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Nitrogen Fixation and Dentrification in Sediments of Eutrophic Mediterranean-Type Estuaries: Seasonal Patterns and Responses to Anthropogenic Nitrogen Inputs

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NITROGEN FIXATION AND DENITRIFICATION IN SEDIMENTS OF EUTROPHIC MEDITERRANEAN-TYPE ESTUARIES: SEASONAL PATTERNS AND RESPONSES TO ANTHROPOGENIC NITROGEN INPUTS

A dissertation submitted in partial satisfaction
of the requirements for the degree Doctor of Philosophy
in Biology

by

Tonya Lynn Kane

2012

ABSTRACT OF THE DISSERTATION

Nitrogen Fixation and Denitrification in Sediments of Eutrophic Mediterranean-type Estuaries: Seasonal Patterns and Responses to Anthropogenic Nitrogen Inputs

by

Tonya Lynn Kane
Doctor of Philosophy in Biology
University of California, Los Angeles, 2012
Professor Peggy M. Fong, Chair

Estuaries are dynamic ecosystems at the interface of terrestrial and oceanic nutrient cycles. Nitrogen (N) fixation and denitrification are key N-cycle components that add or remove N, respectively. In mediterranean-type estuaries, wet and dry seasons affect freshwater inflows and nutrient inputs, and greater coastal development and agriculture increases anthropogenic nutrient inputs, often leading to eutrophication. My research expands understanding of N-cycling in modified mediterranean-type estuaries subject to increasing nutrient influences. Using field surveys and manipulations, I investigated spatial and temporal patterns of N-fixation and relationships with abiotic characteristics and impacts of nutrient inputs on N-fixation and denitrification in unvegetated sediments of intertidal mudflats in southern California estuaries.

A 2-year survey determined N-fixation was influenced by seasonal precipitation, yet played a minor role in supplying N. During dry conditions porewater ammonium (NH_4^+) and nitrate (NO_3^-) were negatively related to N-fixation. In wet conditions, porewater salinity and N-fixation showed a negative relationship, despite simultaneously high NO_3^-. Only a small proportion of N-fixation variability was explained by these factors, possibly because salinity and NO_3^- typically co-vary and have conflicting relationships with N-fixation.

In a microcosm experiment, sediments were subjected to elevated NO_3^- and phosphate (PO_4^{3-}) concentrations, alone and together. NO_3^- concentrations up to 2730μM significantly affected both processes; N-fixation decreased up to 40%, while denitrification increased up to 180-fold. However, denitrification was too low to compensate for high N inputs.

In a small, highly modified estuary, I determined N-fixation and denitrification rates under simulated low and high tide and NO_3^- enriched conditions. There were no differences in N-fixation or denitrification between ambient emerged and submerged conditions, suggesting rates do not vary across tidal cycles. Both processes responded significantly to N-enrichment; N-fixation decreased up to 9-fold and denitrification increased more than 100-fold.

Overall, N-fixation rates were comparable to other eutrophic mediterranean-type systems, though lower than in more oligotrophic systems. Denitrification was lower than in other eutrophic estuaries despite dramatic increases in activity under high NO_3^-. These results provide insight into how N is supplied internally in mediterranean-type estuaries with anthropogenic nutrient impacts and suggest additional nutrient management strategies are necessary to reduce anthropogenic eutrophication.

The dissertation of Tonya Lynn Kane is approved.

Richard F. Ambrose

Richard R. Vance

Peggy M. Fong, Committee Chair

University of California, Los Angeles

2012

I dedicate my dissertation to my parents,

Debbie and Andy Kane,

my first and most influential teachers.

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This research would not have been possible without gas chromatography equipment and technical support provided by Doug Capone and his students at University of Southern California, and by John Duff and Larry Miller at United States Geological Survey in Menlo Park, California. They were all very generous with their time and resources as I learned and executed the techniques necessary to measure nitrogen fixation and denitrification. They also did a great job of providing conversation breaks throughout the uncountable hours of injecting samples and pushing buttons. I cannot thank them enough.

I am beyond thankful to my PhD Advisor, Dr. Peggy Fong, who has been supportive of me in countless ways. She is truly an inspiring mentor with never ending enthusiasm, and I am eternally grateful for her guidance. I am also grateful that she recruited her family to be field assistants! The entire Fong family, including Peggy, Jon, Caitlin, Kendal and Jordan, spent many hours in the field with me. Not only were they integral to my research efforts, but I also consider them family.

In addition to Peggy, I was fortunate to have incredibly supportive PhD committee members, including Drs. Rick Vance, Rich Ambrose, Rebecca Shipe, and Peter Nonacs. I also had fantastic role models as an incoming grad student – thanks to Anna Armitage, Risa Cohen, and Jayson Smith for helping me to navigate my early grad school years in the Fong lab and continuing to demonstrate that academic success is possible! And no student in our department would be able to navigate UCLA without Jocelyn Yamadera. You are the best, Joce!

I had many friends and colleagues who were willing to help me with these projects and didn't mind getting VERY muddy. Without them, the scale of this study would have been impossible. For outstanding field and/or laboratory assistance, thanks to Gwen Noda (especially for her help with the night-shift!), Sarah Bryson, Rachel Kennison, Lauri Green, Jayson Smith, Mathew Lurie, Matt Wartian, David Lin, Sarah Bittick, Ranjan Muthukrishnan, Sara Painter-Kappus, Rachel Clausing, Vanessa Gonzales, Sarah Lombardo, Laura Haas, Christina Chung, Matt Orique, Jennifer Boyer, Chia Lin, Maylen Perez, Dana Gulbransen, and many more undergraduate student volunteers. Thank you all for playing in the mud with me!!!

I am also appreciative for the year I spent away from my PhD as a NOAA Sea Grant Knauss Marine Policy Fellow in Washington, DC. My fellowship year exposed me to wonderful opportunities and tremendous amounts of new knowledge. I learned a great deal about myself and I built strong friendships with amazing people. You are all fantastic and I miss you!

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Now, let's celebrate!

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Kane T & Fong P. 2007. *Quantifying rates and mechanisms controlling sediment nitrogen fixation in a eutrophic estuary.* Oral presentation at Estuarine Research Federation meeting, Providence, RI

Kane T & Fong P. 2007. *Sediment nitrogen fixation in Upper Newport Bay Estuary, southern California.* Poster presentation at Ecology & Evolutionary Biology Research Symposium, UCLA

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Kane T & Fong P. 2012. *Extreme nitrogen enrichment suppresses nitrogen fixation and enhances denitrification, but not enough.* Oral presentation at Ocean Sciences mtg., American Society of Limnology & Oceanography, Salt Lake City, UT

Kane T, Kwon P & Fong P. 2012. *Adventure in the South Pacific: UCLA's Marine Biology Quarter and COSEE-West develop strong research and communication skills in young scientists.* American Society of Limnology & Oceanography, Ocean Sciences mtg., Salt Lake City, UT

INTRODUCTION

Estuaries link terrestrial and oceanic nutrient cycles and function as a filter of nutrients, polluted run-off, and sediments from coastal communities to the near shore waters; thus, they are dynamic locations for the study of nutrients. Estuaries are highly productive ecosystems that are a haven for juvenile fish and migratory birds, and provide critically important habitat to endangered or threatened plants and animals, as well as commercially important species. In southern California, little estuarine habitat remains (Zedler 1996). Studying patterns of nutrient cycling is a powerful tool for assessing large-scale ecosystem functions and may be important for conservation of remaining estuaries, especially in light of global climate change and accelerating anthropogenic impacts.

With increasing development and human population growth in coastal areas, and expansion of agriculture in coastal watersheds, many estuaries are experiencing greater nutrient inputs via runoff enriched by wastewater and fertilizers (Bricker et al. 2008, Galloway et al. 2004, Valiela & Bowen 2002, Vitousek et al. 1997). The magnitude and relative proportions of the nutrient source can depend on land use patterns in the watershed (Valiela & Bowen 2002). Shifts in land-use practices result in greater nutrient loading; modifications of watersheds resulting from urbanization reduce natural land and increased coverage by impervious surfaces and lawns, which accelerates delivery of runoff to coastal systems (Valiela & Bowen 2002). Greater understanding of nutrient inputs and cycling in these highly anthropogenically impacted systems is needed, especially as nutrient regimes may be continuously changing.

Estuarine productivity is driven largely by nitrogen (N), which tends to be the primary limiting nutrient (Howarth & Marino 2006, Paerl 2009). However, anthropogenic activity has resulted in dramatic changes to the N cycle (Vitousek at al. 1997). Globally, anthropogenically created N sources now exceed natural biologically fixed N and are expected to increase (Gruber & Galloway 2008). Anthropogenic impacts on coastal nutrient cycles are a serious concern in many estuarine systems world wide, as excess nutrients can lead to eutrophication (Nixon

1995). Symptoms of eutrophication, including algal blooms and hypoxia, can alter ecosystem function in estuaries (Howarth & Marino 2006, Bricker et al. 2008). Incidences of eutrophication are increasing globally (Diaz & Rosenberg 2008) and it is one of the most important problems to consider in the protection of coastal marine ecosystems (NRC 2000). We must understand the impacts of increased nutrient inputs on estuarine nutrient cycling, especially in estuaries adjacent to highly developed urban areas where anthropogenic impacts are already a major concern.

Estuarine sediments can act as a buffer for organic matter and nutrients in coastal ecosystems, and are very sensitive to eutrophication (Jørgensen 1996). Mudflat habitat can comprise as much as 70% of the area of estuarine habitats in California systems (e.g. Rysgaard et al. 1996, Joye & Paerl 1994) and have the potential to influence much of the nutrient dynamics and potential export of nutrients to coastal waters from natural and anthropogenic sources.

Two key processes in the estuarine N cycle that can be affected by increased supplies of inorganic nutrients are N-fixation and denitrification occurring in sediments. N-fixation and denitrification interact directly with the atmosphere to create new N or remove N from a system. N-fixation is carried out by autotrophic cyanobacteria and heterotrophic bacteria, and provides a source of new biologically available N through the conversion of atmospheric N_2 to ammonium (NH_4^+). Denitrifying bacteria convert NO_3^- into the atmospheric gasses N_2 and nitrous oxide (N_2O), rendering it unavailable to primary producers and effectively removing N from the system. If N is limiting, N-fixation could provide necessary N for primary producers. However, if N is present in excess, denitrification could be a mediating factor in eutrophication by removing a portion of N inputs. How N-fixation and denitrification function to add and subtract N from the budget are important to the health of the estuary and surrounding habitats.

The majority of N-fixation research in marine systems has taken place in the water column, with fewer studies focused on benthic habitats (Carpenter & Capone 2008). Of the studies on benthic sediments, the majority have been in vegetated habitats (e.g., Piehler et al. 1998, Bagwell & Lovell 2000, McGlathery et al. 1998, Capone 1982); only a small number have investigated unvegetated intertidal mudflats. Where rates have been measured, sediment N-fixation has been shown to be a minor contribution to N-budgets (Howarth et al. 1988a). However, N-fixation was found to be important in some estuarine systems (Gardner et al. 2006, Tyler et al. 2003). We have limited understanding of environmental controls on N-fixation in natural systems (Vitousek et al. 2002), and responses are not consistent. NH_4^+ can decrease N-fixation activity (Howarth et al. 1988b), as was found in a southern California marsh experiment (Moseman-Valtierra et al. 2010), but not in eelgrass sediments of an Atlantic system (McGlathery et al. 1998). High concentrations of NO_3^- can also suppress N-fixation activity, as in Tomales Bay, northern California, where N-fixation in a microbial mat decreased significantly during high-NO_3^- runoff following a storm event and returned to pre-runoff levels when water column NO_3^- decreased (Joye & Paerl 1994).

The importance of sediment denitrification varies across estuarine systems. It has been found to remove as much as 50% of N input in some estuaries (Seitzinger 1988), though Nowicki (1994) concluded denitrification might not be significant in reducing eutrophication in one system. Denitrification activity is dependent on NO_3^- availability, either from water column and pore water sources or from NO_3^- produced by nitrification, but some studies suggest denitrification efficiency (i.e. proportion of NO_3^- removed) may decrease with higher N-loads (Mulholland et al. 2008). Denitrification rates increased dramatically with NO_3^- enrichment in San Francisco Bay (Oremland et al. 1984) and Tomales Bay (Joye & Paerl 1994), and denitrification decreased water NO_3^- during and after a storm event in Tomales Bay (Joye & Paerl 1993). The ability to respond to nutrient pulses with an increase in denitrification activity might help to reduce high N inputs to coastal waters.

Southern California has a mediterranean-type climate with distinct wet and dry seasons. The majority of freshwater input to estuaries occurs during the wet season (November to April), and the quantity of N-enriched runoff following rainstorm events can be high (Zedler 1996). These estuaries are in areas of dense human population where watersheds have extensive agricultural and urban/suburban development, resulting in large pulses of freshwater with high concentrations of nutrients and other pollutants to the system. High nutrient levels can lead to increased macroalgal blooms (Kamer et al. 2001, Kennison et al. 2003, Boyle et al. 2004) that alter food webs (L. Green, unpublished data). In the summer dry season, estuaries can function as marine embayments with oceanic salinity, though with increasing urbanization and inland agriculture, nutrient inputs may extend into the dry season (Zedler 1996). Southern California estuaries are representative of mediterranean-type estuaries worldwide that experience similar anthropogenic stresses. Among areas of the world with a mediterranean-type climate, few studies have investigated the impact of nutrient inputs on N-fixation and denitrification in estuary sediments (but see Cabrita & Brotas 2000). However, because changes in N loading may alter rates of N-fixation and denitrification, it is important to study these processes across seasons and under different levels of N pulses. In southern California, only four studies have measured N-fixation in salt marsh sediments in San Diego County (Langis et al. 1991, Moseman 2007, Moseman-Valtierra et al. 2009, Moseman-Valtierra et al. 2010), and no published studies have measured denitrification rates. Our study is the first to address both processes simultaneously in southern California estuarine systems.

I investigated microbial processing of N via N-fixation and denitrification in intertidal mudflat habitats of eutrophic Southern California estuaries. Specifically, I addressed the following questions: 1) What are the spatial and temporal patterns of N-fixation and denitrification rates?, 2) What relationships exist between N-fixation and abiotic factors?, and 3) How are N-fixation and denitrification rates impacted by nutrient inputs? These questions were answered through a series of field surveys and experiments. In Chapter 1, I determined spatial and temporal patterns of sediment N-fixation rates and relationships with abiotic characteristics through a series of field surveys over two years in intertidal mudflats along tidal creeks of Upper

Newport Bay Estuary, an ecological reserve in Orange County, California. In Chapter 2, I performed a microcosm experiment to determine the effect of extremely high water column nutrient concentrations on sediment N-fixation and denitrification rates in a southern California estuary by simulating a nutrient pulse event. In Chapter 3, I measured sediment N-fixation and denitrification rates in Famosa Slough, a small, highly modified estuary in San Diego County, quarterly over one year and investigated how these rates varied tidally, between wet and dry seasons, and in response to N-enrichment of the water.

My research contributes to understanding the microbial N cycling processes of N-fixation and denitrification in a range of estuarine conditions over time, and the response of these processes to anthropogenic nutrient inputs in estuarine ecosystems with a mediterranean-type climate. As southern California estuaries, like many mediterranean-type estuaries, are highly impacted by nutrients due to intense urbanization and agriculture, studying biogeochemical processes that remove or add N is vital to increase our understanding of N-cycling in these systems, and may aid in making more informed decisions regarding management of watersheds and estuarine habitats. This is especially important as nutrient loading and subsequent eutrophication is expected only to increase in the future.

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CHAPTER 1

FRESHWATER PULSES FROM SEASONAL PRECIPITATION ENHANCE SEDIMENT NITROGEN FIXATION ACTIVITY DESPITE HIGH NITRATE CONCENTRATIONS IN A EUTROPHIC MEDITERRANEAN-TYPE ESTUARY

ABSTRACT

Nitrogen (N) fixation may be important to N dynamics of estuaries in mediterranean-type climates, which experience distinct wet and dry seasons that affect freshwater and nutrient inputs. N-fixation may provide new N to primary producers when N-inputs are low in the dry seasons, while in wet seasons high inorganic N concentrations from anthropogenic influences in the watershed may decrease N-fixation activity dramatically. We performed a 2-year survey of sediment N-fixation activity in unvegetated intertidal mudflat habitat of tidal creeks across wet and dry seasons, including a relatively high and low water year. Across all dates, mean N-fixation rates by site ranged from 0 to 39.9 μmol N m^{-2} hr^{-1}, with 95% of site means <16 μmol N m^{-2} hr^{-1}, suggesting N-fixation plays a minor role in supplying N to this estuary. Rates were similar to those measured in other eutrophic mediterranean-type estuaries. During wet season periods, salinity was lowest and nitrate (NO_3^-) was highest in water column and porewater samples, with phosphate (PO_4^{3-}) highest in the water column, implying freshwater runoff due to precipitation was a source of nutrients to the estuary. Sediment characteristics showed spatial differences between creeks, with higher organic matter, clay content, and total N in the upstream creek. Porewater characteristics in our sites grouped together and were statistically different from sediment characteristics based on principle components analysis. Multiple regression analyses of porewater and sediment abiotic characteristics on N-fixation activity showed a strong positive relationship between lower porewater salinity and N-fixation during the wettest period, when highest N-fixation rates occurred despite high NO_3^- inputs. During dry conditions porewater ammonium (NH_4^+) and NO_3^- were most related to N-fixation. Though significant, none of the relationships with the abiotic factors we measured explained a large proportion of the variability in N-fixation, possibly due to the strong relationship between low salinity and high NO_3^-, and their conflicting relationships with N-fixation. Our results highlight the influence of seasonal precipitation dynamics on factors regulating N-fixation, and provide much needed insight into how N is supplied internally in mediterranean-type estuaries with anthropogenic nutrient impacts.

INTRODUCTION

Nitrogen (N) fixation, a key process in the N-cycle, may be an important source of N in estuary systems where primary productivity is often limited by N availability (Vitousek et al. 2002, Kamer et al. 2001); however, the role of N-fixation is understudied in estuaries with mediterranean-type climates. Diazotrophic cyanobacteria and heterotrophic bacteria can produce new bioavailable N by converting atmospheric N_2 to ammonium (NH_4^+) via the nitrogenase enzyme in anaerobic conditions (Capone & Montoya 2001). During fixation, N is incorporated into the bacterial cell as organic N and remineralized to NH_4^+ upon death of N-fixers (Joye & Anderson 2008); fixed N may also be leaked to the surrounding environment as NH_4^+. Traditionally, N-fixation has been thought to be unimportant in estuaries and shallow marine environments (Howarth et al. 1988a, Galloway et al. 2004), but some studies have found it provides an important N source in N-limited or restored estuaries (Gardner et al. 2006), and in young and restored salt marshes (Piehler et al. 1998, Tyler et al. 2003). However, understanding the importance of N-fixation in mediterranean-type systems, especially those that are highly eutrophic, requires more study.

