organic matter degradation rate constant value of $k_{DOM} = 1 \times 10^{-5} \text{ s}^{-1}$, where shallow burrows lead to ejection of injected solutes. Filled symbols indicate SWI-dominated systems (the percentage of denitrification in the upper 1 cm > 50% of total denitrification), and open symbols indicate models dominated by the burrow feeding pocket.

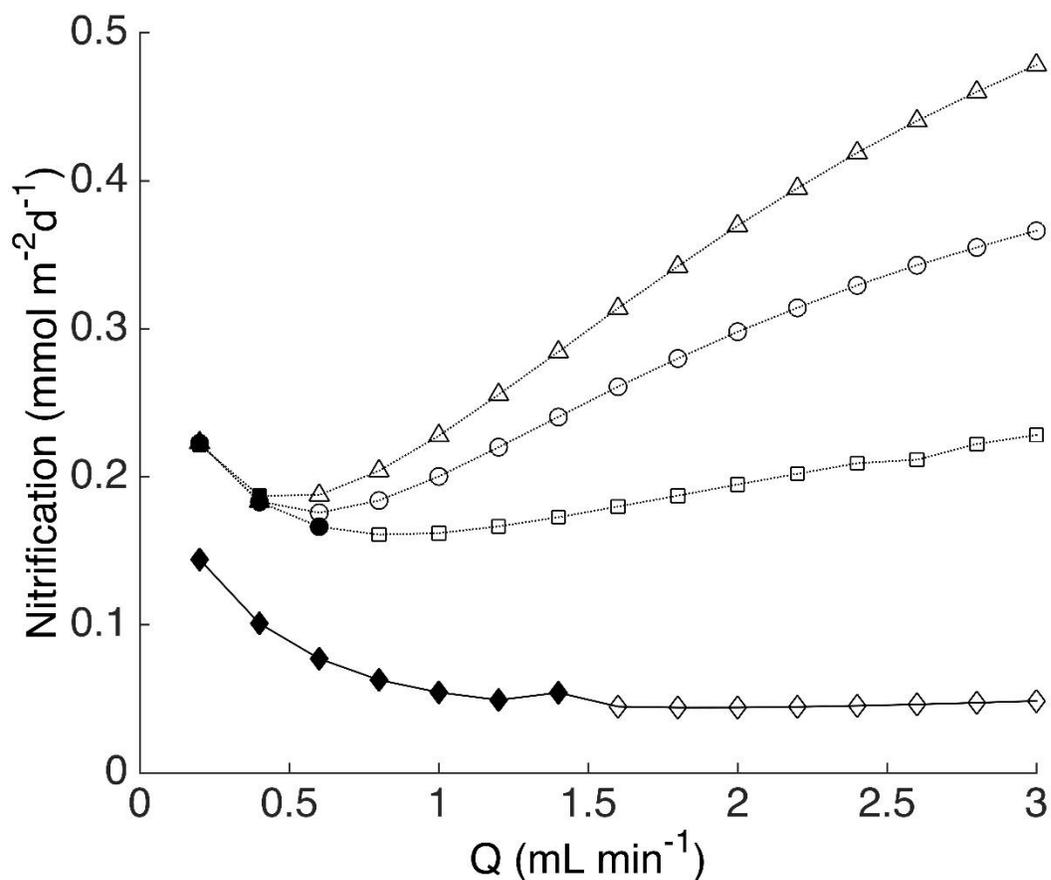


Figure 3.3: Calculated nitrification rates as a function of burrow irrigation intensity.

Dashed lines represent $k_{DOM} = 10^{-5} \text{ s}^{-1}$ and solid lines indicate $k = 10^{-3} \text{ s}^{-1}$. Triangles indicate 15 cm burrow depth, circles indicate 10 cm, and squares indicate 5 cm. Note that diamonds represent all depths for $k_{DOM} = 10^{-3} \text{ s}^{-1}$ because all burrow depths generate identical results. Filled symbols indicate conditions in which surface sediments dominate nitrification (the percentage of nitrification in the top 1 cm > 50% of total nitrification), and open symbols indicate a burrow-dominated environment.

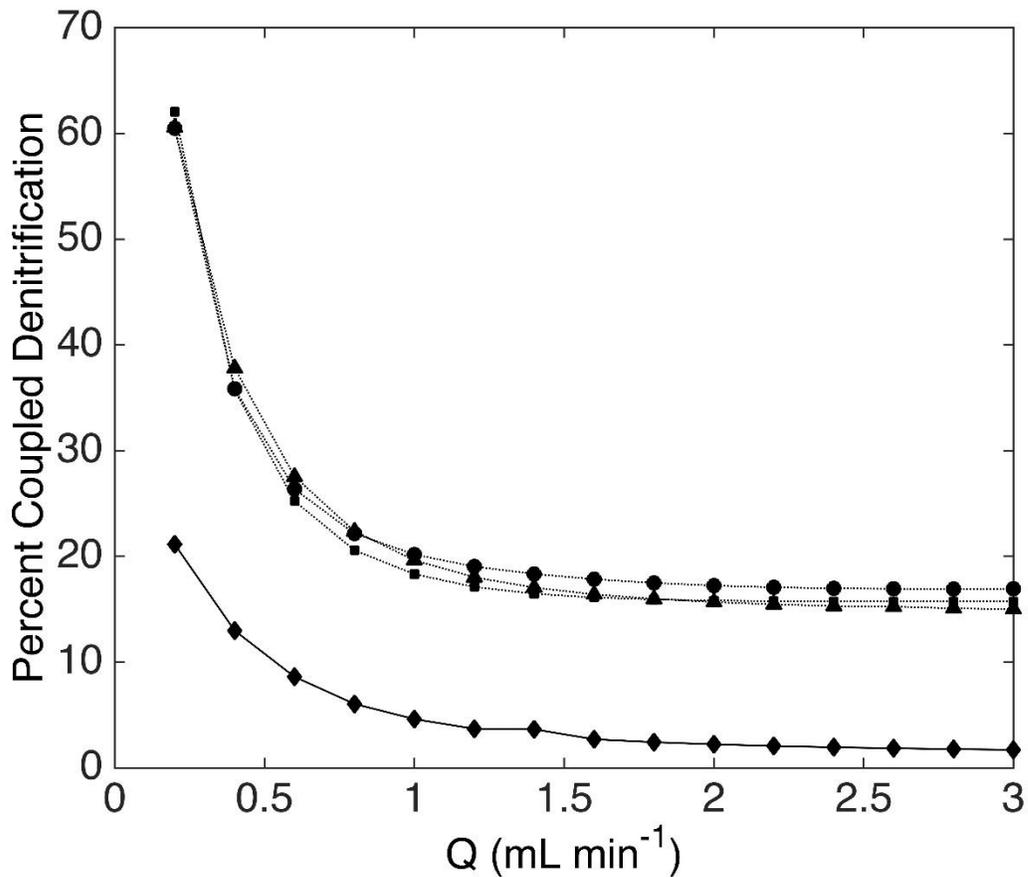


Figure 3.4: Percentage of total denitrification that is supported by coupled nitrification (defined as nitrate produced in nitrification divided by the amount consumed in denitrification) as a function of irrigation intensity Q . Dashed lines indicate sediments with low organic matter reactivity, with squares, circles, and triangles representing 5 cm, 10 cm, and 15 cm deep feeding pockets, respectively. The solid black diamonds indicate all burrow depths in sediments with high organic matter reactivity.

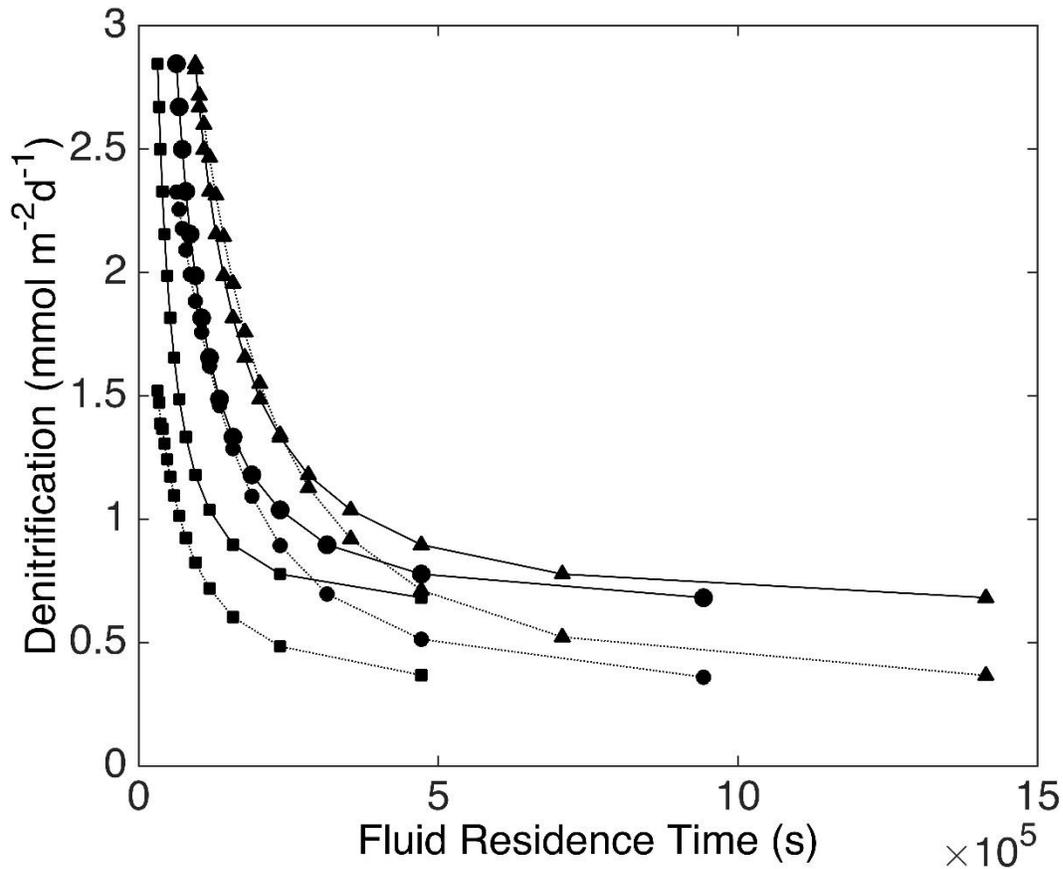


Figure 3.5: Calculated denitrification as a function of transport time (defined as the burrow depth times the microcosm area, divided by the volumetric flow rate). Dashed lines indicate $k_{DOM} = 1 \times 10^{-5} \text{ s}^{-1}$, solid lines indicate $k_{DOM} = 1 \times 10^{-3} \text{ s}^{-1}$, triangles indicate a 15 cm burrow depth, circles indicate 10 cm, and squares represent burrows at 5 cm depth. Note that in low- k_{DOM} sediments, predicted denitrification rates are lower for vigorously irrigated shallow burrows (dashed line with squares) relative to deeper burrows, indicative of ejection of reactive nitrogen into the overlying water.

CHAPTER 4

INFAUNA AFFECT ESTUARINE DENITRIFICATION: INSIGHTS FROM ORGANISMAL AND SYSTEM-WIDE ANALYSIS³

³ Thomas Michael Dornhoffer and Christof Meile, to be submitted to *Frontiers in Ecology and the Environment*.

Abstract

Numerous studies have documented the impacts benthic fauna can have on nitrogen cycling, but extending individual observations to nitrogen transformation at the ecosystem scale remains challenging. We combined estuarine denitrification data with large-scale benthic fauna and water quality databases to shed light on the relationships between the benthic community and system nitrogen removal. This analysis showed a general positive trend between diversity/abundance and denitrification, but this relationship was not significant across all estuaries studied. We then used organism-scale denitrification studies to parameterize a model used to predict estuarine denitrification as a function of nutrient availability and organism abundance. This model provides reasonable estimates of nitrogen removal in 11 out of 12 estuaries studied, emphasizing the importance of benthic fauna for estuarine N removal.

Introduction

Estuaries impact the coastal ocean in ways disproportional to their relatively small area. Estuaries are important environments for the processing of riverine nitrogen loading to the coastal ocean, which has more than doubled since the industrial revolution (Gruber & Galloway 2008), and is projected to continue increasing through the 21st century (Galloway et al. 2004). This dramatic increase is one of the main drivers of a growing eutrophication and hypoxia problem worldwide (Diaz & Rosenberg 2008), and mitigating this problem requires a thorough understanding of the ways nitrogen moves through estuarine systems.

One of the most important processes controlling nitrogen export to the coastal ocean is estuarine denitrification, with areal rates averaging roughly $6,000 \text{ kg N km}^{-2} \text{ yr}^{-1}$ (Seitzinger et al. 2006). Denitrification is regulated by the distribution of oxygen (Kristensen & Blackburn 1987), and so in shallow coastal systems it is typically confined to sediments where the consumption of oxygen leads to the formation of suboxic conditions that are conducive to nitrate reduction. Depth-integrated rates of denitrification are then controlled by the availability of organic matter and the supply of nitrate to suboxic sediment, which is supplied to the benthos from both the overlying water and from nitrification of ammonium produced by organic matter degradation (Seitzinger 1988). In oligotrophic environments, denitrification fueled by nitrification is the dominant sink of nitrogen, but reduction of nitrate supplied from the overlying water becomes more important in eutrophic environments such as many polluted estuaries (Seitzinger et al. 2006). Production of N_2 by anaerobic ammonium oxidation (anammox) is an important process globally, but in shallow, near-shore environments its contribution to N_2 production

tends to be relatively small (Thamdrup & Dalsgaard 2002, Dalsgaard et al. 2005).

Therefore, factors that impact sediment nitrate uptake and production are major controls of estuarine denitrification.

In many coastal environments, the benthic infaunal community substantially influences sediment biogeochemistry, particularly elemental cycling that is sensitive to redox conditions. Benthic fauna can stimulate nitrogen cycling (Aller 1988, Aller 2001, Mermillod-Blondin 2011), as the formation and irrigation of burrows introduces oxidants into otherwise reduced sediments. This not only directly increases the supply of nitrate, but also influences the temporal and spatial distribution of oxygen (Fenchel 1996, Timmerman et al. 2006, Volkenborn et al. 2012), which has cascading effects on sediment nutrient cycling by bringing oxic and anoxic sediments into close proximity (D'Andrea et al. 2002). Despite the rich body of literature documenting the importance of benthic fauna for sediment biogeochemistry at the plot scale, quantifying the effects at the ecosystem scale remains challenging. Extensive studies have provided support for several possible drivers or important benthic community aspects, especially faunal density (Marinelli & Williams 2003, D'Andrea & DeWitt 2009, Braeckman et al. 2010). Faunal diversity (Emmerson et al. 2001, Waldbusser et al. 2004, Michaud et al. 2009), and faunal functional diversity (Norling et al. 2007, Waldbusser & Marinelli 2009, Braeckman et al. 2014) are also significantly related to ecosystem function, though the mechanisms underlying these relationships are less clear. Nevertheless, both organism abundance and community composition are impacted by eutrophication and hypoxia (e.g. Diaz & Rosenberg 1995, Cloern 2001, Conley et al. 2007, Diaz & Rosenberg 2008, Levin et al. 2009), so it is critical to understand how ecosystem function might change as a result of decreases in diversity and abundance.

In this paper, we combine literature data on estuarine denitrification rates with data on estuarine benthic community characteristics and water column nutrient concentrations. We present a model that combines a large-scale data set with detailed organismal and plot-scale observations to shed light on possible system-wide drivers of nitrogen removal and to demonstrate the links between organismal and system-wide scales.

Methods

Data Sets

Data on the benthic community composition, sediment properties and chemical composition, and water quality were obtained from the National Coast Assessment database (NCA, <http://www.epa.gov/emap/nca/index.html>) maintained by the US Environmental Protection Agency (EPA). Data represent a time scale of roughly 15 years, from 1990 to 2005, with the majority of measurements occurring 2000 – 2005 (see Figure A1 for maps of data collection sites). Where available, data for estuaries with sparse data coverage were supplemented with data from the literature (Table 4.1). All water quality and sediment composition data were averaged treating each sample as a discrete data point; benthic community data were analyzed for each sampling grab before being averaged over space and time. Data were collected and analyzed according to established EPA protocols as described in the supporting documentation of the NCA database (<http://www.epa.gov/emap/nca/html/docs/index.html>). In brief, nitrate in the water column was determined using standard colorimetric techniques, and sediment total organic carbon (TOC) was determined with loss-on-ignition. Benthic infauna were sampled

using a Van Veen grab with a typical area of 440 cm². Infaunal species richness (R) was determined as the number of discrete taxa that were identified in the benthic community.

Denitrification rates were obtained from the literature (Table 4.1). Studies were restricted to those that measured denitrification *in situ* or with intact sediment cores, and individually measured denitrification rates were averaged over space and time to provide a single estimate for each system. Data collection sites were evenly spread over the estuaries (Figure A1), so averages were not weighted by area.

Principal component analysis (PCA) was used to identify similar estuaries on the basis of water quality, sediment, and physical characteristics. PCA were performed using NO₃⁻, sediment TOC, and water residence time. This was done in order to reveal what, if any, estuarine characteristics could be used to classify groups of similar systems for further analysis.

Modeling Approach

A model for benthic denitrification was formulated in which N₂ production is limited by the availability of labile organic matter and nitrate. The majority of denitrification in soft-sediment communities is associated with burrow structures in bioturbated sediments (Kristensen et al. 1991, Dornhoffer et al. 2015), stimulated by the enhanced nitrate supply due to bioirrigation. In such settings, which excludes oyster reefs and other epifaunal filter feeders, or sandy sites with wave-driven benthic exchange, we relate the bulk depth-integrated denitrification rate (DNF) to organismal density and substrate availability, reflected in the nitrate concentration in the overlying water and sediment TOC concentration:

$$DNF = \rho \cdot r_{\rho} \cdot \beta_l \cdot TOC \cdot \left(\frac{NO_3}{NO_3 + K_m} \right) \quad (1)$$

where ρ is the total organism density in individuals m^{-2} , r_{ρ} is a per-organism rate of denitrification, β_l is the labile fraction of sediment carbon (assumed to be approximately 10 (5-15) %; Keil et al. 1994, Berg et al. 2003), and K_m is the half-saturation constant of nitrate, set to 10 μM (Wang & Van Cappellen 1996). To establish r_{ρ} , data from individual benthic faunal stimulation of nitrogen cycling was obtained from a comprehensive review by Stief (2013), and the references therein. The experimental microcosm studies used in our analysis were typically amended with fresh organic matter or exposed to freezing-induced cell lysis, so β_l was assumed to be 1 for the laboratory data unless a study used both unamended and unfrozen sediment, in which case a labile portion of 10% was assumed. The per-organism denitrification rate was determined for each organismal study, and averaged to provide a rate constant that could then be applied to system-scale data to predict estuarine denitrification as a function of NO_3^- , TOC, and organism density (Eq. 1).

The model described above does not explicitly include organism functional traits, so an expanded model was established to test the importance of organism traits:

$$DNF = (\sum \rho_i r_i) \cdot \beta_l \cdot TOC \cdot \left(\frac{NO_3}{NO_3 + K_m} \right) \quad (2)$$

where ρ_i and r_i are the density and characteristic stimulation rate respectively of each functional group as determined from the laboratory studies (Table A1, Figure A2); functional designations were assigned at the family level. The lack of biomass data in most systems prevented the inclusion of a biomass term in the models; the potential impact of this exclusion was investigated using a detailed analysis of Chesapeake Bay benthic community data.

Model Application

Equations 1 and 2 were applied in two ways. First, TOC, nitrate, and infaunal abundance were averaged to yield a single value of each for each estuary. Averaging was used because denitrification rate measurement sites and benthic community analysis sites were typically separated by hundreds to thousands of meters, and nitrogen dynamics can vary considerably over such distances (e.g. Hopkinson et al. 1999, Maciolek et al. 2004, Fear et al. 2005).

In addition to computing estuarine denitrification using averaged values for the variables, a bootstrap analysis was performed on the subset of estuaries that were not supplemented with literature data (see Table 4.1), because of the availability in these systems of full data distributions as opposed to single reported values. TOC, organism density, the per-organism rate and nitrate concentrations were assumed to be independent from each other. In each estuary, 100,000 DNF rates were then computed (Eq. 1), using values randomly selected from the measurements of each of the variables. This approach leads to a distribution of DNF rates, providing information on the expected spatial variability and the average DNF rate.

Additionally, two estimates were performed: (1) station matching, wherein only values for variables collected at the same location and time were used to estimate denitrification rates, and (2) an adjusted bootstrapping approach, where a minimum denitrification rate was imposed to reflect nitrogen cycling associated with the sediment-water interface in situations with low organism abundance. This is because conceptually, Eq. 1 implies DNF dominated by irrigation, and does not reflect DNF that takes place in

absence of benthic macrofauna. Thus, a minimum DNF rate was imposed using a value of $0.25 \text{ mmol m}^{-2}\text{d}^{-1}$ times the TOC availability (using the denitrification rate observed with no irrigation by Dornhoffer et al. 2015, Chapter 3, and adjusting for carbon availability in those models).

Statistical Model Approach and Evaluation

To provide a point of comparison for this mechanistic modeling approach, denitrification rates were also modeled using a linear regression based on the base form $DNF = a \cdot X + b$, where a and b are a fitted coefficient and intercept, and the term X is one of the following: 1) the product of the abundance, POC, and nitrate terms in Equation 1, 2) the product of all possible pairs of those terms, or 3) those terms individually. In order to compare results regardless of modeling approach, a goodness of fit index (FI) was

calculated for each model based on the relative residuals: $FI = \frac{1}{n} \sum \frac{|DNF_{predicted} - DNF_{measured}|}{DNF_{measured}}$,

where n is the number of estuaries used in the model.

Results

Estuary Classification

The estuaries selected represent a wide variety of environments, including urbanized harbors (Boston Harbor), large rivers associated with highly urbanized watersheds (e.g. the Potomac and Delaware Rivers), and tidally flushed coastal bays with relatively pristine shorelines (e.g. Plum Island Sound, MA). The data coverage also varies considerably between systems, from extremely well described annual budgets (e.g. Boston

Harbor, Narragansett Bay, and Chesapeake Bay) to relatively sparse coverage in e.g. Little Lagoon, AL.

Principal component analysis revealed three major groups of estuaries (Figure 4.1). Qualitatively, Group I (see Table 4.1) tends to have large amounts of undeveloped shoreline and relatively small watersheds. Group III estuaries are marked by very large watersheds relative to the size of the estuary, which drain either heavily urbanized areas (Scheldt, Potomac, and Delaware Rivers) or mixtures of agricultural and urbanized development (Neuse River).

Benthic Infaunal Community and Water Quality

Major water quality and ecological indicators are summarized in Table 4.1. In general, all measurements varied substantially - both between and within estuaries - with respect to space and time, with coefficients of variation > 1 for TOC and NO_3^- within estuaries. The highest nitrate concentrations were observed in the eutrophic rivers draining large watersheds, with NO_3^- levels exceeding $30 \mu\text{M}$ in all four Group III estuaries, up to a maximum of $200 \mu\text{M}$ in the Scheldt River estuary. The lowest levels were observed in Narragansett Bay ($< 1 \mu\text{M}$), and were below $10 \mu\text{M}$ for all other estuaries. A substantial portion of the observed variability in nitrate concentration within each estuary can be attributed to the transition from riverine to marine concentrations along the salinity gradient of the estuaries (e.g. Hopkinson et al. 1999 and Fear et al. 2005).

Crustaceans and polychaetes made up the vast majority of the benthic community in all but one estuary: Narragansett Bay was dominated largely by bivalve communities, though crustaceans and polychaetes were still major contributors to the Bay's benthic

diversity and abundance (see Table A2). The polychaete families Spionidae and Capitellidae were the most widely distributed, and were among the most dominant groups in all estuaries except Plum Island Sound and Waquoit Bay. Surface deposit feeders were the most abundant, and were present in great numbers in all estuaries. Head-down deposit feeders were also relatively abundant; other main functional groups were present but relatively rare. Narragansett Bay has the highest functional diversity with almost all functional groups represented; other estuaries were typically dominated by 2 – 3 main functional groups (see Table A2).

There were large variabilities in organism abundance, nitrate, and TOC at all scales (Figure A3), with adjacent sites having as much difference between them as sites located across an entire estuary. Organism density, TOC availability, and nitrate concentrations were positively skewed in all estuaries, with relatively few incidents of high nutrient concentrations or very abundant infauna (see Figure A4). There was no clear correlation between these variables when considered sample by sample (Figure A5) or averaged across an entire system (Figure A6).

System-wide Patterns

Increased species richness is generally associated with increased denitrification, but there was no significant relationship between species richness (R) and system wide denitrification across all estuaries considered (Figure 4.2A). However, when the Scheldt River was excluded, there was a moderately strong ($R^2 = 0.41$) and significant relationship between diversity and denitrification ($p = 0.033$). When estuarine groups from the PCA are considered individually, there is a relatively strong relationship between richness and

denitrification in Group I ($R^2 = 0.76$, $p = 0.032$). There is also a relationship between organism density and estuarine denitrification (Figure 4.2B, closed circles). This relationship is moderately strong ($R^2 = 0.54$) and significant when considered across all estuaries ($p = 0.006$).

Multi-scale Linkages and Model Results

At the microcosm scale, the relationship between organism abundance and denitrification in experimental microcosms (Stief 2013 and sources therein) varied considerably (Figure 4.2B, open circles). Accounting for the amount of TOC and nitrate in the literature-reported microcosm experiments (Table A1) and solving Eq. 1 for r_ρ yielded a value of 0.004 ± 0.01 mmol N ind⁻¹ d⁻¹. Using this value and a K_m of 10 μ M, Eq. 1 produced estimates of denitrification rates that were within 30% of the measured rates for 11 of the 12 estuaries when using averaged values for the variables (Figure 4.3A). The model slightly overestimated denitrification in the Scheldt River (17.1 mmol N m⁻² d⁻¹ vs. 14.9 mmol m⁻² d⁻¹) and substantially underestimated denitrification in Little Lagoon, AL (0.02 vs. 1.5 mmol m⁻² d⁻¹). The model explicitly incorporating functional diversity (Eq. 2) provided slightly more accurate denitrification estimates (FI = 0.19 as opposed to FI = 0.3, Figure 4.3A), though this model also underestimated denitrification in Little Lagoon, AL.