While it is known that much of the microbial N-cycle in estuaries occurs in sediments (Joye & Anderson 2008), the majority of studies in marine environments have focused on water column N-fixation (for a recent review see Carpenter & Capone 2008). Of the few studies on benthic habitats, most occurred in vegetated sediments where N-fixation rates tend to be high due to plant associations (e.g. Piehler et al. (1998) and Bagwell & Lovell (2000) in saltmarshes; McGlathery et al. (1998) and Capone (1982) in sea grass beds). Additionally, as most studies to date have taken place in non-mediterranean climates, there is a knowledge gap in the understanding of N-fixation in mediterranean-type systems. Published studies of N-fixation in unvegetated intertidal mudflats of estuaries in Mediterranean-type climates are sparse (but see Joye and Paerl 1994, Magalhaes et al. 2005, Moseman-Valtierra et al. 2010). Intertidal mudflats can comprise as much as 70% of estuarine habitat in Mediterranean systems (e.g. Rysgaard et al. 1996, Joye and Paerl 1994), and the majority of information we have on N-fixation in this habitat has been extrapolated from other habitats in temperate estuaries. In order to develop a clearer understanding of N processes in estuaries with mediterranean-type climates, we need more information on the role of N-fixation in unvegetated intertidal sediments.

Within estuarine sediments, several abiotic water and sediment characteristics may influence sediment N-fixation rates, though relationships are not always clear and interactions of multiple abiotic factors may make relationships more complex. While the physiology of N-fixation is fairly well characterized, we have limited understanding of ecological controls in natural systems (Vitousek et al. 2002), and most of what we do know is from temperate locations. Organic carbon additions increased N-fixation in vegetated salt marsh (Langis et al. 1991), eelgrass (McGlathery et al. 1998), and unvegetated subtidal sediments (Fulweiler et al. 2007). Fine grain sediments tend to have higher organic content and lower O_2 penetration, and generally exhibit higher N-fixation than course grained sediments (Capone 1988). High salinity has been shown to correlate with lower N-fixation (de Souza and Yoch 1997). High concentrations of inorganic N can affect N-fixation activity negatively. NH_4^+ inhibits synthesis of nitrogenase and can keep N-fixation rates low (Howarth et al. 1988b). NH_4^+ has decreased N-fixation in both vegetated and unvegetated marsh sediments (Sundareshwar et al. 2003, Moseman-Valtierra et al. 2010), though McGlathery et al. (1998) found no effect in eelgrass sediments. Nitrate (NO_3^-) can be transformed to NH_4^+ in sediments via dissimilatory nitrate reduction (An & Gardner 2002), potentially increasing NH_4^+ concentrations. Some N-fixers might take up available inorganic N directly rather than fix N_2 (Tibbles & Rawlings 1994, Paerl & Piehler 2008). Phosphorus (P) enrichment has increased water column N-fixation by planktonic cyanobacteria in a variety of habitats (Howarth et al. 1988b, Sanudo-Wilhelmy et al. 2001, Schindler et al. 2008), though the only experiment that directly investigated P-enrichment in marsh sediments found N-fixation decreased (Sundareshwar et al. 2003). Due to the number of factors that potentially affect N-fixation, and the complexity of interactions between these factors, predicting N-fixation activity is difficult. In order to understand the role N-fixation plays in mediterranean-type estuaries, we must better understand the relationships between N-fixation and the suite of changing abiotic characteristics within these dynamic systems.

While N-fixation may be an important N source in N-limited estuaries (Gardner et al. 2006), the role of N-fixation in eutrophic systems is unclear. Many eutrophic estuaries are adjacent to dense human populations and experience impacts from coastal development, inland agriculture, as well as increased N flows due to modification of estuaries and adjoining watersheds (e.g. Flindt et al. 1997, Cabrita & Brotas 2000, Sfriso et al. 2003, Caffrey et al. 2010, Schiff & Kamer 2000). We would expect N-fixation activity to be low or non-existent in eutrophic environments where sediments are enriched and water column N is high. However, despite high N loads and eutrophication, many primary producers are still N-limited in California estuaries (Kamer et al. 2004), therefore N-fixation may still be important. As eutrophication is already a major concern in coastal waters worldwide (NRC 2000) and anthropogenic N inputs are expected only to increase (Diaz & Rosenberg 2008, Galloway et al. 2008), we must understand the role of N-fixation and how it may be impacted by increased eutrophication.

Southern California estuaries, like other mediterranean-type estuaries, can experience tremendous temporal and spatial variability in salinity and nutrient inputs due to strong wet and dry seasonality (Zedler 1996). During wet seasons estuaries may receive intense pulses of anthropogenic nutrients and freshwater during precipitation events (Page et al. 1995, Boyle et al. 2004), though strength and duration of storm events varies within wet seasons and total precipitation can fluctuate from one year to the next (Zedler 1982). In the estuary featured in our study, wet season water column NO_3^- concentrations as high as 2000μM (Kennison et al. 2003) to 3000 μM (Cohen & Fong 2006) have been reported. In the dry season, low stream flow decreases terrestrial N loads to the estuary, though remineralization in sediments may increase NH_4^+ concentrations (Boyle et al. 2004). Wet/dry seasonality may also affect spatial patterns of abiotic factors. Salinity gradients may be episodic within wet seasons and nearly absent during dry periods when freshwater inputs are minimal. In contrast, nutrient gradients may exist year-round due to coastal development that can extend anthropogenic N-inputs into the dry season. While spatial and temporal patterns may affect abiotic factors that regulate N-fixation activity, little information exists on spatial and seasonal patterns of N-fixation in mediterranean-type estuaries (but see Joye & Paerl 1994). In southern California, just four studies have measured N-fixation in estuaries to date (Langis et al. 1991, Moseman 2007, Moseman-Valtierra et al. 2009, Moseman-Valtierra et al. 2010), of which only one included non-vegetated sediments and none included seasonal comparisons. Understanding the effects of seasonal variability on N-fixation activity is necessary to determine how N-fixation in eutrophic estuaries in mediterranean-type climates, like those in southern California, contributes to the N-cycle, especially during periods of increased N inputs.

To quantify temporal and spatial variability of N-fixation rates and investigate relationships between rates of N-fixation and abiotic environmental parameters in a eutrophic southern California estuary, we performed semi-annual field surveys in intertidal mudflats of tidal creeks over 2 years. We expected N-fixation would be lowest during periods of high inorganic N, especially NH_4^+. As inorganic N concentrations tend to vary with inputs of N-enriched runoff during wet season storms, we expected lowest N-fixation rates in wet seasons, and highest rates in dry periods. However, salinity and N inputs typically have a negative relationship during precipitation events, and as lower N-fixation can correlate with high salinity, we were unable to predict whether low salinity or high inorganic N would influence N-fixation activity more strongly. This study provides information on N-fixation in southern California estuarine sediments, especially the effects of wet/dry seasonality. This information is essential for understanding how watershed influences impact microbial N-fixation activity southern California estuaries and anthropogenically impacted estuaries in mediterranean-type climates worldwide.

METHODS

Field surveys took place in Upper Newport Bay (UNB) estuary, an ecological reserve in Orange County, southern California, USA (N 33^{o} 38.01, W 117^{o} 53.18). At 288 ha, UNB is the second largest estuarine embayment in southern California and is critical habitat for many plant and animal species, several of which are threatened or endangered (Sutula et al. 2006). Like many estuaries, UNB has a highly urbanized watershed. Urbanization has altered the quantity, quality and timing of freshwater inflows, and dredging and filling within the estuary has impacted hydrology (Schiff & Kamer 2000). UNB is comprised of a deep, dredged main channel surrounded by extensive broad mudflat and tidal creek habitat. The mouth of the estuary (Lower Newport Bay) is permanently open to the ocean due to extensive modifications including a large marina. San Diego Creek, the main source of freshwater, enters at the head of the estuary and drains 85% of the 400km^{2} watershed, though there are also several additional small drainage creeks entering mid-estuary (Sutula et al. 2006). Southern California has mixed semi-diurnal tides, with a tidal range of up to 2m in UNB (Nezlin et al. 2009). Intertidal mudflat habitat comprises up to 65ha during low tide (Nezlin et al. 2007). UNB is a eutrophic system with a history of nutrient enrichment from both point and non-point sources (Boyle et al. 2004, U.S. EPA 1998) and is subject to large macroalgal blooms (Kamer et al. 2001) and periods of hypoxia (Nezlin et al. 2009). Due to the mediterranean-type climate, UNB experiences extended dry seasons with no rainfall, and wet seasons with intense rainfall events that bring nutrients into the system in large pulses, when water column NO_3^- concentrations of up to 800μM have been reported (Boyle et al. 2004). Water residence times can be variable, from as little as 1.0 – 3.3 days (Nezlin et al. 2009) up to 30 days (Pednekar et al. 2005).

Surveys were performed within intertidal mudflats of 2 tidal creeks in both wet and dry seasons across 2 years in order to quantify spatial and temporal changes in N-fixation rates. Boyle et al. (2004) identified these intertidal mudflat habitats as important areas for nutrient cycling. We surveyed creeks in two areas, including a “low” creek (L) nearest to the mouth and a “middle” creek (M) in the mid-estuary closer to freshwater inputs (Figure 1). Along with water from San Diego Creek, the middle creek site receives some freshwater input from Big Canyon Wash (McLaughlin et al. 2007). In general, sediment % organic matter, % sand and water column nutrients tend to be highest at the head of the estuary and decrease toward the mouth, while % clay, % silt and water column salinity are lowest at the head and increase toward the mouth (Kennison et al. 2003, Boyle et al. 2004). Surveys took place on 4 March and 18 September 2005, and 6 February and 9 September 2006. Daily precipitation totals were obtained from Orange County Public Works station #165 in Costa Mesa (33^{o}40’16.1”N, 117^{o}53’25.9”W) (www.ocwatersheds.com/RainfallData.aspx).

In each creek, five sites were selected in the intertidal mudflat. During low tide on each sampling period, a 5m transect was deployed along the mudflat at each site parallel to the water line and 1m from the vegetated marsh. Along each transect, samples (n=10) were collected at pre-determined random points. On each date, transects were in the same location, though collection points were re-randomized. Dates were chosen to capture tides that were as low as possible to allow enough time to collect samples at all sites during one low tide. Sampling began as the tide was flowing out and sediments became exposed. At each point, we collected one sediment core for N-fixation measurement (2.1cm diameter, 5cm depth) and four cores (3.6cm diameter, 5cm depth) to determine water content, grain size, organic matter content, and sediment total N. Following collection, all samples were transported on ice to the laboratory at University of California, Los Angeles.

Sediment water content was determined by loss of mass after drying at 60°C. To measure sediment grain size and organic content, sediment was dried at 60°C to a constant weight, ground using a mortar and pestle and sieved to <2mm. Grain size was measured using the hydrometer method (Bouyoucos 1962), and organic matter content determined from loss of mass on ignition in a muffle furnace after 10 hours at 400 °C. Sediment was sent to the Analytical Laboratory at University of California, Davis for quantification of total N determined using the combustion gas analyzer method (AOAC International 1997).

Porewater and creek water were sampled concurrently with sediment core collection. At each site, porewater that pooled in holes left by coring was collected using a syringe at each point along the transect (n=10), and creek water (n=3) was collected from the center of the tidal creek just downslope from each transect. All water was stored in acid-washed plastic sample bottles and transported on ice to the lab where it was immediately vacuum filtered (Whatman GF-C), measured for salinity using a handheld refractometer, and frozen. Samples were sent to the Marine Science Institute (MSI) Analytical Laboratory at University of California, Santa Barbara for analysis of NO_2^+ + NO_3^- (hereafter called NO_3^-), NH_4^+, and phosphate (PO_4^{3-}) concentrations via Flow Injection Analysis.

N-fixation measurements were determined using the acetylene reduction technique (Capone and Montoya 2001, Capone 1993). Acetylene gas acts as a substitute for N_2 in N-fixation, so by quantifying the production of ethylene (C_2H_4) gas from the reduction of acetylene (C_2H_2) gas, nitrogenase activity is measured and N-fixation is indirectly quantified. Sediment cores were extruded into a 125ml Erlenmeyer flask, sealed with a recessed rubber stopper and electrical tape to form an airtight vessel, and purged with N_2 gas to create anaerobic conditions. No additional water was added to incubations, therefore measured N-fixation activity reflected low tide conditions of sediments with associated porewater. Rates may be different during high tide conditions when sediments are submerged and have greater access to surface water (Kane & Fong, unpublished data). To the headspace of each incubation vessel we introduced 20ml of lab-created acetylene gas. We sampled each vessel over 24hrs (at 0, 6, 9, 12, 15, 18, 24hrs) by removing 1.5ml of gas from the headspace using a gas-tight syringe, and storing gas samples in glass blood collection tubes until analysis. Samples were analyzed for ethylene using a gas chromatograph (GC) with Flame Ionization Detector to determine acetylene reduction rates. The production of ethylene from 0-18hrs was used to determine N-fixation rates, and the production of N_2O from 0-6hrs was used to determine denitrification rates; these time points were chosen as they captured the maximum rates observed for the majority of samples. Rates were calculated using equations in Capone and Montoya (2001) and presented as μmol N m^{-2} hr^{-1}.

Statistical Analyses

N-fixation rates, creek water, porewater and sediment characteristics for each date are reported as the creek means of the transect means at each of the five sampling sites per creek. To determine if there were significant differences in water salinity and nutrients, sediment characteristics, and N-fixation rates associated with temporal and spatial factors, we used 2-way full-factorial ANOVAs with date and creek as the factors, and the site means of each variable as the response variables. Site mean data were transformed to meet the assumptions of ANOVA for each response variable. Generalized linear model (GLM) analysis was performed if the variable could not be transformed to meet ANOVA assumptions. Principal component analysis (PCA) was used to determine how abiotic site characteristics related to each other across the whole data set. To determine if N-fixation had significant relationships with key sediment or water characteristics, we performed multiple regression analysis including all variables (except where noted below) for all sites on all dates. We also used multiple regression analyses for each date separately to determine if relationships varied across dates. N-fixation was transformed as necessary to meet assumptions of multiple regression. Site M2 was not included in regression analyses due to missing data; we were unable to collect porewater at this location on any sampling date. Additionally, porewater PO_4^{3-} was not measured on the February 2006 samples, thus PO_4^{3-} was not included in multiple regression analysis of the full data set, though it was included in multiple regressions for all dates except February 2006. Due to strong correlations between % clay, % sand and % silt, we chose % clay to represent sediment grain size. Whenever multicolinearity between variables occurred in the multiple regression analyses, we ran the multiple regression again after removing the appropriate colinear variables. This occurred most often with % organic matter and total sediment N, which tended to be highly correlated; in these cases we removed total sediment N from the model.

RESULTS

Upper Newport Bay has a typical mediterranean-type rainfall pattern with a strong wet/dry cycle and considerable interannual variability (Figure 2). Most rainfall occurred during the wet winter months and virtually none during the dry summer months. Further, the wet seasons were variable; total rainfall during the 2004/2005 water year (July-June) was more than 3-fold greater than in 2005/2006 (64.6cm and 19.2cm, respectively). While March 2005 and February 2006 samplings both fell within the wet season, only in March 2005 was there appreciable rain prior to sampling, with 15.1cm over the 16 days before, including 0.25cm over the 48hrs immediately preceding sampling. In contrast, there was no rainfall for 22 days prior to the February 2006 sampling. During the dry season samplings in September 2005 and September 2006 there was no precipitation for at least 3 months preceding sampling dates.

Creek surface water reflected the difference in precipitation across sampling dates, with lowest salinity and highest NO_3^- and PO_4^{3-} coinciding with periods of rainfall (Table 1A). Two-way ANOVA showed salinity differed significantly by date ($p<0.0001$), but not by creek, and there was no significant interaction. Salinity was lowest in March 2005 followed by February

2006, both wet season dates. Concentrations of NO_3^- and PO_4^{3-} were consistent with the history of NO_3^- and PO_4^{3-} being flushed from terrestrial sources and entering the estuary during precipitation events (Boyle et al. 2004, Cohen & Fong 2006). GLM analysis for NO_3^- showed significant effects of date ($p<0.0001$) and creek ($p=0.0023$), and two-way ANOVA for PO_4^{3-} also showed significant effects of date ($p<0.0001$) and creek ($p=0.0001$), with no significant interaction for either nutrient. Both NO_3^- and PO_4^{3-} were greatest during the wet seasons, with highest values in March 2005 and second highest in February 2006. For all dates, NO_3^- and PO_4^{3-} were higher in the middle creek, closer to sources of terrestrial nutrients, verses the low creek. NH_4^+ was similar between creeks, but significantly different by date ($p=0.0407$). The effect of date was driven by the difference between September 2005 and February 2006, when the lowest and highest NH_4^+ concentrations, respectively, were measured.

Porewater salinity and NO_3^- concentration reflected the patterns observed in the creek water (Table 1B), while NH_4^+ and PO_4^{3-} did not. Both porewater salinity ($p=0.0009$, 2-way ANOVA) and NO_3^- ($p<0.0001$, GLM) were significantly different by date; creek was not significant and there was no interaction for either variable. During the rainfall period in March 2005, high levels of precipitation coincided with the lowest porewater salinity and highest NO_3^- concentrations, with NO_3^- as much as 60-fold higher than on any other date. However, during the February 2006 wet season, when precipitation was lower, these relationships appeared to be weakened, suggesting that February 2006 was more similar to dry season rather than the other wet season date. NH_4^+ was significantly different by date ($p=0.0068$, 2-way ANOVA), with highest concentrations in February 2006; creek was not significant and there was no interaction. Across all dates and in both creeks, NH_4^+ was 2 to 10-fold higher in the porewater as compared to surface water (Tables 1A & 1B), suggesting NH_4^+ was being generated (via N-fixation and/or dissimilatory nitrate reduction) and/or regenerated (via remineralization of organic N) within sediments. PO_4^{3-} concentrations were variable across dates and creeks with no significant patterns. For greater spatial detail of porewater measures, see Appendix A.

Sediment characteristics within a creek were consistent across dates, though showed significant differences between creeks (Table 2) for some variables, and no interaction effects. Two-way ANOVAs showed % organic content (p=0.0212), % clay (p=0.0123) and % total N (p=0.0323) were significantly higher in the middle creek compared to the low creek. There was no significant difference in % sand across dates or creeks based on GLM analysis. See Appendix B for additional spatial detail.