Model results were highly sensitive to the choice of modeling approach (Figure 4.3A, Table A4). The general linear model using TOC, nitrate, and infaunal abundance provided reasonably accurate results (FI = 0.61), but the most accurate GLM was the one which consisted solely of infaunal abundance and nitrate concentrations (FI = 0.35), the accuracy of which approached that of the base model with averaged values. General linear models

that did not include infaunal abundance were less accurate, and models that included nitrate availability were generally more accurate than those including TOC availability (see Table A4). Denitrification models incorporating nitrate alone, TOC alone or TOC and nitrate concentrations provided very poor fits to measured denitrification rates. Bootstrap analysis of the subset of seven estuaries yielded results that were highly variable depending on the approach used (Fig. 4.3B), with goodness of fit indices ranging from 0.47 to 0.75, compared to a fit index of 0.19 for the averaged-variable model of the same estuaries (Table A4). Bootstrap models that included consideration of a minimum denitrification rate were most accurate, and the use of simultaneously collected data points provided more accurate predictions than a purely random distribution.

Discussion

Principal component analysis revealed distinct clusters of estuarine systems based on physical, chemical, and sediment characteristics (Figure 4.1). A few systems, such as the Patuxent River, share characteristics between Groups I and III. However, the relationship between diversity and denitrification in Group I (Figure 4.2A) is significant regardless of whether such intermediate systems are assigned to Group I or not. Such categorization is therefore useful for determining major system-wide relationships in different environmental conditions.

Drivers of Nitrogen Removal

Our results show that both benthic community diversity and benthic faunal density are overall loosely correlated with estuarine nitrogen removal (Figure 4.2). Data did not

reveal a significant relationship between TOC or nitrate availability and diversity or abundance when considering either estuarine averages (Figure A6) or individual samples (Figure A5), which suggests that the observed relationships between infaunal density or richness and denitrification is not the result of substrate availability driving both aspects of the ecosystem simultaneously.

Experiments have shown a relationship between organism density and nitrogen cycling (Pelegri et al. 1994, Marinelli & Williams 2003, D'Andrea & DeWitt 2009, Braeckman et al. 2010), and our analysis suggests that this effect is also apparent at the scale of an entire estuary (Figure 4.2B). In general, this stimulation is due to an increase in the supply of oxidants (including oxygen and nitrate) to the sediment, whether by increased surface area for diffusive exchange (Aller 1988, Kristensen et al. 1991, Kristensen 2000) or by pumping of nitrate into the sediment (Meysman et al. 2005, Meysman et al. 2006, Dornhoffer et al. 2015). The importance of this enhanced benthic-pelagic coupling is most pronounced in systems with abundant nitrate ($> 10 \mu\text{M}$) in the overlying water, where direct denitrification of overlying water nitrate is most prominent (Seitzinger et al. 2006).

The observed relationship between infaunal diversity (R) and denitrification (Figure 4.2A) in Group I estuaries is substantially more difficult to explain mechanistically. Many studies have shown an impact of faunal diversity on ecosystem function, but these relationships are not always consistent (Waldbusser et al. 2004, Norling et al. 2007, Michaud et al. 2009); furthermore, even when clear directional relationships are described, the precise mechanisms underlying those relationships are unclear. One possible explanation is that increasing diversity changes oxic volume and distribution as shown in

Michaud et al. (2009), but mechanistically linking experimental diversity-ecosystem function studies to the system scale is problematic due to differences in complexity and environmental conditions, as well as emergent properties that may not manifest at the experimental scale as a result e.g. of complex organism interactions (Snelgrove et al. 2014).

Model Evaluation: Justification and Accuracy

The model used here to predict system-scale denitrification provides reasonable estimates of denitrification in 11 of 12 estuaries (Figure 4.3A). Denitrification is driven by the availability of both organic matter and nitrate, as reflected in the rate expressions used in early diagenetic models (e.g., Wang & Van Cappellen 1996, Berg et al. 2003, Na et al. 2008). Our model builds on these approaches, but because the model is applicable at a much larger scale, the biological transport that drives sediment nitrate uptake is expressed as the product of an intrinsic denitrification rate (r_ρ) and infaunal abundance (ρ). The formulation of denitrification as a sum of such “organismal stimulations” represents conditions in which the majority of nitrogen cycling is associated with burrow structures in bioirrigated sediments (e.g. Kristensen et al. 1991 and Dornhoffer et al. 2015), but to our knowledge this has not been empirically tested at whole-system scales.

The association of a rate of denitrification with infaunal density does not account for functional and size differences, and it relies on a parameter (r_ρ) based on controlled experiments. Yet denitrification measured in laboratory settings can exceed ambient environmental rates substantially. Reported rates as high as 11 - 12 mmol N m⁻² d⁻¹ have been measured in experimental microcosms (Svensson & Leonardson 1996, Nizzoli et al. 2007, Kristensen et al. 2011). By comparison, measured estuary-wide denitrification rates

were typically between 0 – 2 mmol N m⁻² d⁻¹ (Table 4.1). The discrepancy between laboratory and estuarine measured denitrification rates can at least partially be explained by differences between laboratory and natural conditions, including organism behavior and acclimatization. For instance, Na et al. (2008) measured N₂ production rates of 7.7 ± 0.9 mmol N m⁻²d⁻¹ in microcosms with fresh *Arenicola marina*, compared to 4.7 ± 3.4 mmol N m⁻²d⁻¹ in microcosms with acclimated ones, which would result in an overestimation of estuarine denitrification by a factor of 2. Another common difference between the laboratory and estuarine conditions is the availability of substrate. In our model, the common excess of nitrate in microcosms compared to the natural environment was accounted for with the Monod formulation of our model, and differences in carbon availability were reflected in β_i .

The lack of accuracy in Little Lagoon is likely related to the sparse benthic data coverage in this system (Figure A1), which leads to a poor estimate of both the state of the benthic community and nutrient availability across the entire estuary. It is also notable that Little Lagoon is unique among the estuaries studied here in that the nutrient input is dominated by submarine groundwater discharge rather than riverine input or tidal flushing, and denitrification rate measurements were carried out at seepage dominated sites (Bernard et al. 2014).

Model Evaluation: Functional Traits and Biomass

The accuracy of the predictive model using organism abundance as the only infaunal community characteristic is notable given that it does not explicitly include consideration of organism biomass and functional traits. However, the parameter r_ρ does implicitly reflect functional differences because of the inclusion of organisms from multiple functional

groups in the laboratory data set (Table A1, Figure A2). Distinguishing between functional groups in the large scale benthic data sets provided slightly more accurate denitrification estimates (Fig. 4.3A), but this slight increase in accuracy must be weighed against a substantial amount of uncertainty: functional traits for poorly studied organisms were assigned based on family identification, and often had to be deduced from organism appearance. Although biomass can have important impacts on denitrification stimulated by individual organisms, detailed analysis of Chesapeake Bay data revealed that the vast majority of organisms are relatively small, numerous, and individually have small effects on denitrification (Figure A7). Larger organisms have locally strong impacts on nitrogen cycling, but their relative rarity means that these organisms do not play a major role in determining system-wide denitrification, as evidenced by the distribution of predicted denitrification stimulation rates (Figure A7).

Model Evaluation: Comparison of Approaches

Denitrification rate measurement sites and benthic community analysis sites were typically separated by hundreds to thousands of meters, a scale over which both nitrogen dynamics and organism abundance can vary considerably (e.g. Hopkinson et al. 1999, Maciolek et al. 2004, Fear et al. 2005, Oehler et al. 2015). The large variability in TOC, nitrate, and infaunal density across these scales means that proximal sites may not reliably provide a reasonable estimate of benthic community state or substrate availability. Therefore, in the basic model approach estimating estuary-wide dynamics, data were averaged over space and time in each estuary.

In comparison to the model in Eq. 1, general linear models were substantially less accurate except for the GLM that consisted of nitrate and infaunal density, which did approach the accuracy of the base model (FI = 0.35 vs. FI = 0.3). The most accurate general linear models were those including infaunal abundance (see Table A4), which is further evidence of the importance of the infauna community in predicting denitrification rates. This is further supported by the finding that GLMs consisting of substrate availability without consideration of infauna were not statistically significant compared to a constant model, even when TOC and nitrate availability were considered together.

Bootstrapping revealed that the large variability in nutrient concentrations and organism density leads to large variability in predicted denitrification rates (Figure A4). Because the distributions of all variables are skewed, the majority of predicted denitrification rates were very low (Figure A4). The predictive model assumes the majority of denitrification is associated with burrow structures, which is not a valid assumption in cases with sparse infauna. The imposition of a minimum denitrification rate in the bootstrap analysis better reflects nitrogen cycling in sparsely populated sediments, and these models provided more accurate estimates (Table A4).

Model Evaluation: Biases

The nature of the data used in this study introduces some important biases. Benthic data and denitrification estimates were based on samples largely from soft-sediment communities, so they may not accurately represent the entirety of a system given the importance of other community types such as oyster reefs. Our approach also assumes that sediment irrigation is solely due to the infaunal community; this is not true in sandy

sediments such as in Plum Island Sound, where abiotic advection can have a significant contribution to benthic fluxes (Huettel et al. 2014). This discrepancy may account for the slight underestimation of denitrification in certain systems (including Plum Island). The relatively shallow grab depth of Van Veen grabs results in a bias towards small organisms over larger deep-dwelling burrowers. In terms of temporal patterns, the majority of data in most estuaries were collected in the months June – October (see Figures A8 – A17), so our analysis is more representative of summer and does not reflect the full seasonal variability. An analysis of monthly – rather than annual – averages for water quality suggests that variation over space accounts for as much or variability as that over time (Figures A8 through A17). The spatial coverage of data in most estuaries is sufficient to account for the spatial variability (Figure A1, Table 4.1), but the low sampling effort in winter represents an important area for future study.

Ecological Context

Small-scale studies have shown the importance of benthic fauna in experimental laboratory or plot-scale studies, and our large-scale analysis confirms the relationship between the benthic community and system-wide denitrification rates. This finding is especially significant within the context of increasing anthropogenic nutrient enrichment and hypoxia in coastal waters. A vast body of literature has documented the impacts of hypoxia on coastal ecosystems in general and the benthos in particular (e.g. Diaz & Rosenberg 1995, Cloern 2001, Kemp et al. 2005, Conley et al. 2007, Levin et al. 2009, Belley et al. 2010), though much of the work has focused primarily on how hypoxia affects the benthic community. A growing number of studies have begun to investigate the broader

ramifications of benthic community changes (Van Colen et al. 2010, Howarth et al. 2011, Kristensen et al. 2014), and our results suggest that these changes will indeed have ramifications at the scale of entire systems.

Lower infaunal diversity and abundance tend to be associated with lower rates of denitrification in natural systems (Figure 4.2, closed symbols), and our model similarly suggests that a decrease in denitrification would be associated with loss of infaunal abundance. This loss in nitrogen removal can then exacerbate eutrophication by enhancing regeneration and recycling rather than removal of excess nutrients (Middelburg & Levin 2009, Testa & Kemp 2012). At the onset of hypoxia, enhanced nutrient recycling resulting from a decrease in denitrification stimulated by infauna would be further compounded by shifts in biogeochemical cycles as a result of decreasing bottom water oxygen content (e.g. Middelburg & Levin 2009). Indeed, these shifts could be further accelerated by the loss of infaunal density, given that infauna themselves can have major impacts on sediment porewater chemistry (e.g. Aller 1988, Volkenborn et al. 2007). Finally, our results strongly suggest that degradation of benthic habitat not related to hypoxia (e.g. dredging) could even play a part in encouraging the onset of hypoxia via the decrease in infaunally-stimulated nitrogen removal.

Conclusions

Although there is a rough trend of increasing denitrification with increasing infaunal species richness there is no significant relationship at the system scale when considered across all systems. There is a significant and moderately strong relationship between organism density and system-wide denitrification. Published organism scale studies show

a strong stimulation of denitrification by benthic fauna, driven by the enhanced supply of nitrate to the sediment environment, and experimental data from these studies can be used to parameterize a model used to predict estuarine denitrification as a function of benthic faunal abundance, nitrate concentration, and sediment TOC. This model provides reasonable accuracy in 11 of 12 sites studied. These results provide strong evidence for the importance of the benthic community for system-wide nitrogen removal.

Acknowledgements. This work was supported by the National Science Foundation (OCE 0751882 to CM). Additional support was received from the Gulf of Mexico Research Initiative Ecological Impacts of Oil and Gas Inputs to the Gulf to CM. We would also like to thank Dr. George Waldbusser for providing the detailed Chesapeake Bay data set, collected and compiled with support from Dr. Roberta Marinelli and Maryland Sea Grant.

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Table 4.1: Estuaries included in this analysis, estimated denitrification and method of measurement, data sources, chemistry and benthic community data obtained from the Environmental Monitoring and Assessment Program (EMAP) and the National Coast Assessment (NCA), acquired from the EPA database at <http://www.epa.gov/emap/nca/html/data/index.html>. Average residence times were estimated based on ranges reported in the respective sources. Roman numerals in parentheses indicate groups as shown by PCA (Figure 4.1), bracketed terms refer to the initials used in Figure 4.1, and plus signs indicate Monte Carlo analysis estuaries. Error is the standard error of the mean.

Estuary	Denitrification (mmol m ⁻² d ⁻¹)	Methodology	Source	DIN (μM)	Sediment TOC (% Weight)	Species Richness	Organism Density (m ⁻²)	Residence Time (months)
Plum Island Sound, MA, USA (I) [PI]	0.89 ± 1.54	Isotope Pairing, 7 cores taken annually 2 years	Hopkinson et al. 1999 & Koop-Jakobsen & Giblin 2010	4.57 ± 6.43 ¹	2.34 ± 3.19	7*	1437*	0.28
Little Lagoon, AL, USA (I) [LL]	1.48 ± 0.117	Isotope Pairing, 3 cores taken monthly 1 year	Bernard et al. 2014	1.00 ± 0.78	0.14 ± 0.13	34 ± 0	3432 ± 2377	0.33
Galveston Bay, TX, USA (I) + [GB]	0.44 ± 0.18	N ₂ Flux, 5 cores taken seasonally 1 year	Zimmerman et al. 1994	2.87 ± 5.00	0.98 ± 0.69	7.43 ± 0.88	1596 ± 512	2.13
Narragansett Bay, RI, USA (I) + [NB]	1.06 ± 0.147	N ₂ Flux, 10 sites sampled seasonally 2 years	Nixon et al. 1995	1.19 ± 1.43	1.54 ± 1.07	29.22 ± 2.16	13727 ± 2409	0.87
Chesapeake Bay, USA (I) + [CB]	0.67 ± 0.23	N ₂ Flux and Mass Balance, 2 cores taken seasonally 11 Years	Boynton et al. 1995	3.31 ± 7.14	1.16 ± 1.38	12.6 ± 1.60	2478 ± 626	7.6
Patuxent River, MD, USA (I) + [Pat]	0.84 ± 0.10	N ₂ Flux, 6 to 8 Cores taken seasonally, 1 year	Boynton et al. 2008	10 ± 17.14	1.61 ± 1.1	8.17 ± 5.28	1688 ± 2132	2
Waquoit Bay, MA, USA (II) [WB]	3.83 ± 1.56	N ₂ Flux, 3 cores taken quarterly 1 year	LaMontagne et al. 2002	3.50 ± 35.70 ²	2.62 ± 2.86 ²	43.2 ± 10.75 ³	15276 ± 9547 ³	0.07
Boston Harbor, MA, USA (II) [BH]	1.895 ± 0.186	N ₂ Flux, 6 cores taken monthly 2 years	Nowicki et al. 1997 & Giblin et al. 1995	8.23 ± 10.00	5.15 ± 3.34	50 ± 5.0 ⁴	2601 ± 2874 ⁴	0.33
Neuse River, NC, USA (III) + [NR]	1.82 ± 0.192	N ₂ Flux and MIMS, 5 cores taken monthly 1 year	Fear et al. 2005	35.70 ± 35.70	2.64 ± 2.71	11.2 ± 1.25	1651 ± 318	1.7
Potomac River, MD, USA (III) + [Pot]	0.9 ± 0.118	N ₂ Flux and Mass Balance, 2 cores taken weekly 11 years	Boynton et al. 1995	30.50 ± 35.70	1.70 ± 1.03	6.57 ± 1.17	2005 ± 338	5
Delaware Bay, DE, USA (III) + [DB]	2.53 ± 0.15	N ₂ Flux, 8 cores taken seasonally 1 year	Seitzinger 1988	50.70 ± 52.10	1.16 ± 1.30	18.63 ± 1.80	6067 ± 1840	3.3
Scheldt River, Netherlands (III) [SR]	14.85	Mass Balance	Billen et al. 1985	200 ⁵	2.40 ± 0.70 ⁶	13.6 ± 6.55 ⁷	18683 ± 13101 ⁷	4.67

* Only one sample was available from the NCA database

¹Supplemented with data from the Plum Island Estuary LTER

²National Coast Assessment supplemented with data from Carmichael & Valiela 2005

³Fox et al. 2009 and Olsen et al. 2013

⁴Maciolek et al. 2006

⁵Vanderborght et al. 2007

⁶Abell et al. 2009

⁷Ysebaert & Herman 2002

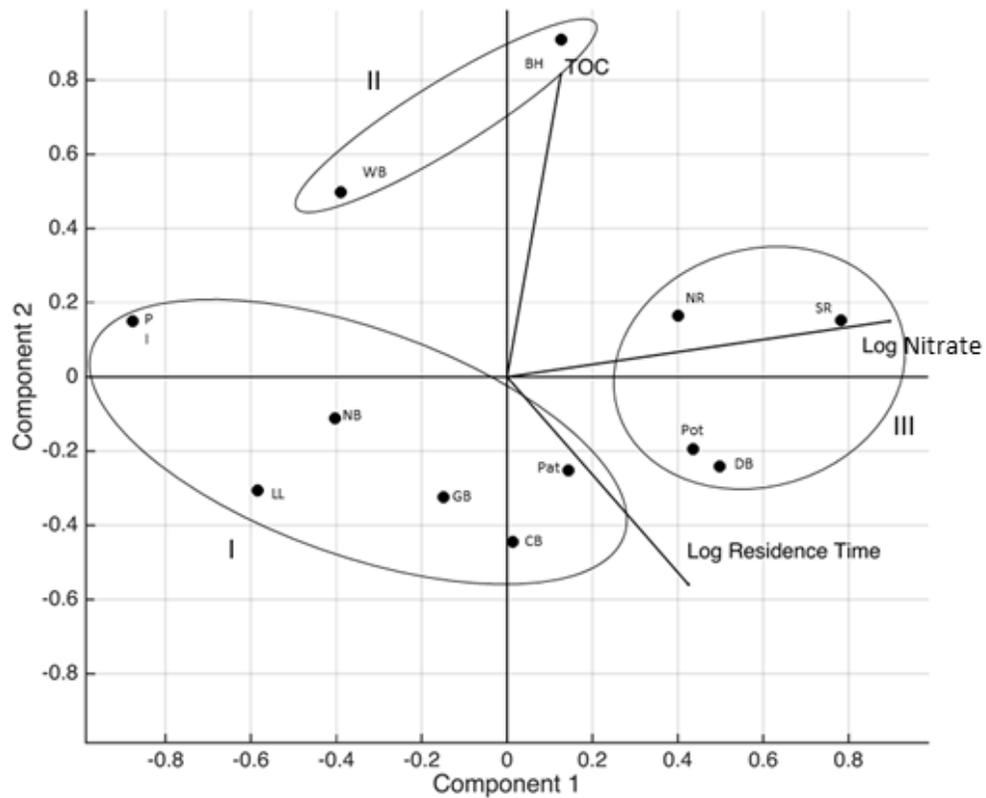


Figure 4.1: PCA of the estuarine nitrogen cycling data set using nitrate, sediment TOC, and water residence time, showing that estuaries fall into three distinct groups based on water quality, sediment, and physical properties. See Table 4.1 for key to abbreviations.

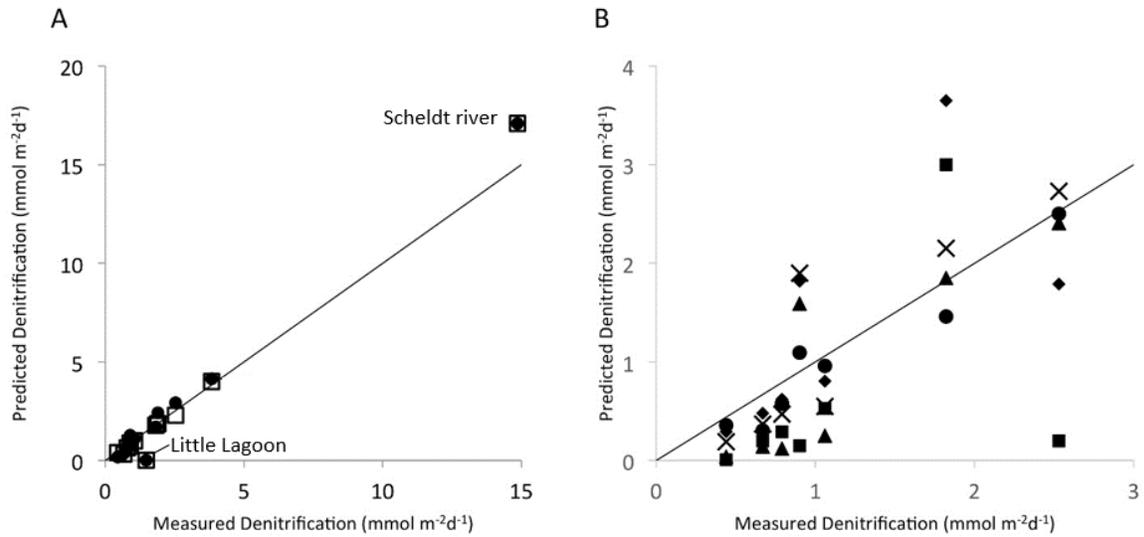


Figure 4.3: (A) Comparison of measured and predicted rates of denitrification using the model $DNF = \rho \cdot r_p \cdot \beta_l \cdot TOC \cdot \left(\frac{NO_3}{NO_3 + K_m} \right)$, circles, and using functional traits, squares, (see methods); the solid line is the 1:1 line. **(B)** Denitrification rates in a subsample of estuaries using the averaged values (circles), random bootstrapping (squares), data matching based on station identity (triangles), randomly selected data with an imposed minimum denitrification rate (diamonds), and the imposed minimum rate with data matching (X); the solid line is the 1:1 line.

Appendix A: Supplementary Tables and Figures

Table A1: Species, denitrification rates, sediment POC and source for laboratory organismal data. SF = suspension feeder, SDF = surface deposit feeder, HDDF = head-down deposit feeder, TS = tube forming scavenger, BC = burrowing carnivore

Species	Denitrification (mmol N m ⁻² d ⁻¹)	Sediment TOC (%)	Organism Density (ind m ⁻²)	Per organism Stimulation Rate (mmol N m ⁻² d ⁻¹ ind ⁻¹)	Source	Functional Group
<i>Upogebia deltarua</i>	0.70 ± 0.34	11	14	0.00454	Howe et al. 2004	SF
<i>Corophium volutator</i>	2.88 ± 1.2	0.7	12000	0.000348	Pelegri et al. 1994	SDF
<i>Cirriformia filigera</i>	3 ± 0.6	5	1000	0.0006	Banks et al. 2013	SDF
<i>Monoporeia affinis</i>	0.15 ± 0.001	0.05	5000	0.0006	Autio et al. 2003	SDF
<i>Abra alba</i>	2.1 ± 0.68	0.6	1273	0.00274	Braeckman et al. 2010	SDF
<i>Lanice chonchilega</i>	3.9 ± 1.98	0.6	764	0.00872	Braeckman et al. 2010	HDDF
<i>Nephtys</i>	3.7 ± 1.65	0.6	382	0.0161	Braeckman et al. 2010	BC
<i>Chironomus</i>	12.0	36	2630	0.000126	Svensson & Leonardson 1996	TS
<i>Chironomus</i>	9.264 ± 1.10	36	1972	0.000130	Svensson 1997	TS
<i>Tubifex</i>	3.0 ± 0.36	36	38567	2.16E-06	Svensson 2001	HDDF
<i>Monoporeia affinis</i>	0.043 ± 0.01	7	5000	1.23E-06	Karlson et al. 2005	SDF
<i>Macoma balthica</i>	0.017 ± 0.002	7	1200	2.02E-06	Karlson et al. 2005	SDF
<i>Marenzelleria viridis</i>	0.016 ± 0.002	7	5000	4.57E-07	Karlson et al. 2005	HDDF
<i>Monoporeia affinis</i>	0.1 ± 0.01	31	6400	5.04E-07	Karlson et al. 2007	SDF
<i>Marenzelleria sp.</i>	1.5 ± 0.1	4	1950	0.000192	Hietanan et al. 2007	HDDF
<i>Marenzelleria viridis</i>	11 ± 1.7	0.55	1200	0.0167	Kristensen et al. 2011	HDDF
<i>Nereis diversicolor</i>	11 ± 0.77	0.55	1200	0.0167	Kristensen et al. 2011	TS
<i>Nereis spp.</i>	12.2 ± 0.03	11	2000	0.000554	Nizzoli et al. 2007	TS

Table A2: Numerically important functional groups (abundance > 10% of total abundance) in the 12 estuaries. Groups are 1: Burrowing carnivore/scavenger, 2: Deep deposit feeders, 3: Head-down deposit feeders, 4: surface deposit feeders, 5: Suspension feeders, 6: tube-building filter feeders, and 7: Tube-building scavengers.