Across all sampling dates, means of site means for N-fixation for creeks ranged from 2 to 16 μmol N m^{-2} hr^{-1} (Figure 3). Variability was high, as mean N-fixation rates for individual sites ranged from 0 to 39.9 μmol N m^{-2} hr^{-1} across dates (Appendix C). Two-way ANOVA showed mean site N-fixation differed significantly by date (p=0.0290), which appears to be driven by the higher rates in March 2005. Across all dates, N-fixation in the middle creek was either similar to or higher than the low creek, though this trend was not significant, likely due to high variability, and there was no interaction between date and creek.

In the principle components analysis (PCA), the first three principle components (PC) explained 81.1% of the variation in the abiotic variables using the full data set, including all sites from both creeks on four sampling dates (Table 3, Figure 4). PC1 alone explained 46.3% of the variation, with sediment characteristic of % organic matter, % clay, and % total N grouped loading positively, while % sand loaded negatively. Porewater characteristics grouped on PC2 and PC3. Porewater NH_4^+ and PO_4^{3-} loading positively on PC2 to explain 19.3% of the variation, and PC3 explained 15.5% of the variation, with salinity loading positively and NO_3^- loading negatively.

Multiple regression analysis of the full data set showed porewater salinity and NH_4^+ had strongly significant negative relationships with N-fixation (Table 4), but explained only 19% of the variation in N-fixation. Breaking the data down by date revealed the relationship between N-fixation and low salinity prominent in the full data set appears to be driven primarily by the March 2005 sampling, which occurred during a period of high precipitation when freshwater input resulted in decreased salinity. However, like across all individual dates, the significant relationship explained only small portion of variation in N-fixation rates (Table 4). In the drier months, when salinity was less variable, porewater nutrients, as well as % clay in February 2006, were the dominant influencing factors on N-fixation. In September 2005 porewater NO_3^- was related significantly to N-fixation rate in a positive direction (Table 4), despite the very small range of porewater NO_3^- concentration on this date (Table 1B). In February 2006, a negative relationship with porewater NH_4^+ and a positive relationship with % clay were significant, while in September 2006, the significant relationships were negative with NH_4^+ and positive with porewater PO_4^{3-}.

DISCUSSION

Episodic rain events during the wet season, typical of mediterranean-type climates, appear to play an important role in regulating N-fixation within the sediments, and may act to supply additional N to the system via N-fixation even during periods of high N-inputs from terrestrial sources. Factors affecting N-fixation, especially nutrients and salinity, are impacted directly by seasonal precipitation events that bring pulses of both freshwater and nutrients, which can act in opposing ways to influence N-fixation and may have complicated the response of N-fixers to storm runoff. In this estuary, highest N-fixation rates coincided with greatest precipitation, despite the simultaneous increase in N from terrestrial sources that has been shown to decrease N-fixation (see Kane PhD Dissertation Chapters 2 & 3). Conversely, N-fixation decreased when terrestrial N sources were low, likely limited by higher salinity and possibly suppressed by porewater NH_4^+ due to internal N recycling within sediments. Thus, salinity was likely the seasonal driver of N-fixation, as low salinity appears to have exerted a stronger influence than high N concentration on N-fixation activity during the wettest period. This relationship implies inorganic N inputs may increase due to internal N-fixation during storm events in addition to already N-enriched runoff from terrestrial sources. Our results highlight the necessity of sampling across seasons and over multiple years in order to capture temporal changes, especially in mediterranean-type climates where seasonal rainfall differences are extreme and rainfall patterns between years can be inconsistent in strength and duration, as occurred during the years we sampled. The specific effects of salinity on N-fixation, especially when interacting with increased N, should be tested explicitly in future experiments, as these relationships have seasonal implications for microbial N processing in these dynamic mediterranean-type estuaries.

N-fixation did not supply a substantial source of N when watershed N sources were low. During the two dry season dates and the drier wet season in February 2006, high porewater NH_4^+ was negatively related to N-fixation. This relationship suggests internal nutrient recycling processes in the sediment that generate NH_4^+, which could include remineralization of organic matter and dissimilatory nitrate reduction to ammonium (DNRA), may be important in the regulation of N-fixation. DNRA was found to be important in some eastern US and Gulf of Mexico estuaries (Tobias et al. 2001, Gardner et al. 2006) and in a Mediterranean estuary in France (Rysgaard et al. 1996). Investigating the role of other sediment N processes, especially DNRA, could be an important next step in understanding N-cycles and effects of eutrophication in these systems. Additionally, as our rate measurements all took place during low tide exposed conditions, it will also be important to measure these processes during submerged periods at high tide to get a more complete picture of microbial N cycling.

Our study demonstrated intertidal sediments contribute fixed N to this mediterranean-type estuary throughout the year, though N-fixation was generally low relative to Tomales Bay, the only other estuary in California where study of N-fixation has focused on intertidal mudflats. When compared to Tomales Bay, a more oligotrophic mediterranean-type system in northern California, our rates were 8 to 50-fold lower than N-fixation associated with an intertidal microbial mat community on those mudflats (Joye & Paerl 1993). However, N-fixation rates in Tomales Bay approached zero when exposed to water column NO_3^- concentrations up to

162μM (Joye & Paerl 1993), while we still measured rates of 5 μmol N m^{-2} hr^{-1} with 90μM NO_3^-, even with a similar reduction in salinity in both studies. The range of N-fixation activity in UNB was less dynamic than in oligotrophic Tomales Bay, which may be partially due to differences in the N-fixer communities (i.e. cyanobacteria vs. hetertrophic bacteria). It may also suggest that the microbial community in UNB, which has been subject to a history of increased N, may maintain low levels of N-fixation activity as an adaptation to boom and bust pulses of inorganic N experienced as a result of extreme wet and dry seasonality. While N-fixation in the mudflats may not be a dominant process in N dynamics of UNB, N-fixation activity was observed year-round and should be included in N-budgets, especially as unvegetated mudflats can comprise large portions of estuarine habitat.

N-fixation rates in UNB were more similar to rates in other eutrophic mediterranean-type systems, including muddy and sandy sediments of the moderately eutrophic Duoro River estuary, Portugal (2.67 umol N m^{-2} h^{-1}) (Magalhaes et al. 2005), and unvegetated marsh sediments in highly eutrophic Tijuana Estuary, southern California (8 umol N m^{-2} h^{-1}, assuming 3:1 C_2H_2:N_2 ratio) (Moseman-Valtierra et al. 2010). While the extent of eutrophication differs among these three systems, N-fixation rates were similar, suggesting N-fixation activity may be suppressed in even moderately eutrophic sediments. Estuary characteristics can be highly variable between, and even within, regions and climates, thus it is important to study N-fixation in a wide range of estuaries in order to get a complete picture of microbial N cycling.

It is not clear if the cycle we documented in these intertidal mudflats is natural or if the patterns were due to the modified state of this particular estuary. While highly significant, relationships with abiotic characteristics were rather weak and did not explain a large proportion of variability in N-fixation. These weak relationships suggest N-fixation rates were not strongly driven by individual abiotic factors, but were likely regulated by co-variation of positive and negative relationships between driving variables. Our study has implications for understanding how microbial N cycling could be affected by future changes in weather patterns due to long term climate change if it is associated with an altered precipitation regime, as well as to short term changes related to El Niño and La Niña events. In southern California, El Niño tends to bring very wet years with high rainfall, while La Niña tends to bring drought conditions (Cayan et al. 1999). Similarly, climate change predictions indicate more extreme weather events are expected to increase in the future. Therefore, by altering precipitation patterns, El Niño/La Niña and climate change are likely to exert strong influences on nutrient and salinity characteristics of the estuary. Our results suggest that N contributed by N-fixation may increase during wet periods and decrease during dry periods. However, additional studies are needed in both eutrophic and non-eutrophic estuaries in mediterranean-type climates in order to understand how N-fixation contributes to N dynamics in these systems and how they might respond to future climate changes, especially as related to precipitation. Future studies must also consider these changes in the context of anthropogenic influences that impact nutrient dynamics and hydrology of these systems.

Table 1-1. Mean (Std Err) by creek of site (transect) means for salinity and nutrient concentrations of A) creek surface water, and B) porewater. nd = no data collected.

A.

	2005		2006	
	March	**September**	**February**	**September**
Salinity (ppt)				
Low creek	21.9 (2.9)	30.3 (2.8)	25.5 (3.4)	31.1 (2.6)
Middle creek	15.3 (3.7)	29.4 (2.9)	22.5 (3.0)	32.3 (1.2)
NO_3^- (μM)				
Low creek	21.9 (5.3)	1.6 (0.5)	7.2 (1.4)	4.2 (2.7)
Middle creek	41.8 (9.4)	6.8 (5.8)	19.3 (4.7)	7.4 (3.7)
NH_4^+ (μM)				
Low creek	8.60 (2.19)	7.87 (1.42)	9.12 (0.69)	8.25 (0.64)
Middle creek	8.08 (2.97)	5.30 (0.99)	18.18 (6.54)	9.86 (1.54)
PO_4^{3+} (μM)				
Low creek	3.49 (0.33)	1.77(0.19)	nd	1.47 (0.33)
Middle creek	7.13 (0.53)	2.77 (0.52)	nd	2.49 (0.16)

B.

	2005		2006	
	March	**September**	**February**	**September**
Salinity (ppt)				
Low creek	28.9 (3.8)	33.7 (1.1)	32.1 (1.3)	34.3 (0.4)
Middle creek	24.3 (0.8)	32.6 (1.2)	30.1 (1.1)	34.3 (0.4)
NO_3^- (μM)				
Low creek	90.8 (18.2)	0.7 (0.2)	1.5 (0.2)	1.4 (0.4)
Middle creek	44.3 (10.7)	0.6 (0.1)	2.4 (1.0)	1.4 (0.1)
NH_4^+ (μM)				
Low creek	17.94 (2.53)	68.87 (10.26)	69.61 (10.65)	51.36 (11.31)
Middle creek	50.46 (16.97)	54.50 (20.43)	175.28 (113.47)	62.95 (27.51)
PO_4^{3-} (μM)				
Low creek	6.38 (2.43)	4.59 (1.35)	nd	1.72 (0.60)
Middle creek	3.63 (1.84)	6.55 (3.11)	nd	4.54 (2.38)

Table 1-2. Mean (Std Err) by creek of site (transect) means for sediment characteristics on all sampling dates.

	2005		**2006**	
	March	**September**	**February**	**September**
% organic matter				
Low creek	2.47 (0.63)	2.33 (0.66)	2.40 (0.64)	2.44 (0.68)
Middle creek	3.71 (0.70)	3.50 (0.84)	3.33 (0.94)	3.50 (0.56)
% sand				
Low creek	59.74 (9.76)	62.29 (12.25)	58.60 (10.42)	57.01 (11.42)
Middle creek	47.07 (8.03)	52.37 (7.30)	54.25 (8.03)	43.96 (7.79)
% clay				
Low creek	16.57 (3.90)	16.45 (4.23)	17.10 (4.22)	24.20 (5.90)
Middle creek	27.65 (5.13)	25.01 (4.06)	24.40 (3.54)	30.47 (4.00)
% total N				
Low creek	0.086 (0.025)	0.070 (0.026)	0.082 (0.026)	0.090 (0.028)
Middle creek	0.110 (0.025)	0.122 (0.029)	0.133 (0.036)	0.134 (0.016)

Table 1-3. Results of PCA showing A) eigenvalues and cumulative % variation explained by the principal components (PC), B) loading of eigenvalues of the variable included in analysis on the first three principal components. Bold values indicate variables that are dominant on that PC.

A. Eigenvalues

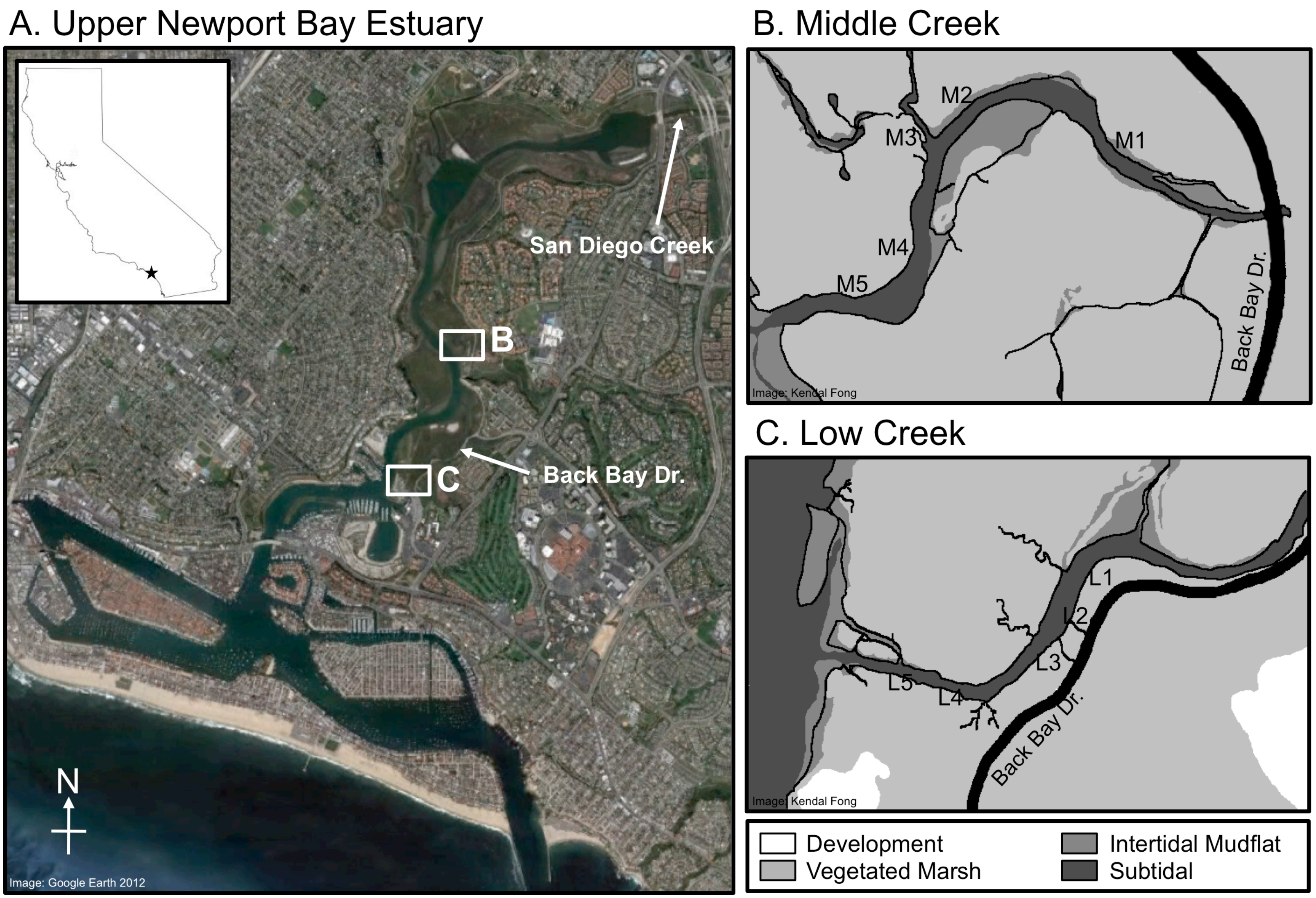
PC number	Eigenvalue	% Variaton	Cumulative % Variation
1	3.7008	46.260	46.260
2	1.5447	19.308	65.569
3	1.2413	15.517	81.085
4	0.6619	8.274	89.359
5	0.4028	5.035	94.394
6	0.2008	2.510	96.904
7	0.1385	1.732	98.635
8	0.1092	1.365	100.000

B. Eigenvector Loading

	PC1	PC2	PC3
salinity	-0.4826	0.1848	**-0.5364**
NO3	0.0122	-0.2701	**0.8670**
NH4	0.4959	**0.7883**	-0.0728
PO4	0.5541	**0.6738**	0.3063
% organics	**0.8666**	0.0036	-0.0028
% sand	**-0.8447**	0.3841	0.1744
% clay	**0.7638**	-0.4632	-0.2550
sediment total N	**0.9312**	0.0036	-0.0645

Table 1-4. Significant variables as indicated by multiple regression analyses.

	Regression Coefficient	Standardized Reg. Coeff.	t Ratio	p-value	R^2	adjusted R^2
all dates combined						
Salinity	-0.0785	-0.03965	-6.77	<0.0001	0.2068	0.1913
NH_4^+	-0.0024	-0.2258	-3.6	0.0004		
March 2005						
Salinity	-0.1141	-0.6198	-4.96	<0.0001	0.3261	0.2487
September 2005						
NO_3^-	0.1878	0.4631	4.76	<0.0001	0.2813	0.2287
February 2006						
% clay	0.057	0.5067	3.59	0.0006	0.288	0.2364
NH_4^+	-0.0027	-0.3723	-2.79	0.0069		
September 2006						
PO_4^{3-}	0.0501	0.3709	2.98	0.0039	0.1983	0.1204
NH_4^+	-0.0043	-0.3427	-2.37	0.0204		



A. Upper Newport Bay Estuary

B. Middle Creek

C. Low Creek

Figure 1-1. Map of Upper Newport Bay Estuary showing A) locations of sampled creeks, B) sites within the middle creek, and C) sites within the low creek.

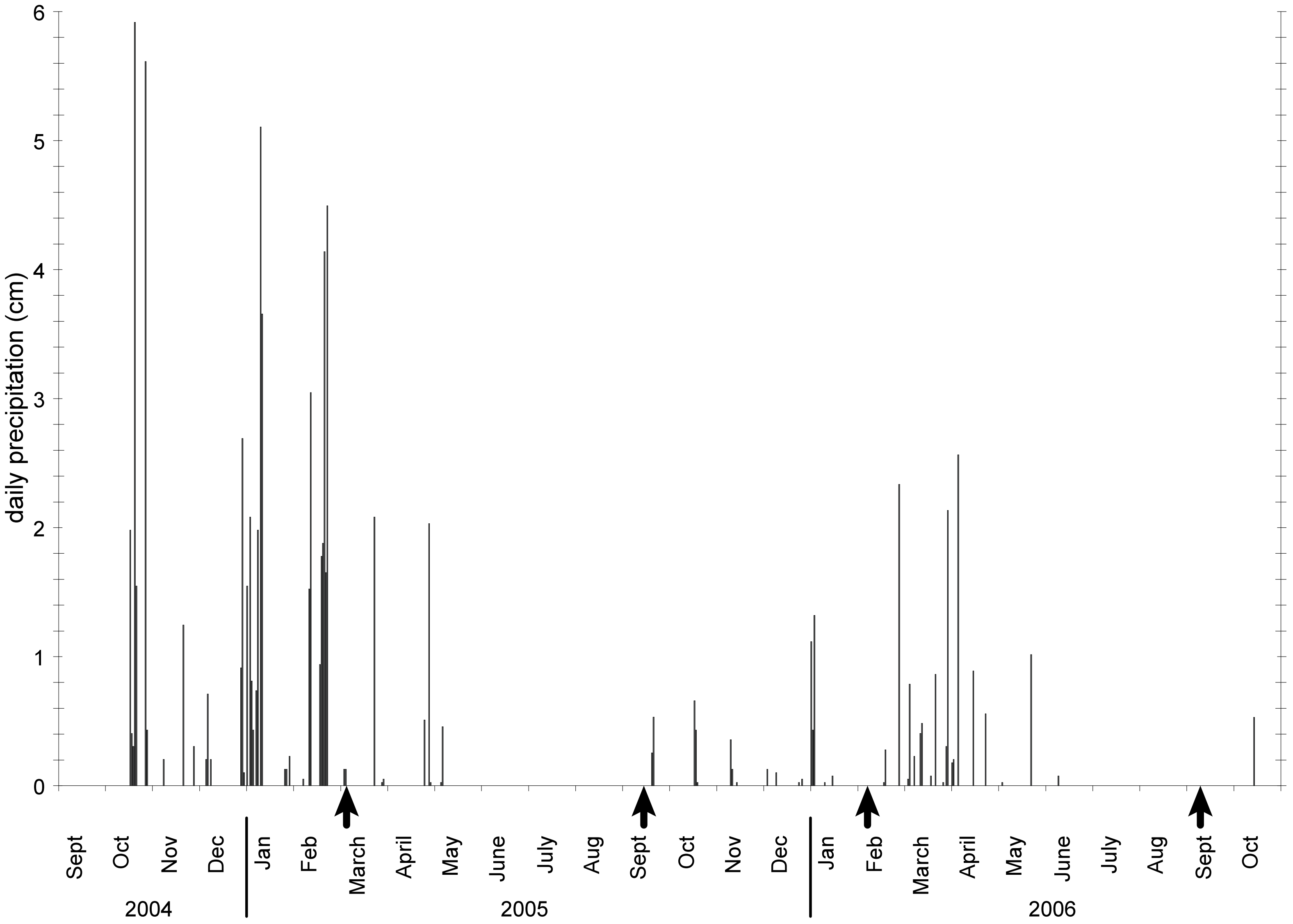


Figure 1-2. Daily precipitation (cm) for Costa Mesa Station #165. Arrows indicate sampling dates.

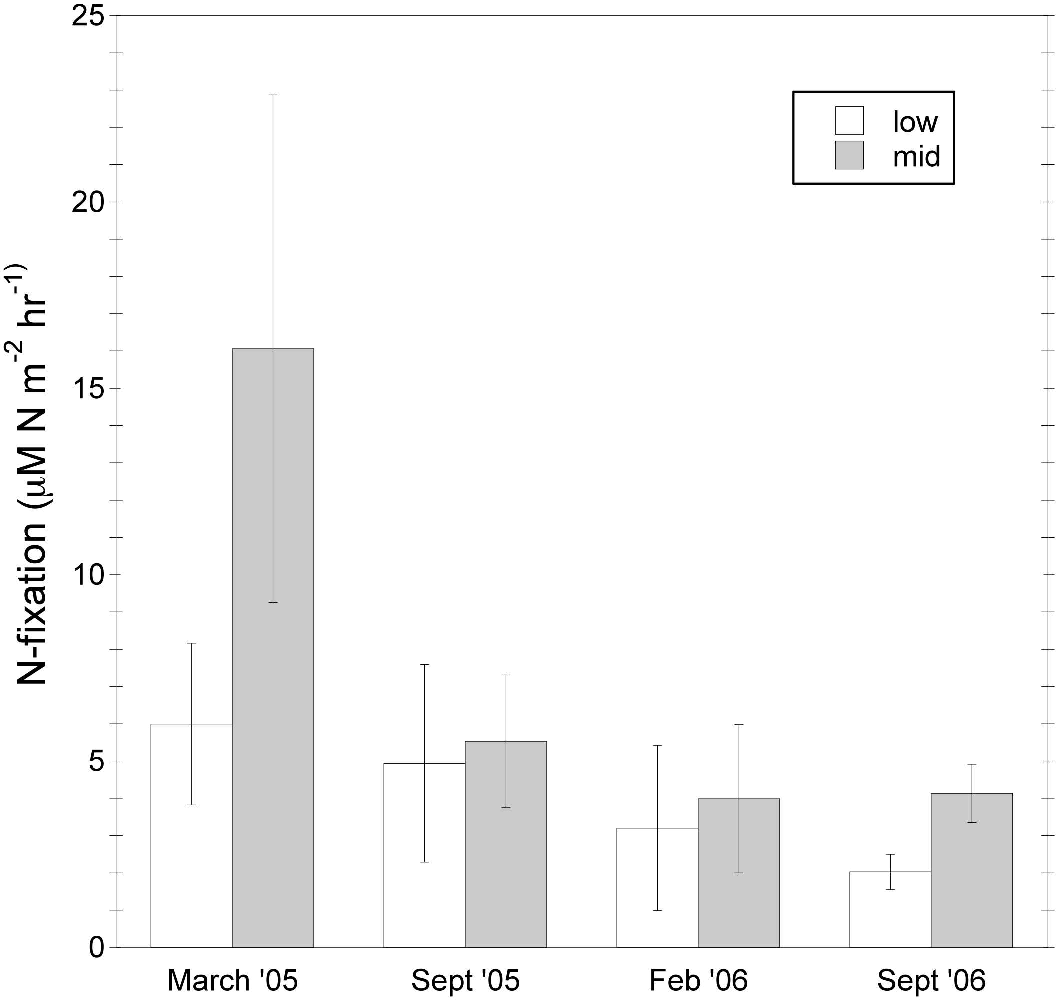


Figure 1-3. Creek means of site (transect) means for N-fixation rates in Low (white bars) and Middle (grey bars) creeks for each sampling date. Error bars indicate standard errors.

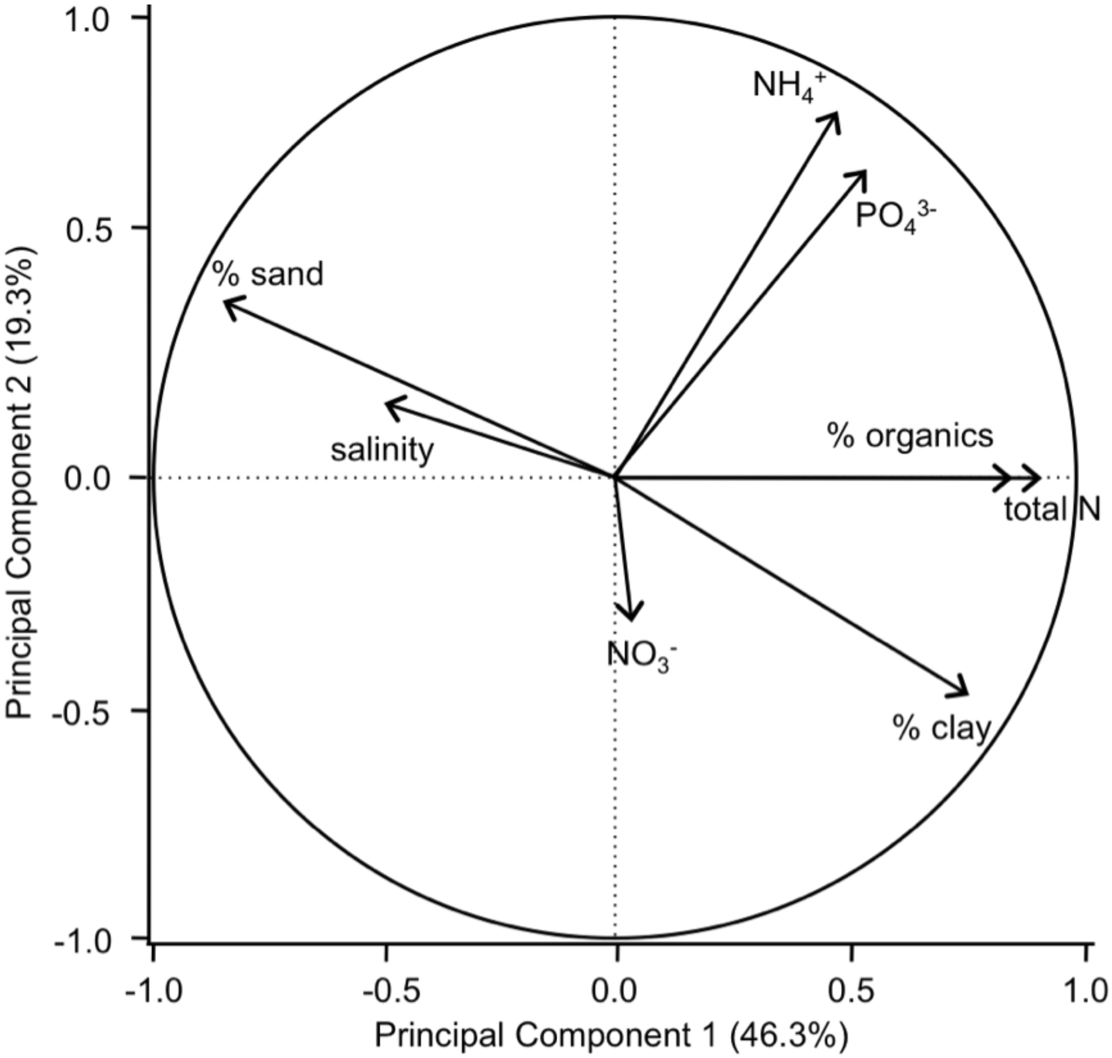


Figure 1-4. Biplot of PCA results showing loading of variables on PC1 and PC2.

Appendix 1-A. Mean (std err) of porewater salinity and nutrient concentrations for each site on all sampling dates. nd = no data collected.

Site	March 2005	Sept 2005	Feb 2006	Sept 2006
Salinity (ppt)				
L1	25.0 (0.4)	31.7 (0.7)	30.6 (0.2)	34.0 (0.3)
L2	32.2 (1.0)	35.7 (0.4)	33.0 (1.1)	35.6 (0.5)
L3	20.6 (1.7)	30.5 (0.5)	27.9 (1.4)	33.2 (0.6)
L4	37.7 (1.7)	35.4 (0.7)	35.4 (0.7)	34.6 (0.4)
L5	nd	35.4 (0.2)	33.5 (0.5)	34.0 (0.3)
M1	24.3 (0.5)	29.6 (0.4)	27.9 (1.5)	34.7 (0.5)
M2	nd	nd	nd	nd
M3	25.5 (0.5)	34.8 (0.1)	31.0 (0.3)	33.1 (0.5)
M4	25.1 (0.3)	34.2 (0.4)	nd	34.6 (0.4)
M5	22.1 (0.4)	31.7 (0.4)	31.5 (0.3)	34.9 (0.2)
NO_3^- (μM)				
L1	100.27 (19.46)	0.47 (0.06)	0.92 (0.10)	0.67 (0.06)
L2	130.92 (35.09)	0.65 (0.09)	1.44 (0.30)	0.90 (0.05)
L3	43.04 (15.09)	0.43 (0.04)	2.02 (0.35)	0.96 (0.09)
L4	88.94 (33.92)	0.66 (0.12)	1.48 (0.10)	2.33 (0.62)
L5	nd	1.35 (0.36)	1.62 (0.17)	2.36 (0.32)
M1	60.25 (24.06)	0.49 (0.11)	4.39 (0.87)	1.74 (0.25)
M2	nd	nd	nd	nd
M3	37.21 (16.80)	0.50 (0.06)	1.13 (0.15)	1.38 (0.04)
M4	17.07 (7.76)	0.66 (0.10)	nd	1.39 (0.09)
M5	62.69 (21.18)	0.76 (0.10)	1.79 (0.22)	1.07 (0.07)
NH_4^+ (μM)				
L1	10.44 (9.21)	85.29 (11.23)	89.31 (6.49)	75.71 (9.26)
L2	19.79 (12.23)	89.27 (4.50)	84.51 (18.32)	19.57 (6.18)
L3	19.95 (10.03)	81.75 (1.66)	86.83 (7.06)	78.74 (13.43)
L4	21.58 (13.36)	41.14 (10.06)	46.49 (7.79)	39.66 (10.22)
L5	nd	46.89 (4.55)	40.88 (6.27)	43.11 (9.66)
M1	100.05 (44.11)	111.33 (29.45)	400.11 (29.41)	29.23 (9.37)
M2	nd	nd	nd	nd
M3	27.52 (12.33)	17.02 (7.11)	36.19 (10.47)	144.80 (23.13)
M4	44.73 (11.49)	35.20 (9.72)	nd	32.22 (6.83)
M5	29.53 (12.15)	54.46 (10.30)	89.54 (9.89)	45.56 (10.82)
PO_4^{3-} (μM)				
L1	13.38 (2.51)	7.06 (1.27)	nd	3.98 (0.81)
L2	5.56 (0.90)	4.43 (0.38)	nd	1.25 (0.28)
L3	2.27 (0.54)	8.16 (0.93)	nd	1.77 (0.42)
L4	4.31 (1.97)	2.10 (1.51)	nd	0.61 (0.10)
L5	nd	1.19 (0.16)	nd	0.98 (0.13)
M1	9.10 (2.69)	15.83 (3.89)	nd	1.93 (0.43)
M2	nd	nd	nd	nd
M3	2.38 (0.96)	2.86 (0.35)	nd	11.69 (3.14)
M4	1.02 (0.33)	3.37 (0.45)	nd	2.14 (0.39)
M5	2.01 (0.92)	4.13 (0.70)	nd	2.40 (0.48)

Appendix 1-B. Mean (std err) of sediment characteristics for each site on all sampling dates.

Site	March 2005	Sept 2005	Feb 2006	Sept 2006
% organic matter				
L1	3.99 (0.23)	3.66 (0.08)	3.08 (0.08)	3.43 (0.12)
L2	2.82 (0.27)	1.41 (0.12)	3.01 (0.29)	2.55 (0.12)
L3	3.53 (0.12)	4.22 (0.33)	4.09 (0.14)	4.34 (0.23)
L4	1.06 (0.05)	1.29 (0.16)	0.98 (0.06)	1.09 (0.03)
L5	0.96 (0.12)	1.08 (0.13)	0.85 (0.06)	0.78 (0.03)
M1	6.16 (0.43)	6.62 (0.23)	6.93 (0.19)	3.64 (0.18)
M2	2.02 (0.09)	2.24 (0.13)	2.11 (0.08)	2.53 (0.16)
M3	3.94 (1.64)	1.86 (0.19)	1.85 (0.08)	2.08 (0.17)
M4	3.72 (0.26)	3.38 (0.10)	3.37 (0.10)	4.03 (0.07)
M5	2.71 (0.18)	3.38 (0.13)	2.39 (0.21)	5.24 (0.48)
% sand				
L1	37.84 (2.06)	33.45 (0.95)	43.20 (0.91)	46.15 (1.31)
L2	60.50 (3.52)	77.95 (1.69)	49.70 (3.42)	51.29 (2.39)
L3	37.65 (10.55)	31.40 (0.84)	33.45 (0.95)	22.81 (2.36)
L4	82.54 (1.10)	84.95 (1.40)	83.20 (0.91)	82.90 (1.00)
L5	80.17 (1.82)	83.70 (1.89)	83.45 (1.69)	81.90 (0.62)
M1	29.32 (1.36)	50.31 (1.46)	41.20 (1.43)	54.16 (1.38)
M2	37.95 (1.73)	58.20 (1.24)	48.95 (2.94)	35.40 (1.80)
M3	72.45 (0.99)	77.20 (1.40)	82.20 (0.76)	69.65 (2.34)
M4	36.70 (1.07)	36.70 (0.93)	37.95 (1.37)	31.39 (1.19)
M5	58.95 (2.39)	39.45 (1.80)	60.95 (4.02)	29.22 (2.16)
% clay				
L1	30.11 (1.13)	28.55 (0.92)	24.80 (0.73)	26.34 (0.67)
L2	17.68 (1.69)	12.05 (0.69)	21.80 (1.39)	28.09 (1.27)
L3	17.20 (6.30)	24.57 (1.36)	25.05 (1.06)	43.24 (2.42)
L4	8.03 (0.68)	8.05 (0.77)	5.55 (0.42)	11.47 (0.64)
L5	9.82 (0.73)	9.05 (0.79)	8.30 (0.55)	11.84 (0.50)
M1	36.80 (0.63)	23.61 (0.90)	25.30 (1.00)	28.21 (1.01)
M2	30.05 (0.92)	23.30 (1.30)	26.05 (1.06)	29.37 (1.77)
M3	11.55 (0.45)	11.80 (0.65)	14.30 (0.53)	17.24 (1.22)
M4	39.05 (1.02)	36.05 (5.80)	35.80 (1.00)	39.16 (0.64)
M5	20.80 (1.07)	30.30 (3.73)	20.55 (2.15)	38.38 (1.72)
% total N				
L1	0.149 (0.003)	0.137 (0.003)	0.120 (0.003)	0.135 (0.005)
L2	0.098 (0.011)	0.039 (0.006)	0.107 (0.009)	0.101 (0.002)
L3	0.127 (0.005)	0.130 (0.005)	0.141 (0.006)	0.164 (0.008)
L4	0.027 (0.004)	0.025 (0.003)	0.020 (0.000)	0.029 (0,005)
L5	0.027 (0.004)	0.020 (0.000)	0.020 (0.000)	0.022 (0.002)
M1	0.198 (0.015)	0.229 (0.017)	0.267 (0.008)	0.150 (0.009)
M2	0.065 (0.003)	0.075 (0.005)	0.084 (0.003)	0.092 (0.005)
M3	0.076 (0.005)	0.061 (0.005)	0.071 (0.003)	0.097 (0.008)
M4	0.129 (0.005)	0.122 (0.001)	0.145 (0.005)	0.161 (0.005)
M5	0.082 (0.005)	0.122 (0.002)	0.096 (0.008)	0.168 (0.008)

Appendix 1-C. Mean (std err) of N-fixation rates (μmol N M^{-2} hr^{-1}) for each site.