Estuary	1	2	3	4	5	6	7
Plum Island Sound (I)				■			
Waquoit Bay (II)				■			
Little Lagoon (AL)(I)			■	■			
Boston Harbor (II)			■	■			
Neuse River (III)		■	■	■			
Potomac River (III)			■	■			■
Galveston Bay (I)			■	■			
Narragansett Bay (I)	■		■	■	■	■	
Chesapeake Bay (I)			■	■			■
Delaware Bay (III)			■	■		■	
Patuxent River (I)			■	■			■
Scheldt River (III)			■	■			■

Table A3: Functional designations of the major families (abundance > 10% of total abundance) identified in the National Coast Assessment data, after D'Andrea & Lopez 1997. Groups are 1: Burrowing carnivore/scavenger, 2: Deep deposit feeders, 3: Head-down deposit feeders, 4: surface deposit feeders, 5: Suspension feeders, 6: tube-building filter feeders, and 7: Tube-building scavengers.

Group	Family	Functional Description
1	Lumbrineridae	Burrowing carnivore/scavenger
	Nephtyidae	Burrowing carnivore/scavenger
2	Haustoriidae	Deep deposit feeder
3	Captitellidae	Head-down deposit feeder
	Maldanidae	Head-down deposit feeder
	Tubificidae	Head-down deposit feeder
4	Ampeliscidae	Surface deposit feeder
	Aoridae	Surface deposit feeder
	Calyptraeidae	Surface deposit feeder
	Chironomidae	Surface deposit feeder
	Cirratulidae	Surface deposit feeder
	Corbiculidae	Surface deposit feeder
	Corophiidae	Surface deposit feeder
	Gammaridae	Surface deposit feeder
	Idoteidae	Surface deposit feeder
	Leuconidae	Surface deposit feeder
	Mactridae	Surface deposit feeder
	Nuculidae	Surface deposit feeder
	Spionidae	Surface deposit feeder
	Tellinidae	Surface deposit feeder
5	Mytilidae	Suspension feeder
	Veneridae	Suspension feeder
6	Chaetopteridae	Tube-building filter feeder
	Sabellidae	Tube-building filter feeder
7	Nereididae	Tube-forming scavenger

Table A4: Goodness of Fit Indices for the Model approaches. Asterisk indicates a non-significant model using the GLM approach.

Model Approach	Goodness of Fit Index
Averaged Variables	
Equation 1	0.3
Equation 2 (inclusion of functional traits)	0.19
<i>General Linear Models</i>	
All Terms	0.61
Nitrate ¹ x POC	1.08*
Abundance x POC	0.67
Abundance x Nitrate ¹	0.35
Nitrate ¹	1.74*
POC	1.34*
Abundance	0.74
Bootstrap Analysis	
Averaged Variables (Equation 1)	0.19
Bootstrap, Random	0.74
Bootstrap, Matched	0.59
Adjusted Bootstrap, Random	0.48
Adjusted Bootstrap, Matched	0.46

¹The nitrate term in the GLM reflects Monod kinetics, i.e. $\frac{NO_3}{NO_3+K_m}$.

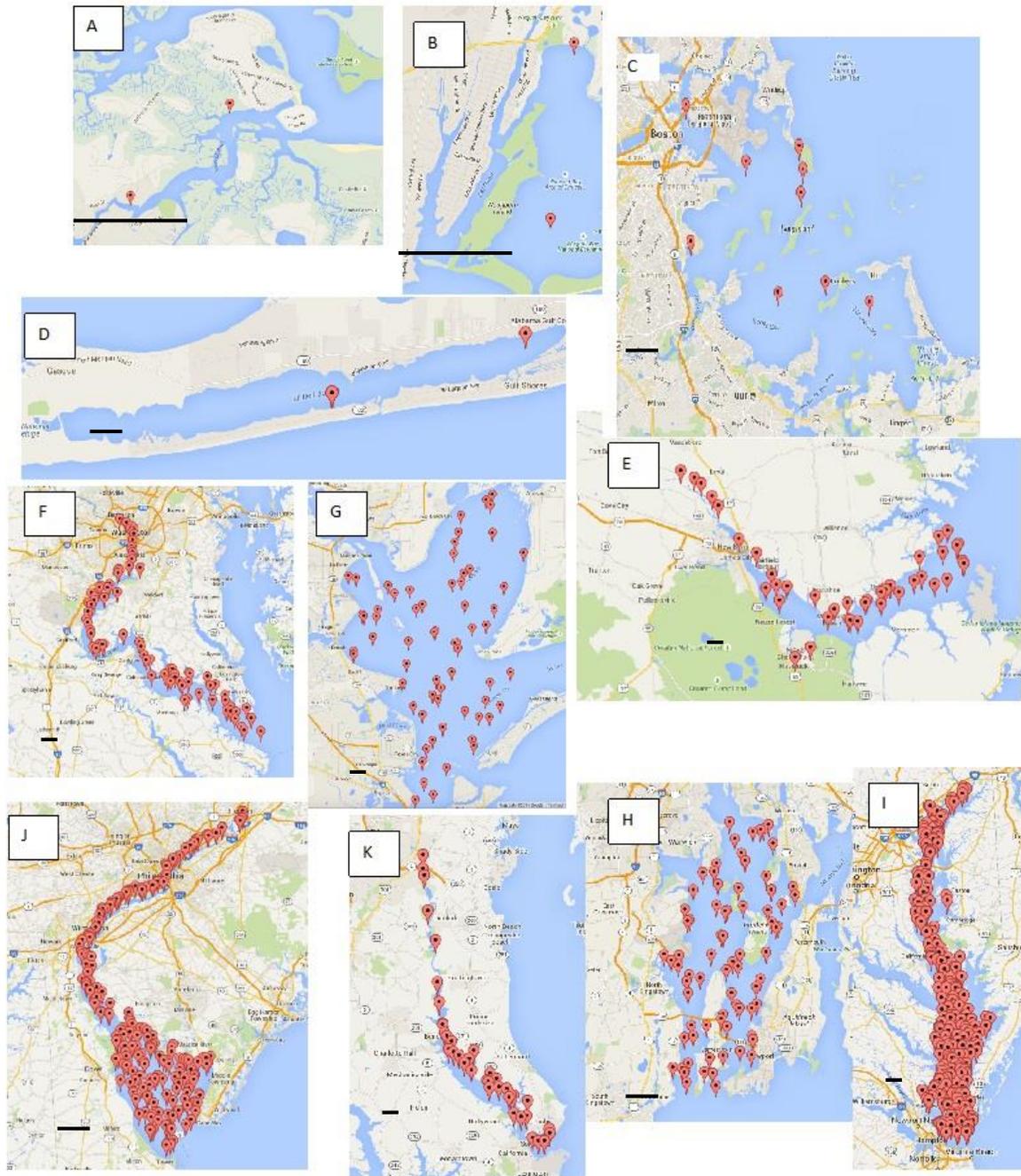


Figure A1: Plots of National Coast Assessment sampling locations in **(A)** Plum Island Sound, **(B)** Waquoit Bay, **(C)** Boston Harbor (redrawn from Maciolek et al. 2004), **(D)** Little Lagoon, **(E)** Neuse River, **(F)** Potomac River, **(G)** Galveston Bay, **(H)** Narragansett Bay, **(I)** Chesapeake Bay, **(J)** Delaware Bay, and **(K)** Patuxent River. See Ysebaert et al. (2002) for data collection locations in the Scheldt River estuary. Scale bars are 1 km except for J, H, and I, where they are 10 km.

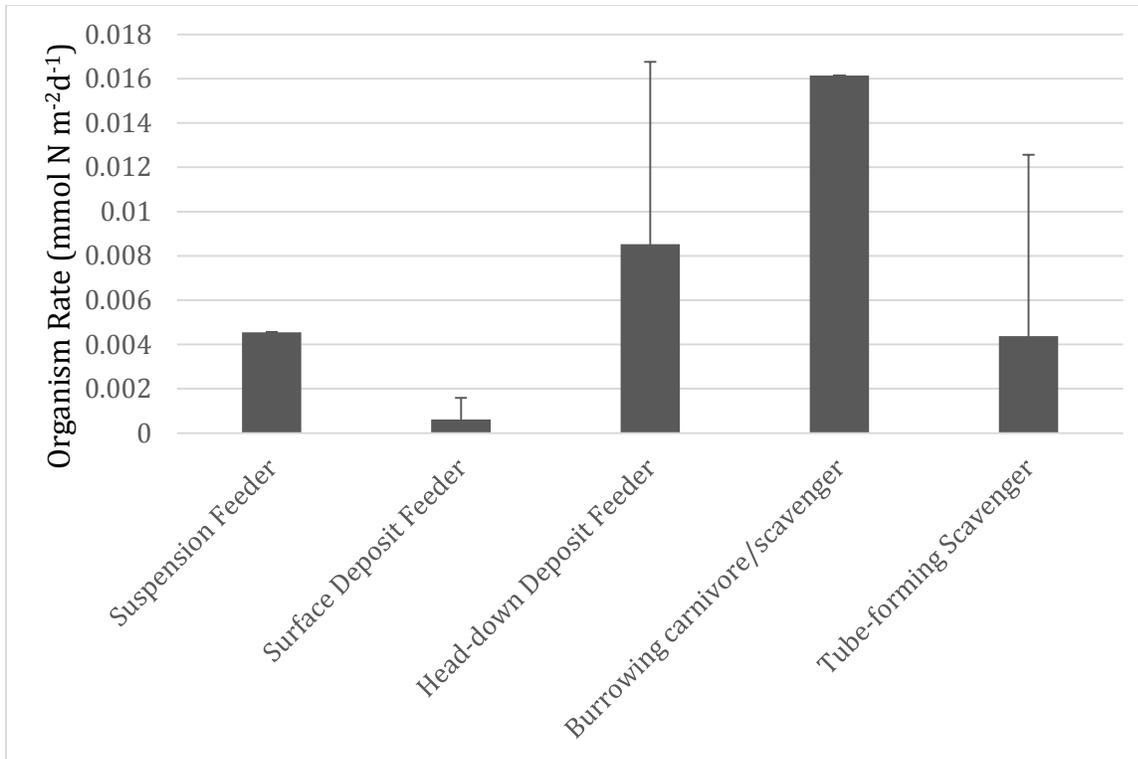


Figure A2: Calculated per-organism denitrification stimulation rates for different functional groups, from the studies reviewed in Stief 2013.

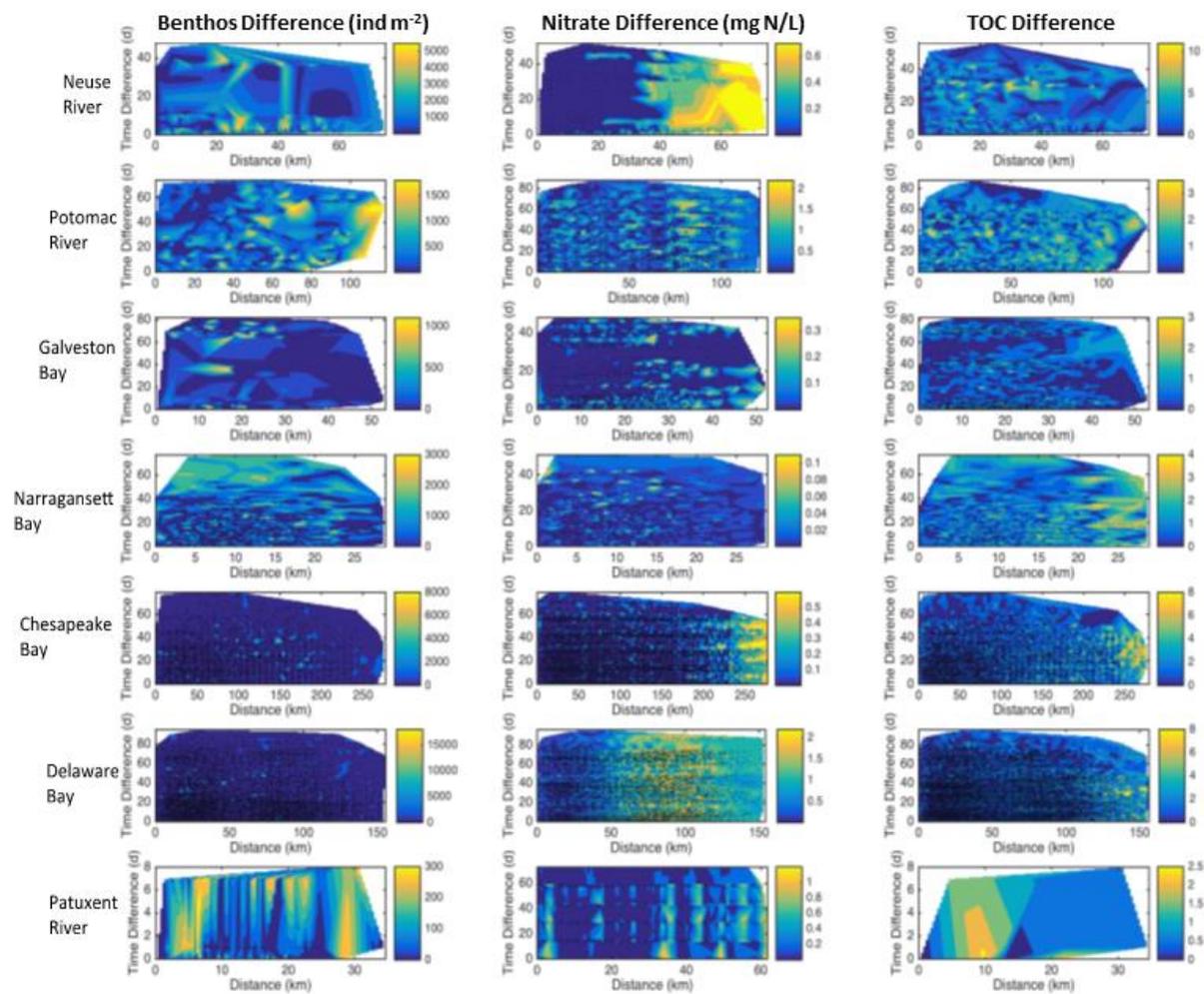


Figure A3: Contour plots of the difference in infaunal density, nitrate, and TOC as a function of distance in both space and time between respective sampling points. Small black dots indicate actual sampling events.

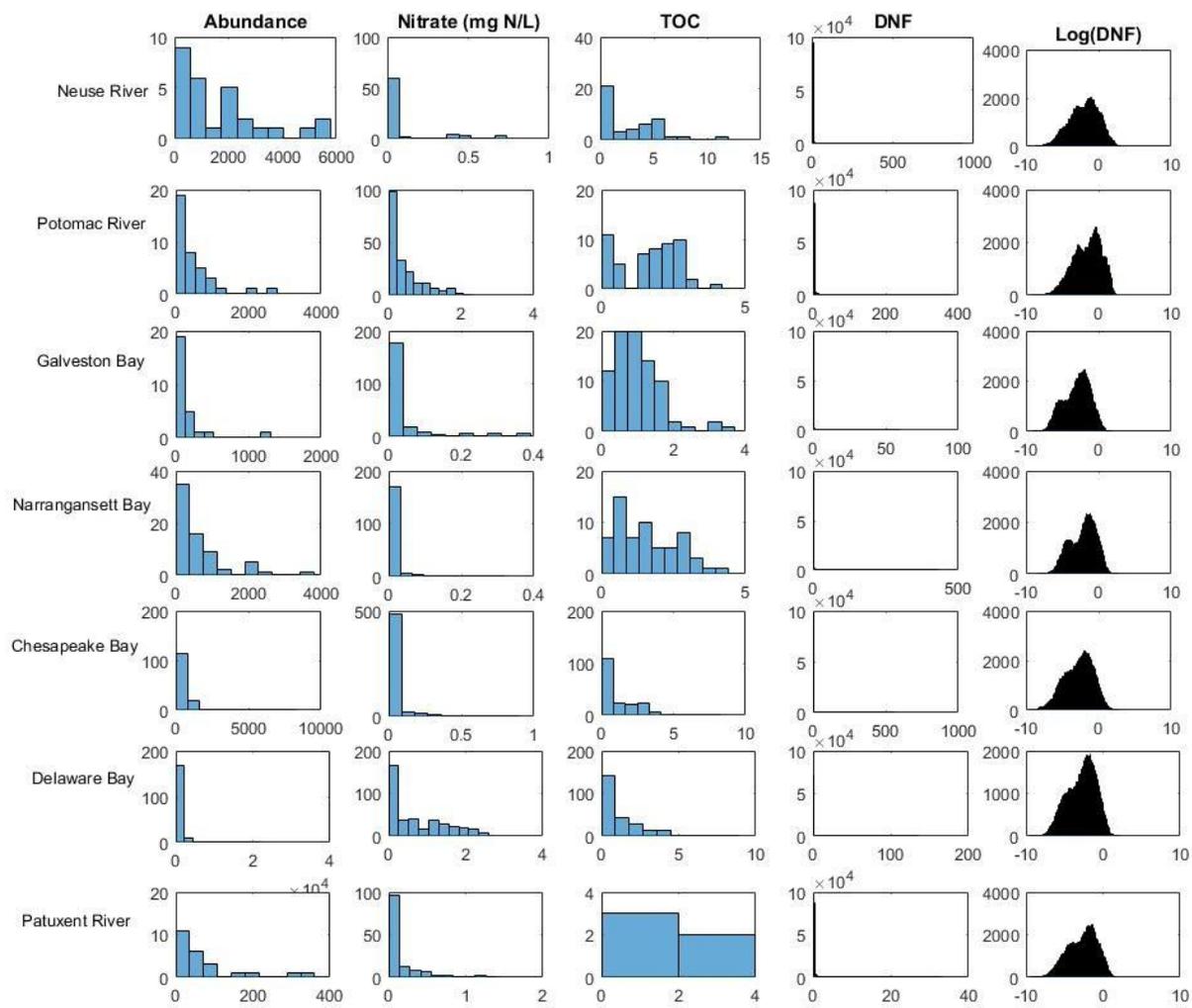


Figure A4: Histograms of organism abundance, nitrate, TOC, and predicted denitrification rates calculated using a bootstrap analysis of randomly selected values for the three variables. X-axes are values, and y-axes are the frequencies. The majority of data is positively skewed, leading to very low predictions of denitrification in most simulations.

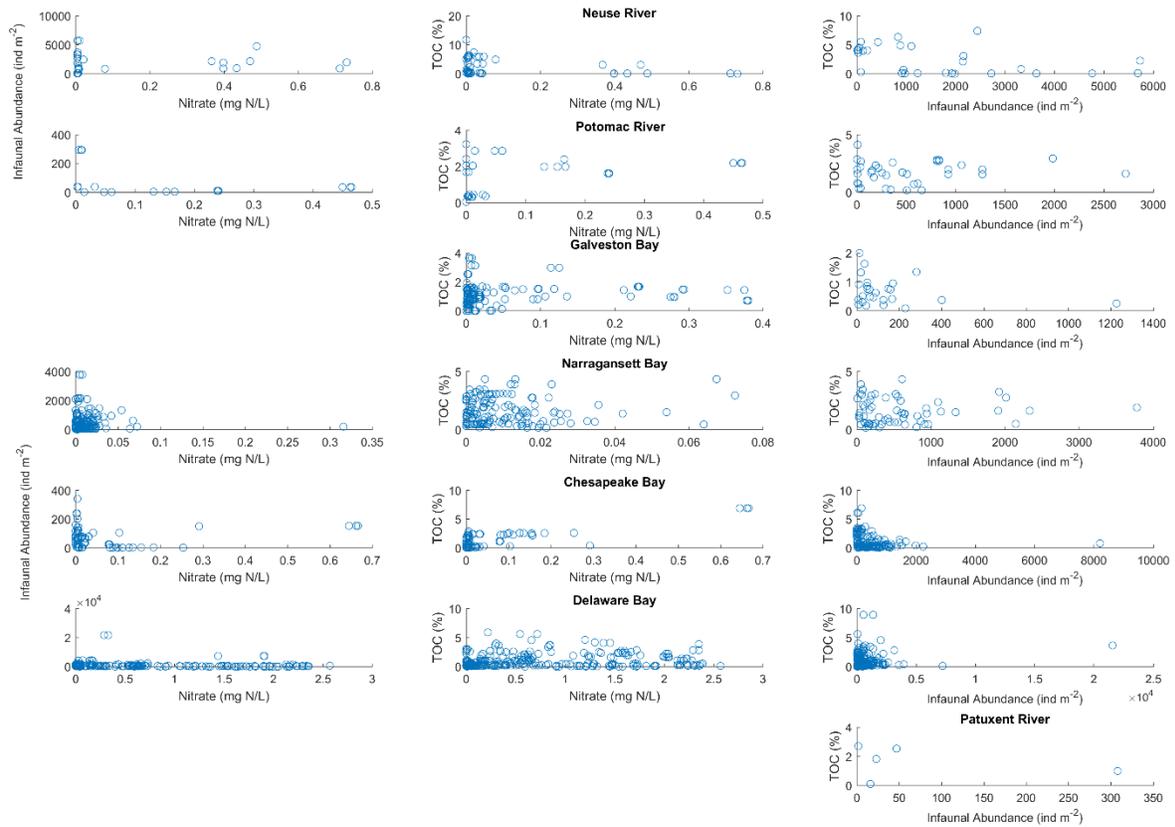


Figure A5: Relationships between variables on the basis of simultaneously collected samples.

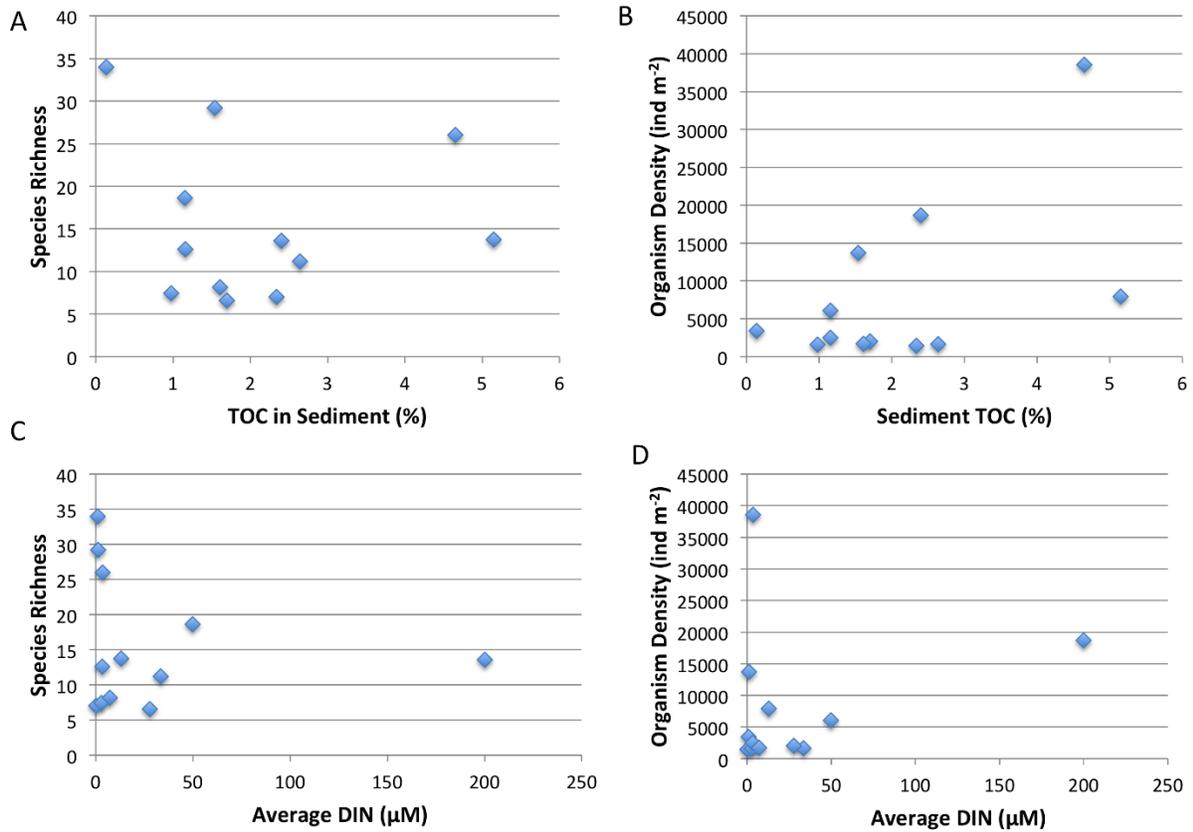


Figure A6: The relationship between averaged values of **(A)** sediment TOC content and species richness, **(B)** TOC content and organism density, **(C)** Nitrogen availability and species richness, and **(D)** Nitrogen availability and organism density in all estuaries.

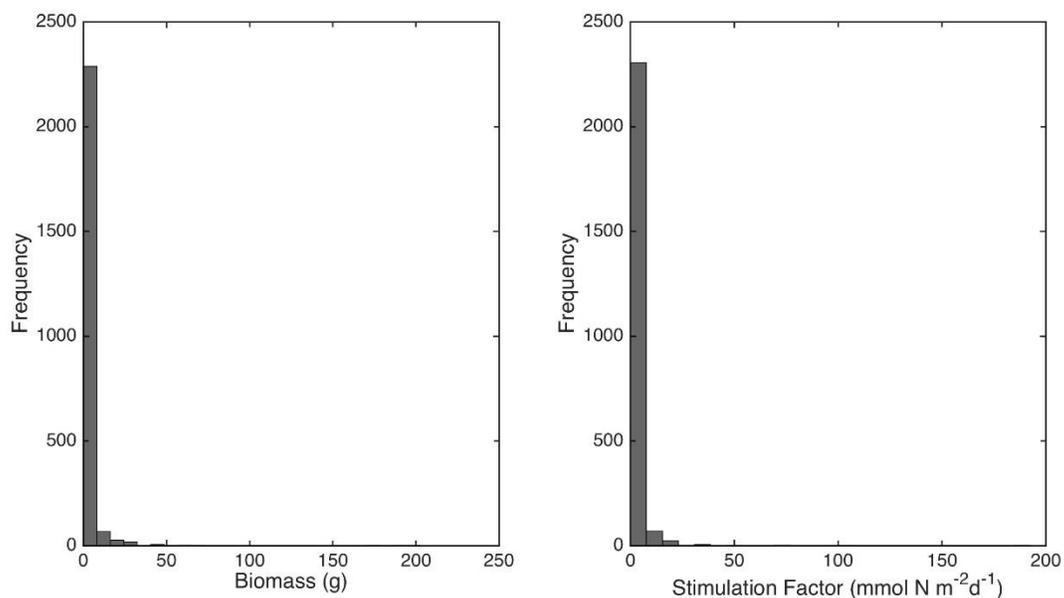


Figure A7: Distribution of biomass and stimulation rates of denitrification based on biomass in the Chesapeake Bay. The large majority of organisms are small and individually have small contributions to denitrification, while large high-impact organisms are rare.