Site	**March 2005**	**Sept 2005**	**Feb 2006**	**Sept 2006**
L1	12.3 (1.3)	2.1 (0.2)	3.6 (0.5)	2.1 (0.6)
L2	4.5 (0.7)	2.8 (0.7)	11.7 (3.1)	3.7 (0.7)
L3	2.8 (1.3)	2.0 (0.3)	0.5 (0.3)	1.3 (0.5)
L4	0.7 (0.4)	2.4 (0.5)	0.0 (0.0)	2.0 (1.0)
L5	9.7 (2.5)	15.5 (3.8)	0.2 (0.2)	1.0 (0.6)
M1	5.2 (0.6)	3.6 (0.9)	0.0 (0.0)	2.8 (0.8)
M2	3.6 (1.1)	12.1 (1.8)	0.0 (0.0)	5.5 (1.8)
M3	22.3 (9.8)	2.3 (0.3)	4.1 (0.5)	6.4 (3.9)
M4	9.2 (0.9)	6.3 (1.7)	5.1 (0.5)	3.5 (0.6)
M5	39.9 (7.7)	3.3 (0.5)	10.8 (2.3)	2.4 (0.4)

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CHAPTER 2

EXPERIMENTAL NITROGEN ADDITIONS ENHANCE DENITRIFICATION AND SUPRESS NITROGEN FIXATION IN SEDIMENTS OF A EUTROPHIC ESTUARY IN A MEDITERRANEAN-TYPE CLIMATE

ABSTRACT

Anthropogenic alterations to coastal ecosystems, especially nutrient inputs leading to eutrophic conditions, can have significant impacts on key nutrient cycle processes, including nitrogen fixation (N-fixation) and denitrification. Alterations to these processes may be especially important in estuaries in mediterranean-type climates adjacent to large human populations where nutrient impacts are of increasing concern, but N-fixation and denitrification are not well studied in mediterranean-type systems. To determine the effects of increased nutrient loads on rates of sediment N-fixation and denitrification, sediments were collected from intertidal mudflat habitat along a tidal creek of one eutrophic southern California estuary and subjected to elevated nitrogen (N) and phosphorus (P) in a laboratory microcosm experiment using a 2x2 factorial design. While P additions did not directly affect N-fixation or denitrification rates significantly, N additions had strong effects. Ambient N-fixation rates of 9.5μmol N m^{-2} h^{-1} were comparable to other eutrophic mediterranean-type estuaries, and much lower than non-eutrophic systems. High concentrations of nitrate (NO_3^-) up to 2730uM, typical of highly enriched conditions observed in southern California estuaries, inhibited N-fixation in surface sediments by up to 40%, though this was a minor reduction in total new N input compared to typically large anthropogenic sources. Denitrification activity was lower than N-fixation in ambient-N treatments, but increased up to 180-fold with the addition of NO_3^- to rates as high as 45μmol N m^{-2} h^{-1}. Even with this dramatic increase, denitrification was lower than in other eutrophic estuarine systems. Although denitrification activity increased in NO_3^- enriched microcosms,

denitrification as the percentage of total dissolved inorganic N was <1%. Therefore, neither the decrease in N-fixation nor the increase in denitrification occurred at a level high enough to compensate for the large dose of N. Other nutrient management strategies are necessary to reduce anthropogenic eutrophication in these systems.

INTRODUCTION

At the interface between freshwater and coastal ocean systems, estuaries are important filters of nutrients to nearshore waters, yet there are concerns this critical function could become overloaded due to human impacts (Cloern 2001, Kemp et al. 2005, Paerl & Piehler 2008). With increased human population growth in coastal areas and expansion of agriculture in watersheds, estuaries are experiencing greater inputs of nutrients via runoff enriched by wastewater and fertilizers (Vitousek et al. 1997, Valiela & Bowen 2002, Galloway et al. 2004, Bricker et al. 2007). Excess nutrients can lead to eutrophication (Nixon 1995) and result in algal blooms and hypoxia, which can alter ecosystem function (Vitousek et al. 1997, Diaz & Rosenberg 2008). Eutrophication is a major issue to consider in protection of coastal marine ecosystems (Smith & Schindler 2009, NRC 2000). Incidences of severe eutrophication are increasing worldwide (Diaz & Rosenberg 2008), and reducing the input of both nitrogen (N) and phosphorus (P) is important for controlling estuarine eutrophication (Paerl 2009).

Sediments play an important role in the storage and buffering of nutrients in coastal ecosystems, and eutrophic conditions can affect the ability of sediments to process nutrients (Jørgensen 1996, Eyre & Ferguson 2009). Typically, N is the primary limiting nutrient in temperate estuaries (Vitousek & Howarth 1991, Howarth & Marino 2006). Two key processes at the foundation of the estuarine N cycle are microbial nitrogen fixation (N-fixation) and denitrification, which occur in the sediments and may act to buffer fluctuations of N in estuaries. N-fixation by cyanobacteria and heterotrophic bacteria provides a source of biologically available N via conversion of atmospheric nitrogen (N_2) to ammonium (NH_4^+). Denitrifying bacteria convert nitrate (NO_3^-) to the atmospheric gases N_2 and nitrous oxide (N_2O). Thus, if denitrification activity increases when high levels of N are present, then denitrification could be a mediating factor in coastal eutrophication by removing N (Seitzinger et al. 2006, Brush 2009), and ultimately protecting coastal water quality. Traditionally, N-fixation has been considered a minor contributor to the N-cycle in estuaries (Howarth et al. 1988a), and denitrification the more dominant process in estuarine sediments (Seitzinger 1988). However, N-fixation has been found to be an important N source in some N-limited estuaries (Gardner et al. 2006) and in young and restored salt marshes (Piehler et al. 1998, Tyler et al. 2003). In some estuaries, N-fixation and denitrification in microbial mats and sediments were each the dominant process at different times, switching on diel and seasonal scales (Joye & Paerl 1994, Gardner et al. 2006, Fulwieler et al. 2007). Since ecosystem response to nutrient enrichment is not the same across all estuarine systems (Cloern 2001), it is essential to understand how N-fixation and denitrification contribute to the N cycle, especially in response to increased nutrient loads of N and P.

N-fixation may supply N during periods of N-limitation, but how sediment N-fixation is impacted by high anthropogenic nutrient inputs requires further study. The majority of studies in marine environments have focused on water column N-fixation (Carpenter & Capone 2008), and we have limited understanding of controls on sediment N-fixation. Inorganic N supply can influence sediment N-fixation rates. NH_4^+ can inhibit synthesis of nitrogenase, the enzyme responsible for fixing N, which may keep N-fixation rates low in sediments (Howarth et al. 1988b), and NH_4^+ has been shown to decrease N-fixation in both vegetated and unvegetated sediments (Sundareshwar et al. 2003, Moseman-Valtierra et al. 2010). Also, NO_3^- can be transformed to NH_4^+ in sediments via dissimilatory nitrate reduction (An & Gardner 2002), potentially increasing NH_4^+ concentrations and leading to decreased N-fixation. In northern California, N-fixation in a microbial mat decreased significantly, approaching zero N-fixation, during a period of moderately high NO_3^- runoff (Joye & Paerl 1994). Since N-fixation is a costly energetic process, N-fixers might preferentially take up inorganic N directly when available rather than fix N_2; thus, under extremely high inorganic N inputs we expect N-fixation activity could cease completely. While water column P enrichment increased water column N-fixation by planktonic cyanobacteria in lakes, an estuary, the open ocean, and in pure cultures (Howarth et al. 1988b, Sanudo-Wilhelmy et al. 2001, Schindler et al. 2008), to our knowledge only one experiment has directly investigated the effect of P on sediment N-fixation. This study found N-fixation in vegetated salt marsh sediments decreased with added P, though this decrease was thought to be related to secondary carbon limitation (Sundareshwar et al. 2003). Understanding the dynamics of sediment N-fixation in estuaries exposed to high N and P conditions will provide important information on the relative importance of N-fixation to the system during periods of anthropogenic nutrient stress.

Our study focuses on the high end of the nutrient load ranges of N and P experienced by some estuaries. It is important to determine the amount of N removed by denitrification during high N loads in order to understand the extent and nature of ecosystem management necessary to mediate eutrophic conditions due to anthropogenic N inputs. The importance of sediment denitrification and the % of N supply removed by denitrification ranges widely across estuaries, from less than 8% in one system (Nowicki et al. 1997) up to 50% of N input in others (Seitzinger 1988), and is influenced by N availability. Denitrification utilizes NO_3^- from water column and porewater sources, and from NO_3^- produced by nitrification (but see Seitzinger et al. 2006). Thus, NO_3^- enriched water can increase denitrification significantly (Oremland et al. 1984, Joye & Paerl 1994, Dong et al. 2000), and denitrification activity can decrease NO_3^- concentration in overlying water (Joye & Paerl 1993). The effects of P are not well understood, though one study found denitrification decreased in high P conditions (Sundareshwar et al. 2003). No similar studies have explored the response of denitrification to nutrient concentrations at levels as high as reported in southern California estuaries (but see Seitzinger & Nixon 1985). To understand the impact of extreme anthropogenic nutrient inputs, it is necessary to determine the response of denitrification to these higher N-loads (Seitzinger et al. 2006).

Eutrophication is a major concern in estuaries in mediterranean-type climates worldwide as many are adjacent to dense human populations with watersheds subject to urban and agricultural development (Flindt et al. 1997, Cabrita & Brotas 2000, Sfriso et al. 2003, Boyle et al. 2004, Caffrey et al. 2010). However, few studies have addressed the effects of nutrient inputs on sediment N-fixation and denitrification in mediterranean-type estuaries (but see Cabrita & Brotas 2000, Magalhaes et al. 2005a in Portugal, Rysgaard et al. 1996 in France, Eriksson et al. 2003 in Italy, Langis et al. 1991, Moseman-Valtierra et al. 2010 in California, USA). Mediterranean-type estuaries can have extensive tidal mudflat areas that comprise a large proportion (up to 70%) of estuarine habitat (e.g. Rysgaard et al. 1996, Joye & Paerl 1994). These mudflats are sites of N-fixation and denitrification (Dong et al. 2000, Joye & Paerl 1994), and may play an important role in estuarine N dynamics.

Like mediterranean-type estuaries worldwide, southern California estuaries experience impacts due to anthropogenic nutrient sources that include agriculture and dense urban and suburban development. Nutrient loads from runoff can be extremely high, especially during the winter wet season, when southern California estuaries experience the majority of freshwater input (Page et al. 1995, Boyle et al. 2004). Due to small estuary sizes and shallow depths, a relatively large amplitude mixed semi-diurnal tidal cycle, extreme high and low flow volumes depending on rainfall events, and hydrological modifications, water residence times can be variable. In one southern California estuary water residence time ranged from as little as 1.0 – 3.3 days (Nezlin et al. 2009) and up to 30 days (Pednekar et al. 2005). N-fixation and denitrification rates may depend on residence time of nutrient-rich water (Joye & Paerl 1993, Seitzinger et al. 2006, Joye & Anderson 2008), as residence time affects the duration of contact between sediments and water column nutrients. Only four studies have measured N-fixation in southern California, and of these, three occurred in vegetated salt marsh sediments (Langis et al. 1991, Moseman 2007, Moseman-Valtierra et al. 2009) and only one measured N-fixation in unvegetated sediments (Moseman-Valtierra et al. 2010). No studies have measured denitrification rates in southern California estuaries. Because estuarine systems in this mediterranean-type climate are already highly impacted by anthropogenic nutrients, we need to it is understand how further nutrient enrichment can affect biogeochemical functioning.

We investigated the effect of increased water column nutrients on sediment N-fixation and denitrification by manipulating water column concentrations of NO_3^- and PO_4^{3-}, alone and in combination, in a microcosm experiment modeling eutrophic southern California estuaries. We hypothesized NO_3^- enrichment would decrease N-fixation, possibly shutting it down entirely, and would increase denitrification activity. Further, we expected PO_4^{3-} enrichment to increase N-fixation and decrease denitrification rates. Understanding how N-fixation and denitrification function to add and remove N has implications for determining the quantity of nutrients that can be processed by the system, and will contribute to the direction of future management strategies. This is especially important, as urban development and nutrient inputs are expected to continue to increase in these impacted estuaries. In order to manage nutrient inputs to these highly impacted estuarine systems, we must better understand how biogeochemical processing responds to anthropogenic inputs of both N and P and the role microbial processes can play in mediating nutrient impacts from coastal development.

METHODS

We investigated the effect of water column nutrient enrichment on sediment N-fixation and denitrification in laboratory microcosms using a 2x2 factorial design with N and P addition treatments. Sediment cores for use in the experiment were collected in Upper Newport Bay (UNB) estuary (N 33^o 38.01, W 117^o 53.18), an ecological reserve in Orange County, California. Like many mediterranean-type estuaries, UNB has a highly urbanized watershed where dredging and filling have altered the estuary and impacted hydrology (Schiff & Kamer 2000). The estuary is comprised of a dredged main channel surrounded by extensive broad intertidal mudflat and tidal creek habitats (Sutula et al. 2006). UNB has a history of nutrient enrichment

(Boyle et al. 2004, U.S. EPA 1998) and is subject to large macroalgal blooms (Kamer et al. 2001) and periods of hypoxia (Nezlin et al. 2009). Southern California estuaries experience wet and dry seasonality, with extended periods with little or no rainfall in the summer months, and several intense winter rainfall events that bring nutrients into the system in large pulses (Boyle et al. 2004, Page et al. 1995).

Twenty-five sediment cores were collected haphazardly at low tide from the exposed intertidal mudflat of a tidal creek of UNB on 27 July 2005 by pushing 9.5cm diameter clear acrylic tubes into the sediment to a depth of 12cm (Figure 1). All sampled sediment had a layer of green macroalgal mat (*Ulva* spp.) growing on the surface (100% cover) that was left intact. Cores were immediately transported on ice to the laboratory at University of California, Los Angeles. Water was permitted to drain from cores during transport, similar to what would take place during low tide, allowing for treatment water to replace porewater during the experiment.

To characterize initial conditions, porewater was sampled concurrently with sediment core collection. Porewater (n=5) that pooled in holes left by coring was collected using a 60cc syringe, stored in an acid-washed 50ml plastic sample bottle, transported to the lab on ice, vacuum filtered (Whatman GF-C), salinity measured using a handheld refractometer, and frozen. Frozen samples were sent to the Marine Science Institute (MSI) Analytical Laboratory at University of California, Santa Barbara for analysis of NO_2^+ + NO_3^- (hereafter, NO_3^-), NH_4^+, and PO_4^{3-} concentrations via Flow Injection Analysis.

Treatment water was created using seawater from the Redondo Beach Power Generating Station, in Redondo Beach, California (N 33° 50.965, W 118° 23.763). Seawater was filtered and divided into four portions, then amended with nutrients to create water treatments of no nutrient addition (ambient), +NO_3^- only (+N), +PO_4^{3-} only (+P), and +NO_3^- and +PO_4^{3-} combined (+N+P). Samples of treatment water (n=1 per treatment) were frozen and sent to MSI for analysis of initial NO_2^+ + NO_3^-, NH_4^+, and PO_4^{3-} concentrations. Nutrient amended treatment water was significantly enriched relative to ambient (Table 1). N in the form of NO_3^- was chosen, as it is often the dominant form of inorganic N entering from watersheds in southern California estuarine systems (Cohen & Fong 2006, Kennison et al. 2003, Page et al. 1995). Concentrations were chosen to simulate a high N pulse that might occur during runoff periods. NO_3^- amendments were within the range of reported maximum values across southern California estuaries, where other studies measured water column NO_3^- concentrations from 2400μM to 3100μM across different estuarine systems in the region (Cohen & Fong 2006, Kennison et al. 2003, Page et al. 1995). P additions were based on an N:P ratio of ≤10, as observed in previous studies of southern California estuaries (Boyle et al. 2004, Kennison et al. 2003).

To begin the experiment, we assembled 20 experimental units consisting of sediment cores with associated benthic macroalgae and treatment water (Figure 1). The bottom 12cm of each acrylic tube was wrapped in opaque tape to prevent light from entering the sides of cores, and the bottom opening was fitted with 50micron mesh cloth held in place with a hose clamp to prevent loss of sediments but allow for water drainage. Each core was numbered haphazardly and placed in an individual 2.5L bucket. One liter of appropriate treatment water was poured slowly down the inside of each core tube so as not to disturb the sediment surface. We chose to use 1L of treatment water in order to equalize the nutrient dose each unit received. All units were placed in large outdoor water baths to maintain temperature at ≤22 ^{o}C and allow access to sunlight. This temperature threshold was based on our measurements of sediment temperature in UNB during previous studies (Kane & Fong, unpublished data). Treatments were assigned to random positions distributed across two pools and covered with one layer of mesh screening to reduce incident light (2200-2500 moles $m^{-2}\cdot s^{-1}$ at mid-day, Kamer et al. 2004) by ~30% to mimic typical cloud cover of local coastal areas (1405-1956 μmoles $m^{-2}\cdot s^{-1}$, Arnold & Murray 1980). The experiment began at 11:30am on 27 July 2005 and units were left undisturbed for 24 hours.

At the end of the experiment, some units (n=4 for ‘ambient’ and ‘+N’ treatments; n=2 for ‘+P’ and ‘+N+P’ treatments) had treatment water remaining above the sediment core (i.e. all water did not drain through). Overlying water was decanted so as not to disturb the sediment surface and a sample was collected. Water that had flushed through the cores (“passed-through water”) was also collected. Water samples were filtered and frozen as above, and sent to MSI for analysis of NO_2^+ + NO_3^-, NH_4^+, and PO_4^{3-} concentrations.

Initial sediment conditions were characterized by randomly selecting five cores prior to the start of the experiment and extruding cores from the acrylic tubes. From the center of each extruded core, we haphazardly collected 3 smaller cores (1.4cm diameter, 5cm depth) for sediment N-fixation and denitrification measurements, 3 cores (1.4cm diameter, 1cm depth) for benthic chlorophyll *a*, which we froze until analysis, and one core (2.1cm diameter, 5cm depth) for sediment water content which was necessary for calculations of N-fixation and denitrification rates. The remaining top 5cm of sediment from each experimental unit was used to measure sediment grain size, organic content and total N and P content. We did not remove the layer of macroalgae from the sediment surface at any stage. Treatment cores were processed in the same way immediately following the end of the experiment. (See below for analytical methods.)

N-fixation and denitrification measurements were determined using acetylene reduction and acetylene block techniques, respectively (Capone 1993, Capone et al. 2001). The three small cores from each of the initial and treatment units were extruded and combined in a 125ml Erlenmeyer flask, sealed with a recessed rubber stopper and electrical tape to form an air-tight vessel, and purged with N_2 gas to create anaerobic conditions. This allowed us to measure both N-fixation and denitrification simultaneously. Experimental units were allowed to drain prior to sample collection and no additional water was added to incubations. Therefore, measured N-fixation and denitrification activity reflected low tide conditions of sediments with associated porewater. To the headspace of each incubation vessel we introduced 20ml of acetylene gas (C_2H_2), which we created in the lab from calcium carbide. Acetylene is reduced to ethylene by the nitrogenase enzyme, giving a measure of nitrogenase activity, an indirect measure of N-fixation. Acetylene also blocks the final step of denitrification where N_2O is converted to N_2, resulting in an accumulation of N_2O and thus a measure of denitrification activity. We sampled each vessel over 24hrs (at 0, 6, 9, 12, 15, 18, 24hrs) by removing 1.5ml of gas from the headspace using a gas-tight syringe, and storing gas samples in glass blood collection tubes until analysis via gas chromatograph (GC). Samples were analyzed for ethylene (C_2H_4) using a GC with Flame Ionization Detector to determine acetylene reduction rates for N-fixation. For denitrification rates, samples were analyzed for N_2O gas using a GC with an Electron Capture Detector. The production of ethylene from 0-18hrs was used to determine N-fixation rates, and the production of N_2O from 0-6hrs was used to determine denitrification rates; these time points were chosen as they captured the maximum rates observed for the majority of samples. Rates were calculated using equations in Capone and Montoya (2001) and presented as μmol N m^{-2} hr^{-1}. For N-fixation we assumed a theoretical reduction ratio of 3:1 (C_2H_2 reduction: N_2 reduction) by nitrogenase (Capone 1983, Joye & Paerl 1993), though this may underestimate rates (Seitzinger & Garber 1987). As nitrification was inhibited in our incubations, our methods detected denitrification based on water column and porewater NO_3^- sources only. *In situ* denitrification may be higher than our measured rates if there is coupled nitrification-denitrification activity, though when water column NO_3^- is high (>60μmol/L) coupled nitrification-denitrification is thought to be a minor component of total denitrification (Seitzinger et al. 2006).

Chlorophyll *a* was determined by sonicating samples in 90% acetone and extracting pigments overnight in a freezer. Extracted pigments were analyzed by spectrophotometer using Standard Methods (APHA 1995). Sediment water content was determined by loss of mass after drying at 60°C. To measure sediment grain size and organic content, sediment was dried at 60°C to a constant weight, ground using a mortar and pestle and sieved to <2mm. Grain size was measured using the hydrometer method (Bouyoucos 1962), and organic content was determined from loss of mass on ignition in a muffle furnace after 10hrs at 400 °C. Sediment was sent to the Analytical Laboratory at University of California, Davis for quantification of total N using the combustion gas analyzer method (AOAC International 1997), and total P by microwave acid digestion and analysis by Inductively Coupled Plasma Atomic Emission Spectrometry (Sah & Miller 1992).

We used two-factor ANOVA with factors N (+, -) and P (+, -) to determine if there were significant differences in mean values of N-fixation rate, grain size, % organic content, water content, chlorophyll *a* concentration, sediment total N and total P, and NH_4^+ concentration of water that passed-through cores, as these data met the assumptions of normality and homogeneity of variance for parametric statistics. For denitrification rate, and NO_3^- and PO_4^{3-} concentrations of passed-through water, variance was heterogeneous and transformations did not result in homogeneity, therefore we used a Generalized Linear Model. No significant differences were detected using two-factor ANOVA for clay content, organic content, chlorophyll *a*, and total sediment P, therefore final values were pooled and compared to initial values using t-tests. To determine if ambient treatment cores drifted from initial core conditions over the course of the experiment, t-tests were used to compare N-fixation, denitrification and total sediment N from ambient treatment cores to initial cores, and pooled treatment sediment characteristics to initial sediment characteristics.

To determine the number of moles added or removed via N-fixation and denitrification for each core, rates were converted from μmol N m^{-2} h^{-1} to μmol N $core^{-1}$ day^{-1}. We used these converted rates to calculate the transformed N as a % of the total dissolved inorganic nitrogen (DIN) load of treatment water (μmol NH_4^+ + μmol NO_3^-) per experimental unit. N-fixation and denitrification as percentages of DIN were compared across treatments using two-factor ANOVA.

Initial Site Characteristics

There were no differences between initial cores and ambient (control) cores for N-fixation, denitrification or sediment total N (Figure 2), showing our mesocosms did not drift from rates measured on initial cores. Sediment characteristics of clay content, organic content, total P, and chlorophyll *a* did not differ between initial and final sediment characteristics across all treatments, therefore all values were pooled (Table 2).

RESULTS

The addition of N-enriched water to cores significantly affected both N-fixation and denitrification (Table 3), though the effects were opposite (Figure 2). N-fixation rates in +N treatments were as much as 40% lower than in ambient cores, demonstrating inhibition of N-fixation by NO_3^-. In contrast to N-fixation, denitrification increased dramatically with NO_3^- addition, up to 180-fold as compared to the ambient treatment. While ambient denitrification rates were lower than all N-fixation rates by more than an order of magnitude, this relationship was reversed with NO_3^- addition; denitrification rates stimulated by NO_3^- addition were up to 4.5x greater than the highest N-fixation rates measured. Neither N-fixation nor dentirification responded significantly to the addition of P, though under high N conditions denitrification activity tended to decrease with added P, resulting in an almost significant interaction (Table 3).

Sediment total N was significantly higher in treatments without added NO_3^- and was not affected by PO_4 addition (Table 3, Figure 2). The treatments where sediment total N was highest were also where N-fixation was highest. As N-fixers incorporate N into their cells via the N-fixation process, we would expect higher N-fixation activity to result in increased sediment N overall. As sediments used for total N measurements also included a layer of macroalgae on the sediment surface, macroalgae could also have contributed to changes in sediment total N if algal tissues were taking up N.

Neither N-fixation nor denitrification comprised a large portion of the total N-load in experimental units. The number of moles added via N-fixation was <2umoles N core-1 day-1 in every treatment (Table 4). In +N treatments, N-fixation as a % of N-load was negligible, though under low N conditions N-fixation accounted for up to ~15% of N-load in the +P only cores. This difference was significant (2-way ANOVA, N*P p=0.0001). Denitrification as a % of DIN was <2% for all treatments, despite removing up to a mean of 13μmoles N core^{-1} day^{-1} in the +N treatment (Table 4). This indicates N-fixation and denitrification did not play a major role in N processing in our cores.

For overlying and passed-through water, NO_3^- remained significantly higher for +N and +N+P treatments than for ambient and +P treatments (Table 3, Figure 3) reflecting treatment concentrations. For all treatments, NO_3^- concentration of overlying water decreased from treatment water (Table 1, Figure 3), suggesting uptake by macroalgae on the sediment surface. Comparison of overlying water with passed-though water showed NO_3^- concentrations increased as water passed-through cores in the ambient treatments, but in the +N treatments NO_3^- concentration appeared to decrease from overlying to passed-through water, though variability is high.

Across all treatments, NH_4^+ concentration of overlying water was comparable to treatment water (Table 1, Figure 3), suggesting little NH_4^+ processing at the sediment-water interface. NH_4^+ concentration of treatment water was low and did not change across treatments, thus it is unlikely it affected N-fixation. NH_4^+ concentration in passed-through water appeared to increase compared to overlying water in all treatments (Figure 3). Comparison of passed-through water across treatments showed a significant effect of added P (Table 3), with lower NH_4^+ in the passed-through water in the presence of high P (Figure 3).

By the end of the experiment, PO_4^{3-} concentration of overlying water decreased by up to 90μM from the initial treatment water concentration in the P-enriched treatments (Table 1, Figure 3), suggesting uptake of PO_4^{3-} at the sediment surface, possibly by macroalgae. Across all treatments, there were significantly higher PO_4^{3-} concentrations in passed-through water with added P, regardless of NO_3^- addition (Table 3), reflecting the treatment dose. In the +N treatment, PO_4^{3-} increased in passed-through water compared to overlying water but there appeared to be no difference in the other three treatments (Figure 3).

DISCUSSION

Our results demonstrated excessive anthropogenic nutrient enrichment will have strong positive effects on denitrification in sediments of eutrophic mediterranean-type estuaries. In our experimental chambers, denitrification increased dramatically with NO_3^- enrichment, indicating the estuary is capable of responding to increased N loads by ramping up denitrification activity. Our results support findings of survey (Cabrita & Brotas 2000) and experimental (Magalhaes et al. 2005a) studies in two eutrophic Mediterranean estuaries in Portugal where sediment denitrification increased with NO_3^- concentration. Taken together, these results indicate mediterranean-type estuaries may have some capacity to ameliorate high nutrient loads from developing watersheds

Our results also make it clear that denitrification is not a solution for the extreme high levels of eutrophication that are found in some mediterranean-type estuaries. During our simulated high nutrient pulses even the highest denitrification rates did not keep up with the extreme levels of N inputs that are experienced by these systems. Despite the significant increase in denitrification rates in the presence of high NO_3^- doses, denitrification only removed a small portion (<2%) of N from our treatment water over 24hrs. In intertidal areas of other mediterranean-type estuaries, denitrification removed 45% of N inputs to the eutrophic Tejo estuary (Tagus, Portugal) (Seitzinger 1988) while in less eutrophic South San Francisco Bay denitrification accounted for <18% of removal of N inputs (Oremland et al. 1984). In both of these systems, sediments were impacted by nearby sewage outfalls, but NO_3^- concentrations were still at least an order of magnitude lower than in our experiment and than frequently occur in southern California estuaries. In a mesocosm enrichment experiment on sediments from temperate Narragansett Bay, where experimental NO_3^- loading ranged from 60 to 3900μmol m^{-2} h^{-1}, Seitzinger & Nixon (1985) concluded removal of N by denitrification would be unable to keep up with large anthropogenic inputs. Similarly, our results demonstrated that while denitrification may help to mediate high N inputs, it will not provide a solution for high levels of eutrophication in these mediterranean-type estuaries.

Even though our denitrification rates increased dramatically with NO_3^- inputs, our rates were lower than could be expected based on the high concentration of NO_3^- to which our cores were exposed, especially when comparing our study to other mediterranean-type systems. However, comparison across studies is challenging due to differences in methods and study conditions. In the studies presented below, all denitrification measurements took place using submerged cores, with some using the acetylene block method and some using the isotope pairing method. Despite the differences across studies, our mean denitrification rate for NO_3^- enriched treatments was 35 μmol N m^{-2} h^{-1}, within the range of denitrification from water N sources (i.e. not coupled with nitrification) reported for other med-type estuaries. Our N-enriched rate was at the low end of the range in Venice Lagoon, Italy, one of the most eutrophic Mediterranean estuaries, where denitrification ranged from 7-200 μmol N m^{-2} h^{-1} (Ericksson et al. 2003) in unvegetated sediments over a year, and was lower than dry season rates in eutrophic Elkhorn Slough in central California with rates of 83-237 μmol N m^{-2} h^{-1} (Caffrey et al. 2010), even though water column NO_3^- was <7μM in both systems. Our rates were also low compared to non-eutrophic mediterranean-type estuaries. In Tomales Bay, northern California, denitrification was up to 60 μmol N m^{-2} h^{-1} during a period of N-enriched runoff due to a precipitation event with NO_3^- concentration up to 162μM (Joye & Paerl 1993), and in South San Francisco Bay rates were >250μmol N m^{-2} hr^{-1} when sediments were experimentally amended with 1000μM NO_3^- (Oremland et al. 1984). Our highest rates were most similar to maximum rates reported in other eutrophic estuaries in Portugal year round, where maximum denitrification ranged from 25 to 35 μmol N m^{-2} h^{-1} (Nizzoli et al. 2007, Cabritas & Brotas 2000, Magalhaes et al. 2005b), though NO_3^- concentrations were only as much as 30% of our N-enriched treatments. Despite our sediments being exposed to a much greater NO_3^- concentration, denitrification rates in other mediterranean-type estuaries tended to be higher than in our study, regardless of eutrophication status of the system and season.

The low denitrification rates measured in our study despite extremely high NO_3^- concentrations may be related to tidal cycle and/or seasonality. While cores were submerged in the denitrification studies in the comparisons above, our acetylene assays were performed on drained cores, simulating an exposed low tide situation. Low tide may be when denitrification from water column sources is lowest, as the only available NO_3^- is in the porewater, and we might expect rates to increase during high tides when sediment is submerged and has greater access to surface water NO_3^-. However, in another study (Kane Dissertation Chapter 3) we did not find significant differences in N-fixation or denitrification activity between exposed and submerged cores under ambient low- NO_3^- water conditions. Seasonality could also have an influence. Our study was performed during a summer dry season when water column NO_3^- concentrations are historically low, which could contribute to low observed rates since we were measuring short-term response to a large nutrient pulse by microbial populations adapted to low NO_3^- conditions. Longer term exposure to high NO_3^-, perhaps during periods of maximum NO_3^- loading associated with winter precipitation events (Schiff & Kamer 2000), could potentially result in different microbial communities better adapted to high NO_3^- conditions. Future research in this and other estuaries in mediterranean-type climates should assess rates during wet and dry seasons, as well as low and high tide conditions across a range of nutrient concentrations in order to untangle the potential relationships between N accessibility, tidal cycle and seasonality.

Inhibition of denitrification activity due to extremely high NO_3^- concentrations may be another possibility to consider in understanding why our rates were relatively low. Studies in temperate estuaries have reported denitrification to be saturated via Michaelis Menton kinetics at NO_3^- concentrations above 200μM (Ogilvie et al. 1997) to 600μM (Dong et al. 2000). If denitrifiers in our study were saturated, we would still expect to see rates more comparable to other moderate to high rates. Therefore, it seems possible that our extreme high levels of NO_3^- were having some type of inhibitory effect or that something other than NO_3^- concentration is regulating denitrification rates in this system. Future studies in highly eutrophic estuarine systems should investigate denitrification across a range of NO_3^- inputs to determine if there is a concentration beyond which denitrification is saturated or inhibited.

Our results showed experimental N enrichment modeling highly eutrophic conditions decreased N-fixation activity though never shut it down completely. While N-fixation was dominant during low NO_3^- conditions, it contributed little to N inputs; under high NO_3^- conditions removal of N via denitrification far exceeded any N added by N-fixation. We do not know the ecological consequences of suppressed N-fixation, how long N-fixation can be maintained during extended periods of high N inputs, and what smaller scale effects decreased N-fixation might have regarding N-fixer diversity, energy flow and food webs. While N-fixation may not be an important N source in already highly eutrophic systems, our results showed that even in the presence of extremely high NO_3^- inputs N-fixation was not shut down completely, indicating a low level of nitrogenase activity is maintained regardless of NO_3^- status. While NO_3^- concentration of treatment water was extremely high, NH_4^+ remained low, and N-fixation may not be influenced as strongly by NO_3^- as it might be by NH_4^+. As NH_4^+ inhibits nitrogenase synthesis, not nitrogenase activity, there may also be a lag in suppression of N-fixation and under longer periods of high N we could see further decreases in N-fixation. Additionally, because N-supply is inconsistent in mediterranean-type systems, perhaps some level of N-fixation activity is always maintained. In highly eutrophic systems, N-fixers may have adapted to increased NO_3^- and N-fixation still occurs regardless of N-status. Further investigation of N-fixation is required to understand the role of this N source in eutrophic mediterranean-type estuaries.

Our N-fixation rates, both ambient and enriched, were comparable to rates in other highly and moderately eutrophic mediterranean-type systems, and much lower than non-eutrophic systems. Our rates were similar to those in unvegetated marsh sediments (8 μmol N m^{-2} h^{-1}, assuming 3:1 C_2H_2:N_2 ratio) in Tijuana Estuary, a highly eutrophic southern California system bordering Mexico (Moseman-Valtierra et al. 2010), and were only slightly higher than in sandy sediments of moderately eutrophic Duoro River estuary, Portugal (2.67 μmol N m^{-2} h^{-1}) (Magalhaes et al. 2005b). Though both of these are eutrophic systems, NO_3^- concentrations were lower than in our experiment, suggesting our system is more similar to other eutrophic systems experiencing a period of low NO_3^- inputs. In Tomales Bay, a non-eutrophic mediterranean-type estuary in northern California, sediment N-fixation associated with a microbial mat community was nearly 30 μmol N m^{-2} h^{-1} (Joye & Paerl 1994) and up to 125 μmol N m^{-2} h^{-1} (Joye & Paerl 1993) when NO_3^- concentrations were low or below detection. These N-fixation rates were 3 to 12-fold higher than our maximum rates. However, rates in Tomales Bay approached zero with exposure to runoff with NO_3^- concentrations up to 162μM (Joye & Paerl 1993), while we still measured rates of 6 μmol N m^{-2} h^{-1} even under NO_3^- concentrations greater than 2000μM. So even during periods of high NO_3^- concentrations, N-fixation in our system is higher than in other eutrophic and non-eutrophic estuaries with lower NO_3^-, supporting the idea that N-fixation is adapted to occur in our system despite extreme N inputs.

Although effects of enrichment on both N-fixation and denitrification rates were influenced strongly by N, increased supply of P did not have a significant effect on either rate. It has been argued that when P concentrations increase, water column N-fixation can increase and provide more bioavailable N (as in some lake systems), thus keeping the N/P ratio in balance (Schindler et al. 2008, Paerl 2009). Based on N-fixation rates, this does not appear to be the case in sediments of our eutrophic mediterranean-type estuary. Since P is often sequestered in and released from sediments, and UNB has a history of sediment P values comparable to other eutrophic mediterranean-type systems (Boyle et al. 2004), all cores may have received sufficient P regardless of treatment. However, there is some evidence to suggest P may have some influence on denitrification, as it appeared to decrease with P when in the presence of high N. While not conclusive, this effect lends support to a study by Sundareshwar et al. (2003) where denitrification was suppressed by P-enriched conditions. Further work is needed to understand the role of P interactions with microbial N-processes in this system.

Additional processes at the sediment–water interface are likely influencing N-cycling in these systems. While our assays accounted for denitrification based only on water column and porewater sources, changes in the water nutrients incorporate all nutrient transformation processes that occurred in the experimental units, which may include uptake by macroalgae, nitrification, and dissimilatory nitrate reduction to ammonium (DNRA). For instance, under low N conditions, denitrifiers could be competing with macroalgae for NO_3^-, as water column NO_3^- in UNB has been reported to be lowest when macroalgae are most abundant (Boyle et al. 2004). Elevated NH_4^+ concentrations in passed-through verses overlying water was greater than N-fixation alone can account for, suggesting either high NH_4^+ initial porewater from saturated cores and NH_4^+ adsorbed to sediment was flushed out with treatment water, or NH_4^+ was generated within cores, and these processes were influenced by P. The generation of NH_4^+ could be the result of both nitrification and DNRA processes. DNRA retains N in the system, potentially enhancing eutrophication in systems where it is more predominant than denitrification (Kemp et al. 1990), and was found to be a significant process producing NH_4^+ in some Gulf of Mexico estuaries (Gardner et al. 2006) and in a Mediterranean estuary in France (Rysgaard et al. 1996). Therefore, investigating the role of other sediment N processes, especially DNRA, could be important to future nitrogen studies aimed at understanding eutrophication effects in these systems.

Table 2-1. Inorganic nutrient concentrations (μM) of porewater (Mean(SE)) from the collection site and initial treatment water.

	n	NO_3^-	NH_4^+	PO_4^{3-}
Porewater	5	0.15 (0.021)	122.8 (20.50)	0.326 (0.075)
Treatment water:				
Ambient	1	13.0	4.08	1.53
+N	1	2730	3.60	1.44
+P	1	8.5	2.40	213
+N +P	1	2620	6.62	223

Table 2-2. Sediment characteristics of initial core conditions (n=5) and final experimental conditions (n=20) were combined to give a grand mean (n=25), as they were not significantly different across treatments.