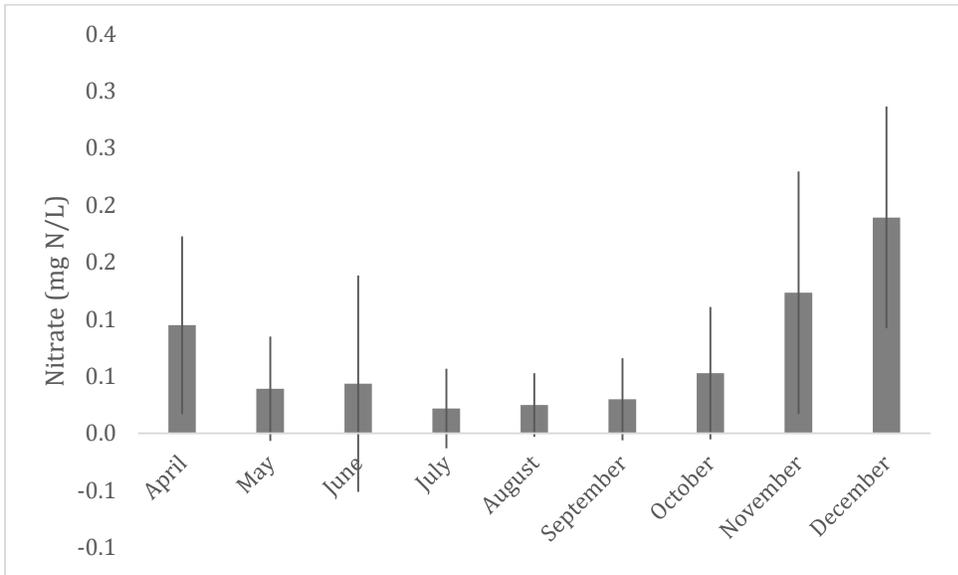


Figure A8: Nitrate concentrations in Plum Island Sound

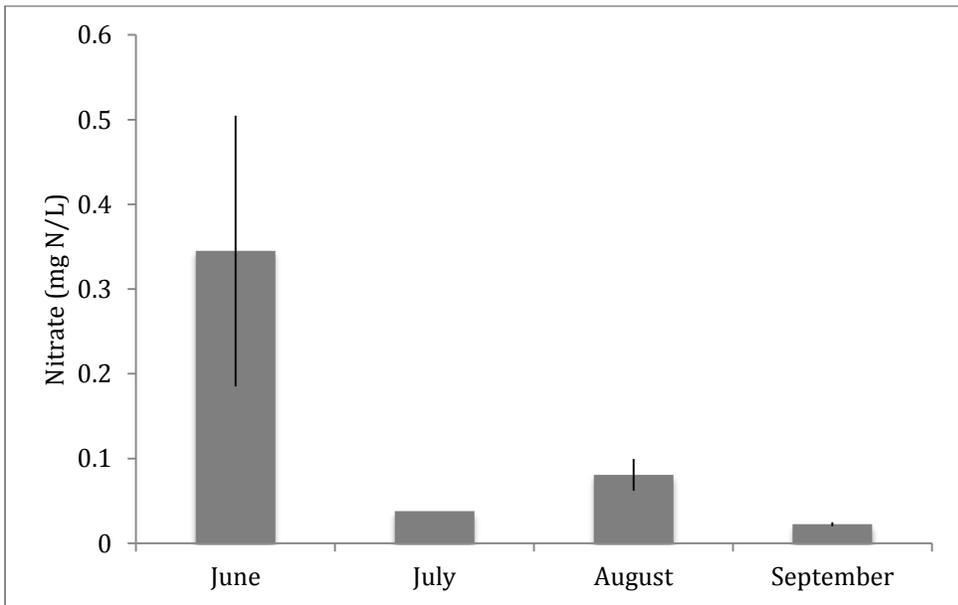


Figure A9: Nitrate concentrations in Boston Harbor

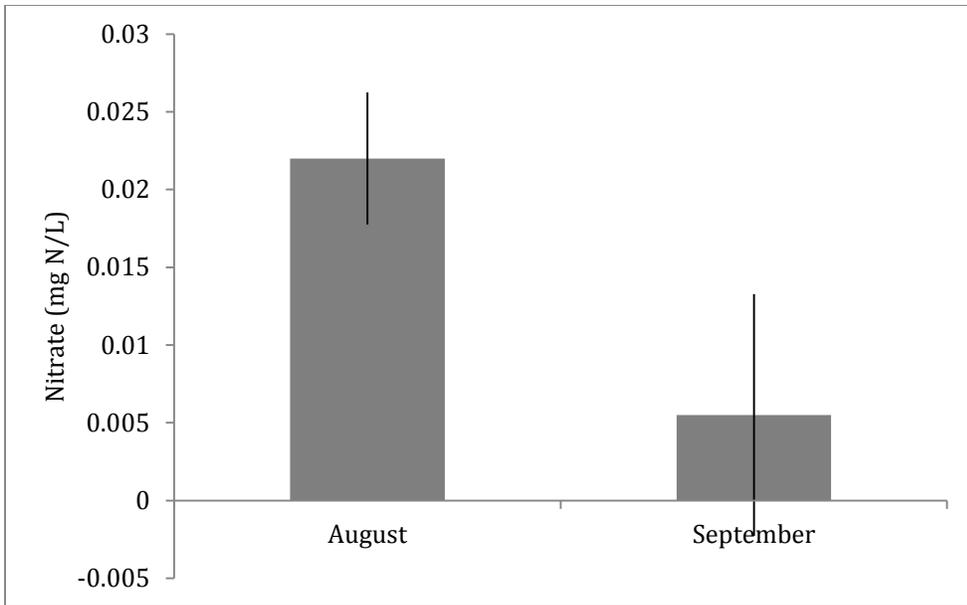


Figure A10: Nitrate concentrations in Little Lagoon, AL.

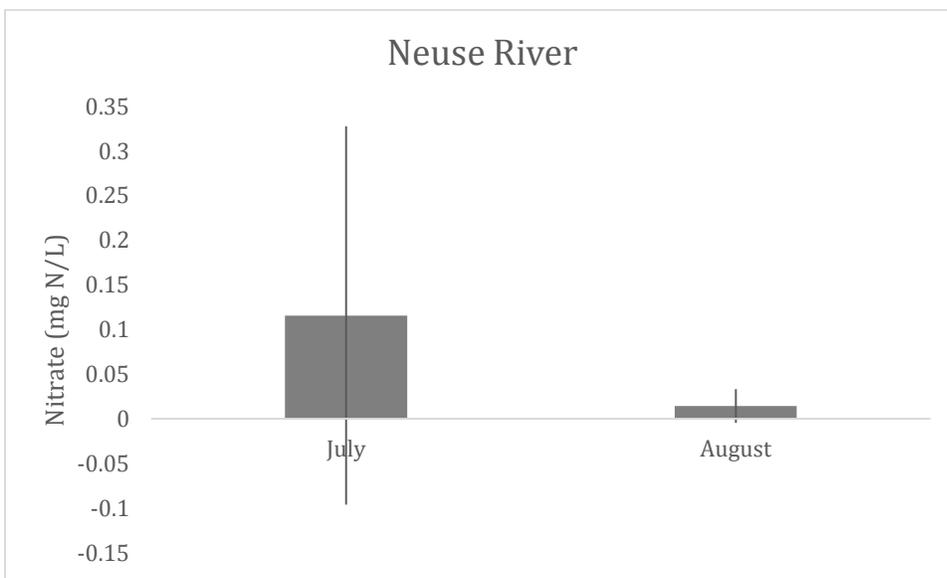


Figure A11: Nitrate concentrations in the Neuse River estuary.

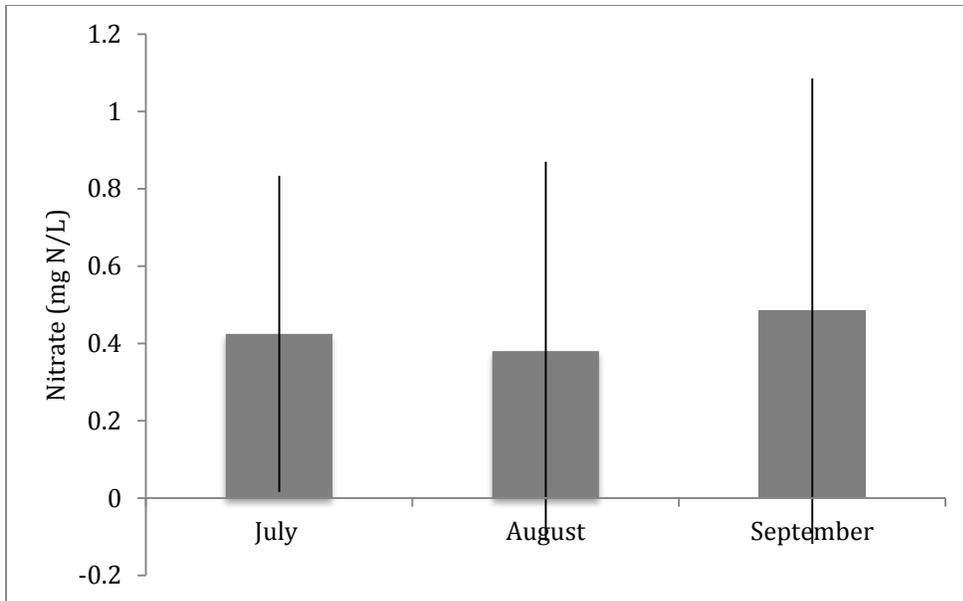


Figure A12: Nitrate concentrations in the Potomac River estuary.