	Mean (SE)
Clay content (%)	28.30 (0.35)
Organic matter content (%)	5.80 (0.08)
Total P (%)	0.069 (0.001)
Chlorophyll *a* (mg/m^2)	43.80 (2.41)

Table 2-3. Statistical tables of sediment and water characteristics for a) variables tested using two-factor ANOVA, and b) variable tested using Generalized Linear Model (GLM). Significant p-values are in bold. Variables with no significant differences detected are not presented.

a. Variables tested using 2-factor ANOVA

Response Variable	Source	DF	Sum of Squares	F Ratio	p-value
Nitrogen fixation	N	1	58.516	21.8734	**0.0003**
	P	1	1.196	0.4469	0.5133
	N*P	1	0.015	0.0057	0.9401
Total N	N	1	0.004	14.0167	**0.0018**
	P	1	0	0.8167	0.3796
	N*P	1	0	2.8167	0.1127
NH_4^+ passed-through water	N	1	3105.032	1.403	0.2535
	P	1	16736.584	7.5625	**0.0142**
	N*P	1	5516.513	2.4927	0.1339

b. Variables tested using GLM

Response Variable	Source	DF	L-R ChiSquare	p-value
Denitrification	N	1	671.4052	**<0.0001**
	P	1	0.0064	0.9359
	N*P	1	3.1027	0.0782
NO_3^- passed-through water	N	1	25832.224	**0.0000**
	P	1	1.0874	0.2971
	N*P	1	1.0709	0.3007
PO_4^{3+} passed-through water	N	1	0.0786	0.7791
	P	1	1967.1275	**0.0000**
	N*P	1	0.4013	0.5264

Table 2-4. Nitrogen fixation and denitrification (Mean (SE)) by treatment for experimental units over the 24hrs of the experiment, reported as μmoles N core^{-1} day^{-1} and as percentage of total DIN load (μmol of NO_3^- + NH_4^+) in 1L of treatment water.

	N-fixation		**Denitrification**	
	μmoles N core^{-1} day^{-1}	**% of N-load**	**μmoles N core^{-1} day^{-1}**	**% of N-load**
Ambient	1.61 (0.11)	9.39 (0.66)	0.05 (0.04)	0.30 (0.22)
+N	1.02 (0.19)	0.04 (0.01)	13.41 (5.63)	0.49 (0.21)
+P	1.68 (0.09)	15.42 (0.82)	0.12 (0.10)	1.09 (0.90)
+N+P	1.11 (0.07)	0.04 (0.00)	6.18 (2.76)	0.24 (0.11)

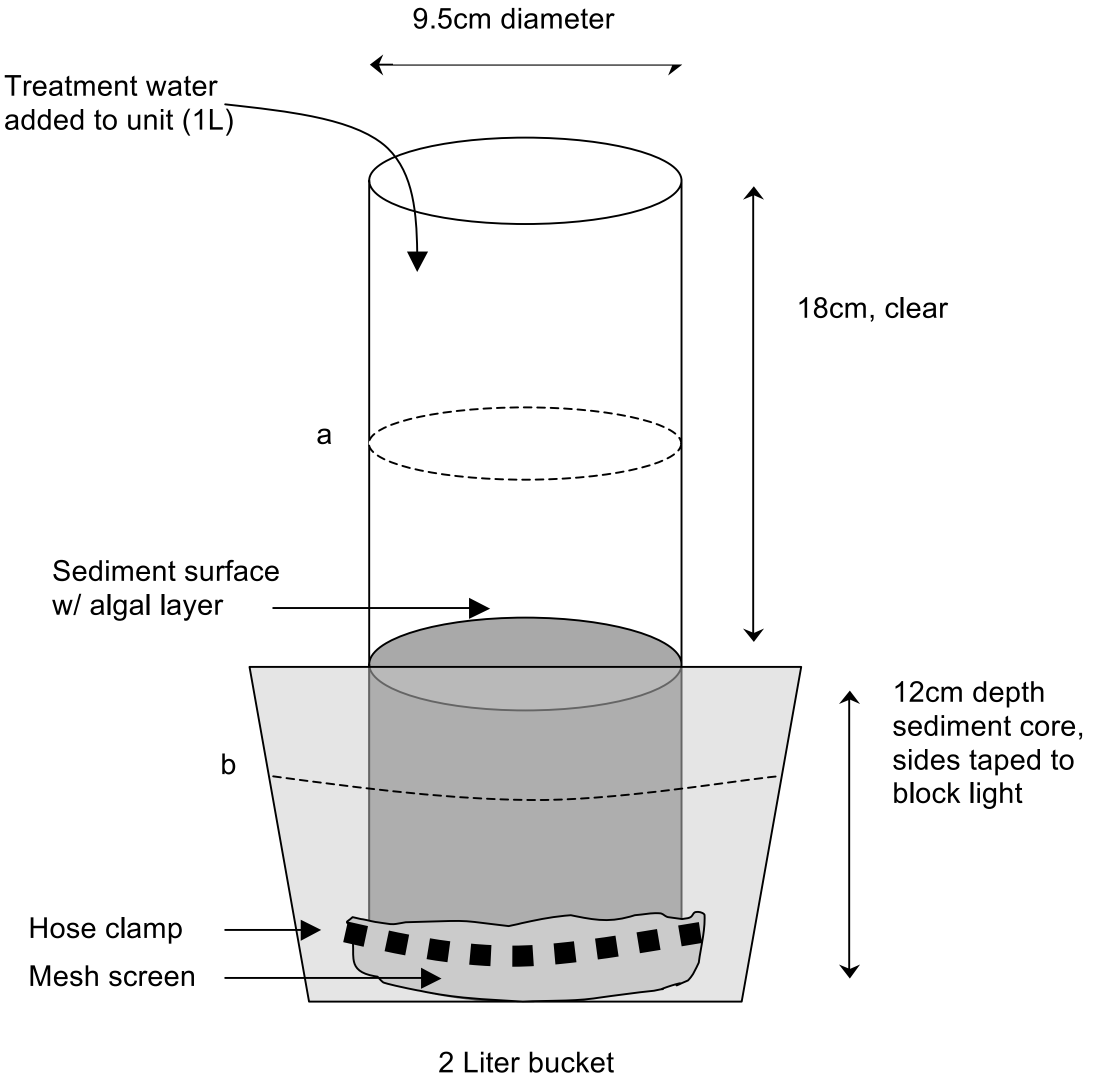

Figure 2-1. Example of experimental unit. Dashed lines indicate a) overlying water, and b) passed-through water remaining at end of experiment.

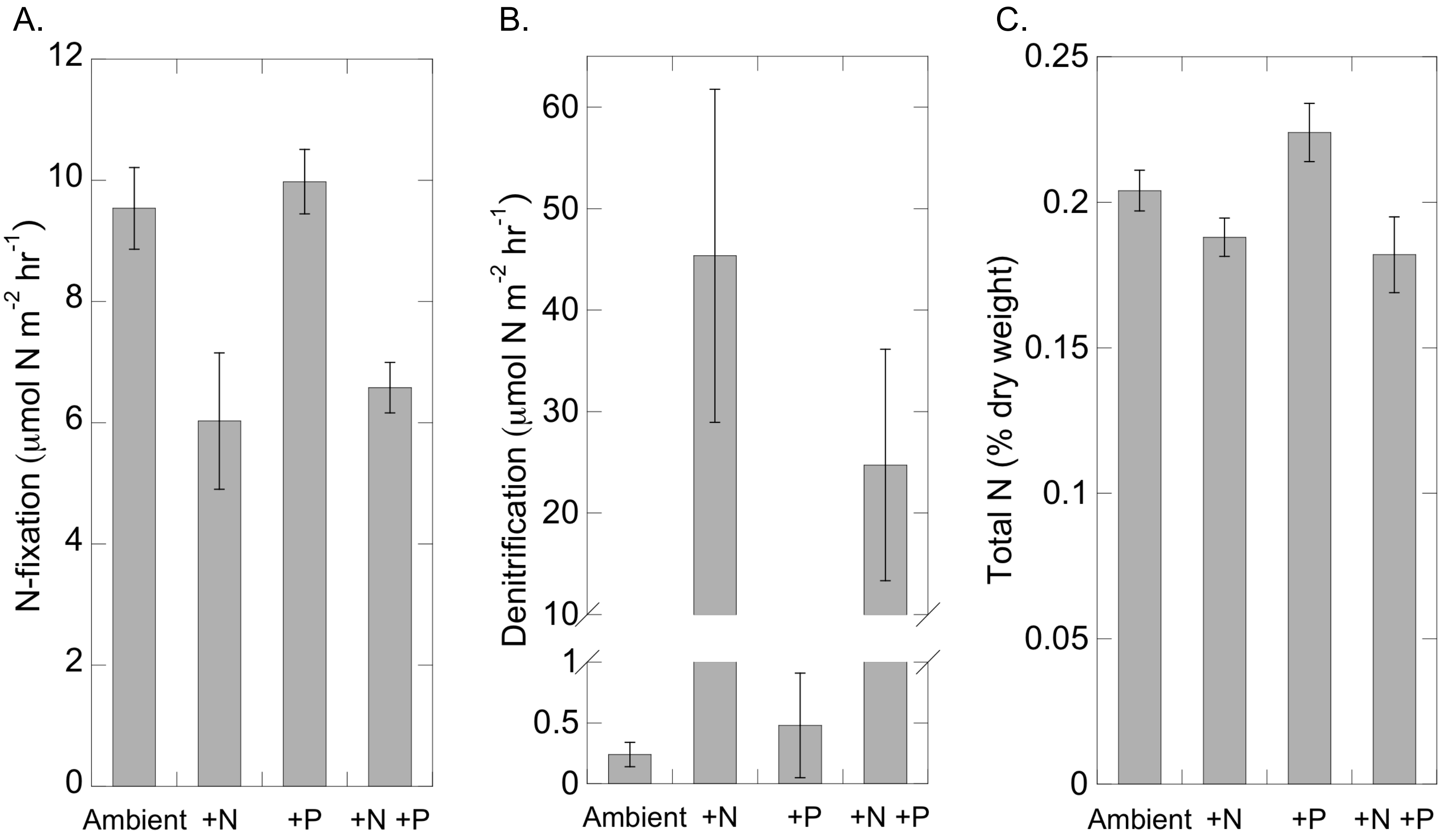

Figure 2-2. Mean (+/-SE) of A) nitrogen fixation (initial = 8.22(0.60)), B) denitrification (initial = 0.43(0.24)), and C) sediment total N (initial = 0.182(0.005)) from experimental microcosms modeling a eutrophic mediterranean-type estuary subjected to high N and P loading.

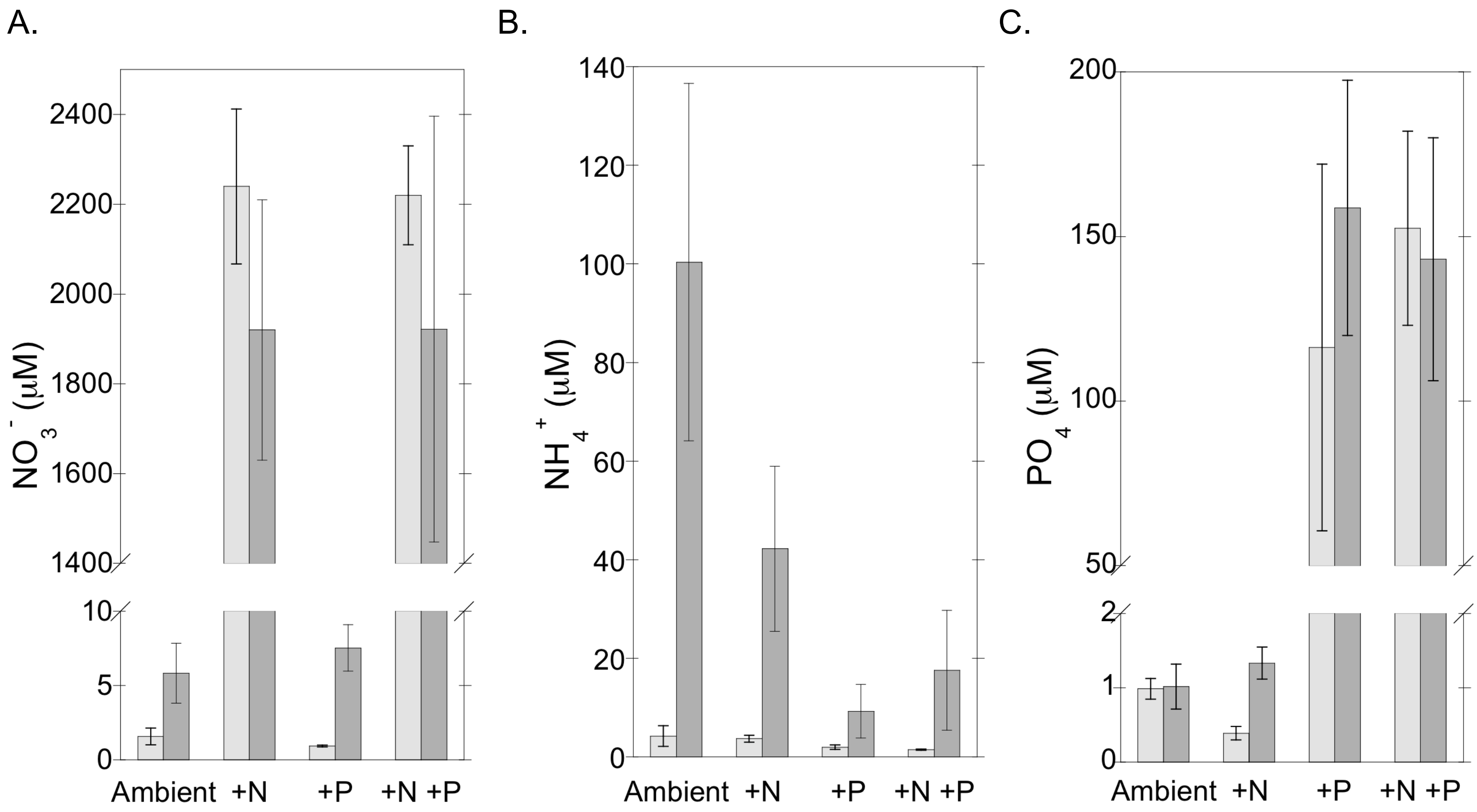

Figure 2-3. Concentration (μM) of A) nitrate, B) ammonium, and C) phosphate of overlying water (light gray bars) and passed-through water (dark gray bars). Error bars indicate standard error. For passed-through water n=5 for all treatments.

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CHAPTER 3

NITRATE INPUTS IMPACT SEDIMENT NITROGEN FIXATION AND DENITRIFICATION RATES IN A SMALL, HIGHLY MODIFIED ESTUARY IN A MEDITERRANEAN-TYPE CLIMATE

ABSTRACT

Estuaries are under increased stress due to excessive inputs of anthropogenic nutrients, which can lead to eutrophic conditions and a suite of associated ecosystem impairments. Thus, it is important to understand nitrogen (N) cycling in these systems, especially microbial N processes such as N-fixation and denitrification, which can respectively add or remove N, and thus affect eutrophication status. N-fixation and denitrification rates were measured in sediments of Famosa Slough quarterly over a 1yr period encompassing wet and dry seasons, including simulated emergent, submergent, and submergent N-enriched conditions. For both N-fixation and denitrification, there were no apparent differences between ambient emerged and submerged conditions, suggesting rates do not vary throughout a tidal cycle. As expected, N-fixation and denitrification responded significantly to N-enrichment; on some dates N-fixation decreased by up to 9-fold and denitrification increased by more than 100-fold. Overall N-fixation rates were comparable to other eutrophic systems in mediterranean-type climates across all N inputs, though lower than in more oligotrophic systems, and activity persisted at low levels despite N enrichment. Denitrification rates of N enriched treatments were within the range of reported values of other mediterranean-type estuaries, regardless of eutrophic status. Studying how N is transformed by N-fixation and denitrification in this system expands our understanding of important global elemental cycles that are subject to increased anthropogenic impacts in this and other highly modified estuaries in mediterranean-type climates.

INTRODUCTION

Estuaries form the interface between freshwater and coastal ocean systems and act as important filters of nutrients to nearshore waters. With increased human population growth in coastal areas and expansion of agriculture in watersheds, estuaries are experiencing greater inputs of nutrients via runoff enriched by wastewater and fertilizers (Vitousek et al. 1997, Valiela & Bowen 2002, Galloway et al. 2004, Bricker et al. 2008). Physical modification of the watershed and estuary can also be extreme, affecting hydrology and further increasing nutrient loading (Schiff & Kamer 2000, Kennison et al. 2003). Excess nutrients can lead to eutrophication (Nixon 1995) which can result in symptoms of impaired ecosystem health including algal booms and hypoxia (Vitousek et al. 1997, Diaz & Rosenberg 2008). Nitrogen (N) is of special concern in coastal waters (Paerl 2009), and anthropogenic N inputs to the global N cycle have risen dramatically since industrialization (Doney 2010, Gruber & Galloway 2008), as a result, incidences of severe eutrophication are increasing worldwide (Diaz & Rosenberg 2008). It is important that we understand the impact of anthropogenic N inputs on the cycling of N in these vital estuarine systems.

At the foundation of the estuarine nitrogen N cycle are the microbial N transformation processes of N-fixation and denitrification. N-fixing diazatrophs, which include heterotrophic bacteria and cyanobacteria, introduce new bioavailable N to the estuary (Howarth et al. 1988a). N-fixers transform atmospheric nitrogen (N_2) to ammonium (NH_4^+) via the nitrogenase enzyme, and incorporate this “new” inorganic N within the cell, as well as release NH_4^+, especially during decomposition. Conversely, denitrification removes inorganic N from the system (Seitzinger 1988). Denitrifying bacteria convert nitrate (NO_3^-) to the atmospheric gasses N_2 and nitrous oxide (N_2O). Traditionally, N-fixation has been considered a minor contributor to the N-cycle in estuaries (Howarth et al. 1988b), though this process has been found to be an important N source in some N-limited estuaries (Gardner et al. 2006), and in young and restored salt marshes (Piehler et al. 1998, Tyler et al. 2003). Even in southern California’s highly eutrophic systems, N can be limiting for primary producers (Kamer et al. 2004) and N-fixation could potentially release this limitation. In contrast, denitrification is considered the more dominant process in estuarine sediments, and may help to mediate N inputs (Seitzinger 1988). However, in microbial mats and sediments of some estuaries, dominance of these two processes has been found to switch on diel and seasonal scales (Joye & Paerl 1994, Gardner et al. 2006, Fulwieler et al. 2007). To better understand the complexities of these biogeochemical N processes, especially in anthropogenically impacted systems, it is imperative that we expand our knowledge on the relative importance of these processes.