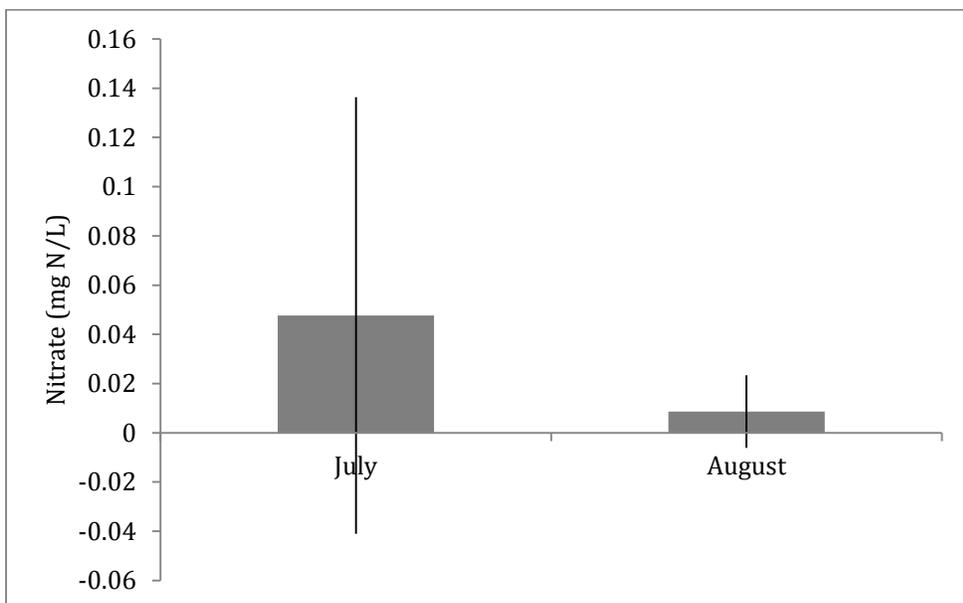


Figure A13: Nitrate concentrations in Galveston Bay

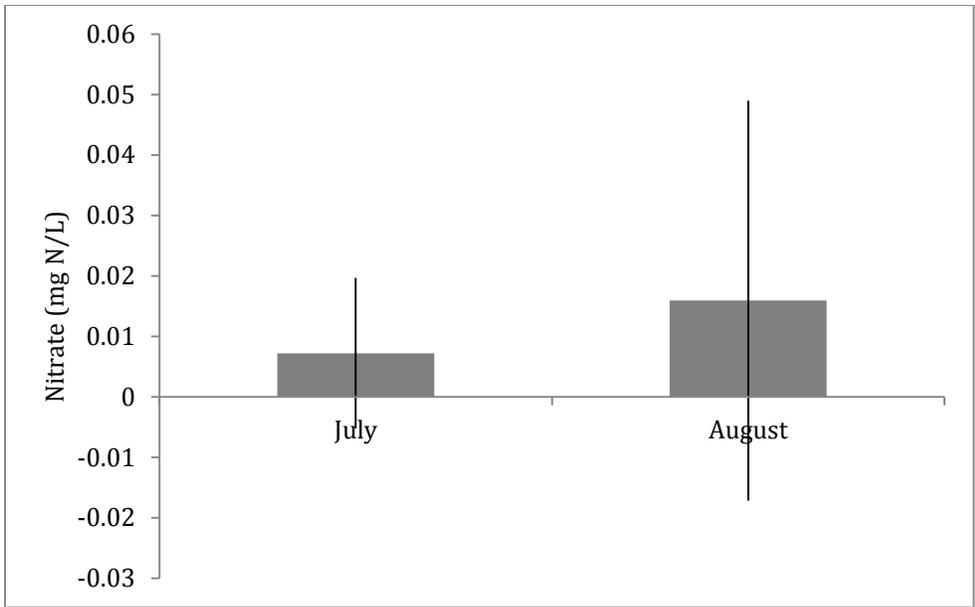


Figure A14: Nitrate concentrations in Narragansett Bay

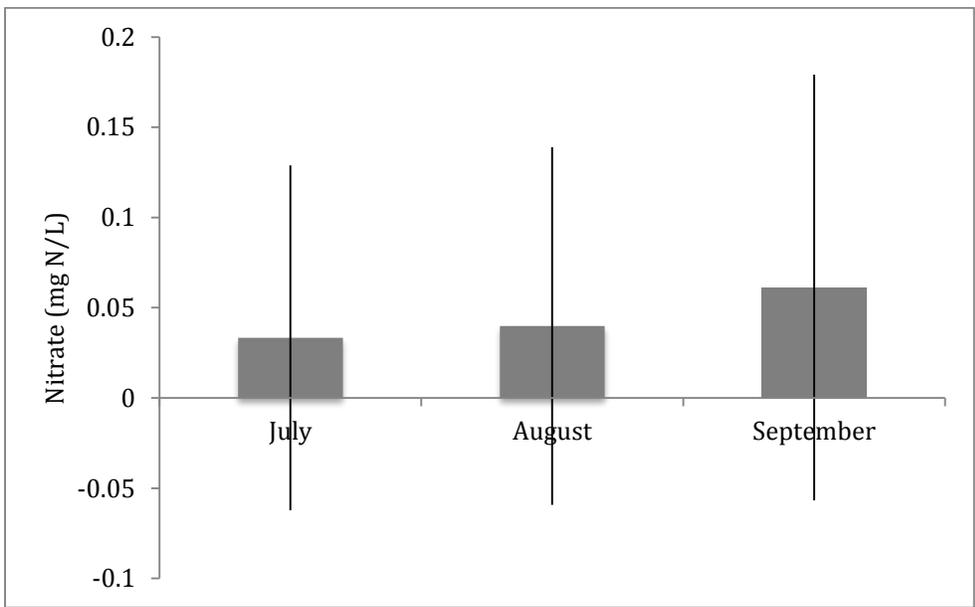


Figure A15: Nitrate concentrations in Chesapeake Bay

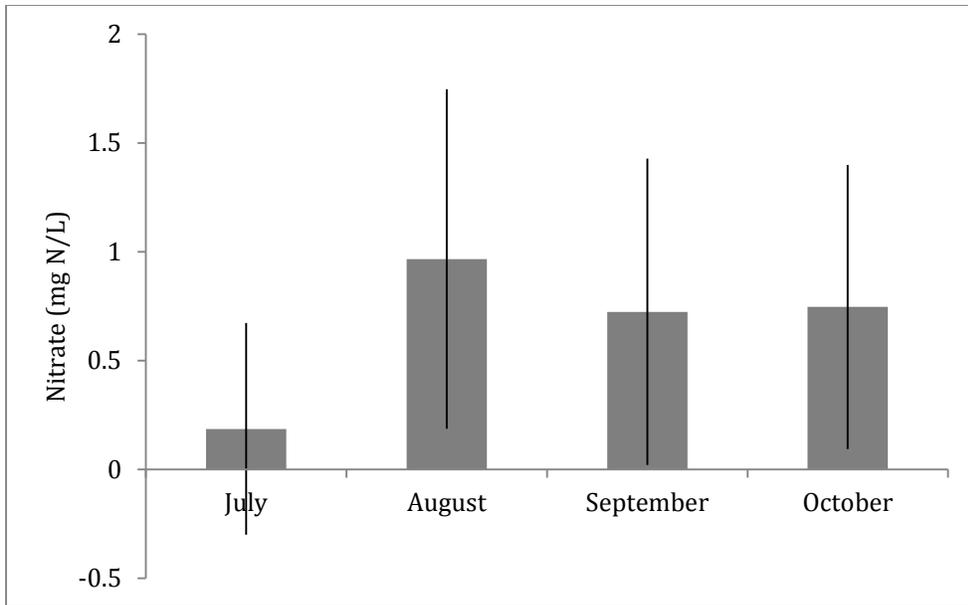


Figure A16: Nitrate concentrations in Delaware Bay.

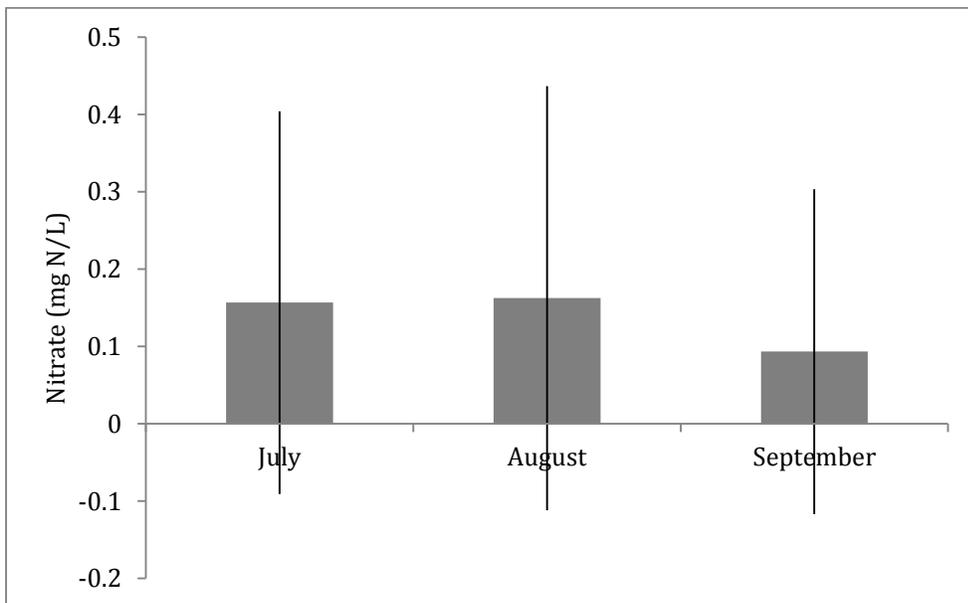


Figure A17: Nitrate concentrations in the Patuxent River estuary.

CHAPTER 5

SUMMARY

Benthic fauna can have large impacts on sediment biogeochemistry, making them a crucial component of nutrient cycling at two major interfaces: the sediment-water interface and the interface between land and sea, where large numbers of benthic fauna impact the removal of nitrogen from the ecosystem. Fauna enhance benthic-pelagic coupling and increase rates of carbon and nitrogen cycling in the benthic habitat (Stief 2013). The impact of some species is so profound that they are considered ecosystem engineers (Meysman et al. 2006), whose presence controls the overall form of their ecosystem. The importance of benthic fauna manifests itself at multiple scales, and the focus of this dissertation has been to describe the benthic community's impact across those scales.

Organism burrowing behavior and nitrogen cycling

The effects of benthic fauna at the organism scale, though highly localized, can extend across communities to have system-wide impacts, so an understanding of organism-level heterogeneity is crucial. In diffusion-dominated environments, flushed burrows act largely as extensions of the sediment-water interface. However, many head-down deposit feeders – of which arenicolid lugworms are widespread examples – irrigate their burrows in such a way as to create porewater pressure gradients and inject overlying water into the surrounding sediments. In this case, burrow irrigation rates have a major impact on benthic nutrient cycling.

Variations in the feeding pocket depth and burrow irrigation rate of lugworms can have substantial effects on nitrogen cycling in the vicinity of individual burrows by increasing the amount of nitrate supplied from the overlying water to the sediment. Irrigation therefore stimulates denitrification as a function of organism pumping rate (Figure 3.2), but shallow feeding pockets can lead to ejection of burrow lumen fluid out of the sediment before consumption of reactive solutes is complete. However, this “short circuiting” is dependent on the environmental context, with the greatest impact being observed in sediments with relatively low oxygen consumption, because slower reaction rates allow the ejection of solute before it can all be consumed.

The impact of burrow irrigation on sediment denitrification is particularly pronounced in eutrophic environments with high levels of nitrate in the overlying water, and the benthic community is a key component of those ecosystems’ function. Depth-integrated irrigation rates of the community are a function of organism density as well as individual organisms’ behaviors and functional traits. Thus, not only do variations in individual organism behaviors have an impact on sediment nutrient cycling, but variance in community characteristics such as organism abundance also have important impacts as well.

Patchiness and benthic fluxes

Whether organism distribution varies as a response to competitive pressures, heterogeneity in food supply, or physical forcing such as water circulation patterns, patchiness in organism distribution is a major structural aspect of the benthic community (Levin 1992, Underwood et al. 2000). This makes patchiness an important consideration

when linking the benthic community to ecosystem function, because of the strong impact that organism behavior has on nutrient cycling as detailed above.

Patchiness of burrow distributions has two major implications, though those implications are heavily dependent on the context in which they are considered. First, tightly clustered burrows decrease oxygen flux relative to the flux observed in evenly distributed burrows, due to the creation of smaller concentration gradients resulting from the overlap of oxic zones. This effect depends on the rates of organic matter degradation, because rapid oxygen consumption – typical of nearshore sediments – leads to very low penetration depth and thus no overlap of oxic zones. On the other hand, very low oxygen consumption rates – typical of deep ocean sediments – lead to the formation of entirely oxidized sediment. Burrow clustering is therefore most important in areas of intermediate oxygen demand, such as the continental shelf slope. Because nitrogen cycling is intimately linked with oxygen dynamics, burrow patchiness could thus impact nitrogen dynamics in these environments; variations in inter-burrow distance are indeed known to impact sediment nitrogen cycling (Gilbert et al. 2003). Although the impact on oxygen fluxes is an important effect, it likely does not apply to the land-sea interface that is the focus of this dissertation, due to the rapid consumption of organic matter and oxygen at this interface that results in very steep gradients and low oxygen penetration depth.

Although patchiness in burrow distributions does not substantially impact modeled benthic oxygen flux in estuarine environments, it has implications for sampling effort and sampling regimens. High organic matter reactivity leads to sediment environments that have large degrees of heterogeneity on the scale of millimeters to centimeters. This implies that measurement instruments with very small sampling volume might miss

biogeochemical hotspots – and thus poorly estimate total reaction rates – unless burrow distribution is taken into account when designing sampling regimes. As the scale of measurement approaches the typical scale of organism heterogeneity (which is dependent on organism density), fewer samples are necessary to appropriately capture the impacts of benthic fauna on fluxes. Although large-scale analysis, such as that presented in chapter 4, averages over much of the heterogeneity to approximate average nitrogen concentrations and budgets, those averaged values are dependent on sampling regimens that properly capture variability at the scale of measurement.

Relationships between benthic fauna and system-scale estuarine nitrogen removal

The impacts of benthic fauna are well documented at the plot and individual level, and the large-scale analysis in Chapter 4 shows that there are significant relationships between organism abundance and denitrification at the system scale; the same can be said of the relationship between organism richness and denitrification, but only for certain groups of estuaries. However, there are large differences in environmental conditions (organic matter and nitrogen availability) between estuaries: substrate availability is a limiting factor controlling denitrification rates, so the lack of a strong relationship between denitrification and organism abundance alone (without consideration of substrate availability) is not entirely unexpected. Moreover, results presented in Chapter 3 show that the majority of denitrification in bioturbated sediments can be attributed to porewater advection caused by burrowing irrigation. This motivated the use of the system-scale denitrification model used in Chapter 4, which includes both substrate availability and the stimulation of denitrification by individual organisms.

This model provides reasonably accurate estimates of estuarine denitrification in 11 of 12 sites studied, and can be used to estimate how system nitrogen removal will change as a response to changing nutrient loading or benthic organism abundance. Decreases in organism abundance will lead to decreases in denitrification, and this in turn can exacerbate eutrophication by favoring nutrient regeneration rather than nutrient removal. This is important not only as impetus for the protection of benthic habitat from destructive forces such as dredging and overharvesting, but also for the prediction of how a system might respond to changing anthropogenic influences. Estuaries and coastal sediments are a critical land-sea interface impacting the transport of nutrients to the open ocean, and this dissertation provides evidence for the importance of benthic fauna in modulating this impact by the stimulation of benthic-pelagic coupling and nutrient cycling.

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