Availability of inorganic N, which has increased due to anthropogenic sources, can influence rates of N-fixation and dentirification. For N-fixation, NH_4^+ can inhibit synthesis of nitrogenase, which may keep rates low in sediments (Howarth et al. 1988a); for example, NH_4^+ has been shown to decrease N-fixation in both vegetated and unvegetated sediments (Sundareshwar et al. 2003, Moseman-Valtierra et al. 2010). NO_3^- can be transformed to NH_4^+ in sediments via dissimilatory nitrate reduction (An & Gardner 2002), potentially increasing NH_4^+ concentrations and leading to decreased N-fixation. N-fixers might preferentially take up inorganic N directly when available rather than fix N_2, thus under extremely high inorganic N inputs we expect N-fixation activity may cease completely. Denitrification utilizes NO_3^- from water column and porewater sources, and from NO_3^- produced by nitrification (Seitzinger et al. 2006). Thus, NO_3^- enriched water can increase denitrification significantly (Oremland et al. 1984, Joye & Paerl 1994, Dong et al. 2000) and this enhanced denitrification activity can decrease NO_3^- concentration in overlying water (Joye & Paerl 1993). As inorganic N concentrations can be dynamic in estuaries, it is important to determine how N inputs affect these two key biogeochemical N processes in already eutrophic systems.

Quantification of N-fixation and dentirification in mediterranean-type systems is sparse and little information exists on seasonal patterns (but see Joye & Paerl 1994) or response to nutrient enrichment. The majority of studies on N-fixation in marine environments have focused on water column N-fixation (Carpenter & Capone 2008) and of the studies in sediments, most have taken place in vegetated and temperate systems. Southern California estuaries, like other estuaries in mediterranean-type climates, can experience tremendous temporal variability in nutrient inputs due to strong wet and dry seasonality (Zedler 1996, Page et al. 1995, Boyle et al. 2004). During precipitation events in the wet season, estuaries may receive high-concentration pulses of anthropogenic nutrients. Low stream flow during dry seasons decreases terrestrial N loads to the estuary, though due to agriculture and dense urban and suburban development sources, nutrient inputs can extend into the dry season and concentrations can be extremely high (Boyle et al. 2004, Kennison et al. 2003). Only four studies have measured N-fixation in southern California, and of these, three occurred in vegetated salt marsh sediments (Langis et al. 1991, Moseman 2007, Moseman-Valtierra et al. 2009) and only one in unvegetated intertidal sediments (Moseman-Valtierra et al. 2010). Studies of sediment denitrification in estuaries seem to be more prevalent than N-fixation, though only a few of these studies have occurred in mediterranean-type systems. In temperate systems, the % of N supply removed by denitrification ranges widely (8-50%) across estuaries (Nowicki et al. 1997, Seitzinger et al. 1988). Based on the few studies available in mediterranean-type systems with which we can compare, the importance of denitrification may also be wide ranging. However, no published studies have measured denitrification rates in southern California estuaries; thus, we have no information on importance of sediment denitrification in these unique systems.

We measured sediment N-fixation and denitrification rates in a small southern California estuary quarterly over one year and investigated how these rates varied tidally, and between wet and dry seasons, as well as in response to N-enrichment of the water. As southern California estuaries have broad expanses of intertidal mudflat that experience mixed semi-diurnal tides with up to ~2m tidal amplitude, it is important to have information on rates under both exposed and submerged conditions. We expected higher N-fixation rates in exposed (bare) vs. submerged (slurry) conditions, as N-fixation depends on the diffusion of N_2 gas to N-fixers.

We also expected N-fixation to be affected negatively by increased N. In contrast, we predicted greater denitrification in submerged vs. exposed conditions since access to porewater NO_3^- would be greater during submergence, and that the addition of N would increase denitrification. We also predicted variability of both N-fixation and denitrification, especially between wet and dry seasons, as salinity fluctuations and nutrient inputs related to seasonal precipitation could influence rates. Specifically, we expected lower N-fixation and higher denitrification during wet season periods when N-inputs to the system were hypothesized to increase as a result of runoff from the urbanized watershed. Investigating microbial N processes in a range of estuarine conditions over time and the response to alterations in nutrient regimes is vital to increase our understanding of N-cycling in anthropogenically impacted, often eutrophic, estuarine systems in mediterranean-type climates.

METHODS

Famosa Slough is a 15ha estuary located in San Diego County, California (32°45’00 N, 117°13’40 W) that drains approx 1.5 km^2 (McLaughlin et al. 2010). It is a remnant of the Mission Bay complex that, due to extensive modification of the surrounding area, has become isolated. A series of culverts isolate it from freshwater sources and most of the surrounding watershed, and tide gates restrict tidal exchange, resulting in muted and delayed tides. Famosa Slough has a history of high inorganic N inputs, especially during wet season rain events (Fong & Zedler 2000) and is on the U.S. federal 303(d) list of impaired water bodies for eutrophication. Water treatment ponds have been added to the south and southeast sides of the slough in order to improve water quality by collecting urban runoff, trash and sediment discharge.

Sediment cores (2.1cm diameter, 5cm depth) were haphazardly collected for N-fixation and denitrification measurements in one location of an unvegetated subtidal mudflat in Famosa Slough on the following dates in 2008: 7 January, 18 March, 1 July, and 12 September. On all dates, cores were collected from a consistent elevation during low tides in the unvegetated subtidal mudflat, then transported on ice to the laboratory at University of California, Los Angeles. Samples were processed for N-fixation and denitrification measurements within 4 to 6hrs of collection. Surface water (1L) was collected at the sediment core collection site and transported to the lab on ice where it was vacuum filtered (Watman GF-C) and used for the treatments.

N-fixation and denitrification rates were determined using the acetylene reduction and block techniques, respectively (Capone & Montoya 2001). Acetylene gas acts as a substitute for N_2 in N-fixation; by quantifying the production of ethylene (C_2H_4) gas from the reduction of acetylene (C_2H_2), nitrogenase activity was measured and N-fixation indirectly quantified. Acetylene also blocks the final step of denitrification; rather than proceeding from NO_3^- to nitrous oxide (N_2O) to N_2, the reactions stop at N_2O. By measuring the accumulation of N_2O over time, we determined the rate of denitrification.

For January 2008, N-fixation and denitrification rates were determined for bare sediment cores (n=5) simulating low tide. For March, July and September sampling dates, N-fixation and denitrification were determined for three treatments: 1) bare sediment core with no additional water added (n=3) as above, 2) sediment core slurried with 50ml of ambient site water (n=3) simulating high tide, and 3) sediment core slurried with 50ml of 300μM nitrate (NO_3^-) amended site water and 1% chloramphenicol (n=3) simulating high tide with N-enrichment. Chloramphenicol inhibits *de novo* protein synthesis (Joye & Paerl 1994), thus potential formation of new nitrogenase enzymes under high N is suppressed, ensuring N-fixation rates are based on activity of the existing population at the time of sampling. Due to low N concentration of the ambient water, formation of new nitrogenase was not a concern over the short incubation period in the ambient treatment.

In the laboratory, each sediment core was extruded into a 125ml Erlenmeyer flask, and where appropriate, treatment water was added. To create the slurry, flasks containing cores and treatment water were gently shaken by hand to disaggregate the sediment and distribute the water. Flasks were sealed with a recessed rubber stopper and electrical tape to create an air-tight vessel, and purged with N_2 gas to create anaerobic conditions. As denitrification is a strictly anaerobic process, initially purging all sediment samples with N_2 gas allowed both N-fixation and denitrification measurements to be performed simultaneously. Preliminary studies showed that adding N_2 gas did not affect N-fixation rates (T. Kane, unpublished data). To the headspace of each incubation vessel we introduced 20ml of purified acetylene created in the lab from the reaction of calcium carbide with dH_2O. One control core, without addition of acetylene, was also sampled for each treatment to account for any natural production of measured gases from the site, and subtracted the natural accumulation of gas from the acetylene-influenced production in the final rate calculations. We sampled each vessel over a 24hr time series, at 0, 6, 12, 18, and 24 hrs, by removing 1.5ml of gas from the headspace using a gas-tight syringe, and stored gas samples in 3ml glass collection tubes for analyses. Sediment water content, required for N-fixation and denitrification calculations, was determined by loss of mass of wet versus dry sediments in the incubation flasks after drying at $60^{o}C$ until weight stabilized.

Gas samples for N-fixation measurements via acetylene reduction were analyzed for ethylene using a gas chromatograph (GC) with a Flame Ionization Detector. For denitrification N_2O was analyzed using a GC with an Electron Capture Detector. The production of ethylene from 0-18hrs was used to determine N-fixation rates, and the production of N_2O from 0-6hrs was used to determine denitrification rates; these time points were chosen as they captured the maximum rates observed for the majority of samples. Rates were calculated using equations in Capone and Montoya (2001) and presented as $\mu mol\ N\ m^{-2}\ hr^{-1}$. For N-fixation, we assumed a theoretical reduction ratio of 3:1 (C_2H_2 reduction: N_2 reduction) by nitrogenase (Capone 1983, Joye and Paerl 1993), though this may underestimate rates (Seitzinger & Garber 1987). As acetylene inhibits nitrification, all denitrification rates were dependent on existing NO_3^- within the incubation. *In situ* denitrification may be higher than our measured rates if there is coupled nitrification-denitrification activity. However, when water column NO_3^- is high (>60 μmol/L) coupled nitrification-denitrification is thought to be a minor component of total denitrification (Seitzinger et al. 2006).

Water samples from each treatment (n=1) were collected, salinity measured using a handheld refractometer, and samples frozen until analyses. All water samples were sent to the Marine Science Institute (MSI) Analytical Laboratory at University of California, Santa Barbara for analysis of nitrate + nitrite (NO_2^+ + NO_3^-, hereafter NO_3^-), ammonium (NH_4^+), and orthophosphate (PO_4^{3-}) concentrations via Flow Injection Analysis.

Daily precipitation totals at the San Diego Sea World station (32.767°N, 117.225°W) adjacent to Famosa Slough were obtained from National Oceanic and Atmospheric Administration National Climatic Data Center (www.ncdc.noaa.gov).

Statistical Analyses

N-fixation and denitrification data were tested for assumptions of parametric statistics. Transformation was not successful in meeting the assumptions of ANOVA. Therefore, we analyzed N-fixation and denitrification response variables using Generalized Linear Model (GLM) analyses by date (March, July, September) and treatment (bare, slurry, slurry+N). January was not included, as only bare core measurements were performed on that date. Statistical analyses comparing salinity and nutrient concentrations across dates and treatments were not possible for treatment water due to lack of replication.

RESULTS

Typical of mediterranean-type climates, the vast majority of rainfall occurred in the winter months (December – March) (Figure 1). During our first wet season sampling in January, Famosa Slough received 5.9cm of rain in the previous 4 days, including 0.9cm in the immediately preceding 24 hours. In our second wet season sampling in March, 0.3cm of rain fell during the 4 previous days, but no rainfall occurred in the 48hrs immediately before our sampling. During the dry season, there was no precipitation between June 5th and the end of September, a period that included our July and September sampling dates.

N-fixation activity was highly variable for all except the September treatments (Figure 2), likely due in part to small sample size. Overall, rates were highest in bare and slurry treatments, and lowest in the slurry+N treatment, resulting in a significant effect of treatment (Table 1A). Relative to the other dates, it appeared as though lower N-fixation activity occurred in September, though date was not statistically significant.

While denitrification rates were extremely low or not detected under simulated exposed (bare) and submerged ambient (slurry) conditions, rates responded dramatically to N inputs (Figure 3). Denitrification rates showed a significant interaction with date and treatment (Table 1B). Rates increased in slurry+N treatments, especially in March and July where mean N enriched rates were up to 2 orders of magnitude higher than ambient treatments. Denitrification rates in the slurry+N treatment were lowest in September. However, rates demonstrated high variability, especially in July.

Treatment water salinity was similar to typical oceanic levels in March, but hypersaline in the dry summer months of July and September (Table 2). Inorganic N concentrations, both NO_3^- and NH_4^+, were extremely low relative to most southern California estuaries (Kennison et al. 2003) in ambient treatments across all dates, even in March when sampling occurred 2 days after a small rainfall event. Ambient NH_4^+ was greater than NO_3^- on all dates, suggesting flux to water from sediment processing may be a source of NH_4^+. Slurry+N treatment water was enriched for NO_3^- relative to ambient site water used in slurry treatments for all three dates, with the highest NO_3^- concentration in July. PO_4^{3-} was lowest in March.

DISCUSSION

Denitrification rates were limited by inorganic N supplies, though the strength of limitation varied across wet and dry seasons. It was clear that a responsive denitrifier community existed year-round in the sediments, as rates always increased with NO_3^- enrichment. Our results support findings of survey (Cabrita & Brotas 2000) and experimental (Magalhaes et al. 2005a) studies in two eutrophic estuaries in the Mediterranean climate of Portugal, where sediment denitrification increased with NO_3^- concentration. Our bare and ambient slurry rates were extremely low or not detected, likely due to the small volume of available low- NO_3^- porewater in the bare treatment and the very low ambient NO_3^- concentration of treatment water even in the wet season. Our N enriched N-fixation rate was much lower in September than on other dates, though this result cannot be explained with available data. As the removal of inorganic N is important in eutrophic estuaries, future research should further investigate seasonal patterns of denitrification under a range of nutrient regimes.

Our highest denitrification rates, measured under N enrichment in March and July, were within the range of other mediterranean-type systems, regardless of eutrophication status, even though our NO_3^- concentrations were 1.5 to 54-fold higher than all studies mentioned below. In non-eutrophic Tomales Bay in northern California, denitrification was up to 60 μmol N m^{-2} h^{-1} during a period of NO_3^- enriched runoff (Joye & Paerl 1993). In eutrophic estuaries, denitrification ranged from 25 to 35 μmol N m^{-2} h^{-1} across systems in Portugal (Nizzoli et al. 2007, Cabritas & Brotas 2000, Magalhaes et al. 2005b) and 83 to 237 μmol N m^{-2} h^{-1} in Elkhorn Slough, central California (Caffrey et al. 2010). In Mediterranean Venice Lagoon, Italy, one of the most eutrophic estuaries in the world, denitrification ranged from 7 to 200 μmol N m^{-2} h^{-1} (Ericksson et al. 2003). In several of these studies, denitrification was measured using the isotope pairing method (An et al. 2001), which incorporates denitrification coupled to nitrification. Even though our method captured denitrification resulting only from water NO_3^- sources, rates were still comparable, indicating the higher NO_3^- concentrations experienced in our treatments relative to other systems was responsible.

N-fixation was severely suppressed by N enrichment. Our bare and ambient rates were comparable to rates in other eutrophic estuaries in southern California, including intertidal mudflats of Upper Newport Bay Estuary (Kane, PhD Dissertation Chapter 1) and unvegetated marsh sediments in Tijuana Estuary (8 μmol N m^{-2} h^{-1}, assuming 3:1 C_2H_2:N_2 ratio; Moseman-Valtierra et al. 2010). However, compared to Tomales Bay, a non-eutrophic estuary with a mediterranean-type climate in northern California, our N-fixation rates were much lower under similar NO_3^- concentrations. Rates in Tomales Bay ranged between 30 and 125 μmol N m^{-2} h^{-1}, an order of magnitude higher than ours, when NO_3^- concentrations were low or below detection (Joye & Paerl 1994, Joye & Paerl 1993); however, high N-fixation activity in Tomales Bay was attributed to a visible microbial mat community, which we did not observe in our system. With the addition of N, our N-fixation rates declined dramatically, as they did in Tomales Bay where rates approached zero with N enrichment from post-storm runoff (Joye & Paerl 1993), and were similar to rates in sandy sediments of moderately eutrophic Duoro River estuary, Portugal (2.67 μmol N m^{-2} h^{-1}) (Magalhaes et al. 2005b), though both Tomales Bay and Duoro River estuary had lower NO_3^- concentrations than we used. While N-fixation in Famosa slough was depressed relative to more oligotrophic systems under low N inputs, our N-fixation community was still active, in contrast to some systems where N-fixation shut down completely during periods of high NO_3^- concentrations. This activity supports the idea that N-fixation is adapted to occur, albeit at very low levels, even during high inorganic N inputs in chronically eutrophic systems.

We found minimal evidence of N-fixation and denitrification patterns related to seasonal precipitation in this estuary. We would expect increased surface water N to coincide with precipitation, though water column nutrient concentrations remained extremely low year-round. Low N concentrations may be a result of the small area of land drained by the watershed in conjunction with isolation of the estuary from the watershed, thus limiting inflows of freshwater that typically deliver anthropogenic N inputs in southern California systems. It could also indicate that the management strategy of adding catchment ponds to intercept any runoff into

Famosa Slough may have been successful. Additionally, oceanic or higher salinity across all dates may have contributed to keeping rates of N-fixation low. In another southern California estuary, we found low salinity correlated with increased N-fixation during large freshwater flows due to a winter precipitation event (Kane, PhD Dissertation Chapter 1). While we did capture some variability of both N-fixation and denitrification throughout the sampling year, a longer study period with greater sample size is necessary to determine seasonal patterns.

The responses of N-fixation and denitrification rates to N-enrichment in our treatments suggest N inputs could influence N-cycling in this estuary. For estuarine systems located in highly urbanized areas where modifications to hydrology can be extreme, is important that future studies aimed at understanding eutrophication effects investigate the role of these and other sediment N processes that have the potential to improve or exacerbate N impacts.

Table 3-1. Statistical results of GLM analysis for A) N-fixation, and B) denitrification. DF = degrees of freedom. Bold p-values are significant.

A. Nitrogen Fixation

	DF	L-R ChiSquare	Prob>ChiSq (p-value)
date	1	2.8113734	0.0936
treatment	2	19.417652	**<0.0001**
date x treatment	2	4.0894917	0.1294

B. Denitrification

	DF	L-R ChiSquare	Prob>ChiSq (p-value)
date	1	5.6269816	**0.0177**
treatment	2	45.470423	**<0.0001**
date x treatment	2	15.053129	**0.0005**

Table 3-2. Salinity and nutrient concentrations of ambient and enriched treatment water for each sampling date (n=1).

	March		**July**		**September**	
	ambient	**enriched**	**ambient**	**enriched**	**ambient**	**enriched**
Salinity (ppt)	33	33	37	37	36	36
NO_3^- (μM)	0.58	263.00	0.61	382.00	0.80	253.00
NH_4^+ (μM)	3.67	3.81	6.94	4.66	3.68	3.92
PO_4^{3-} (μM)	0.35	0.39	2.33	2.59	1.66	1.67

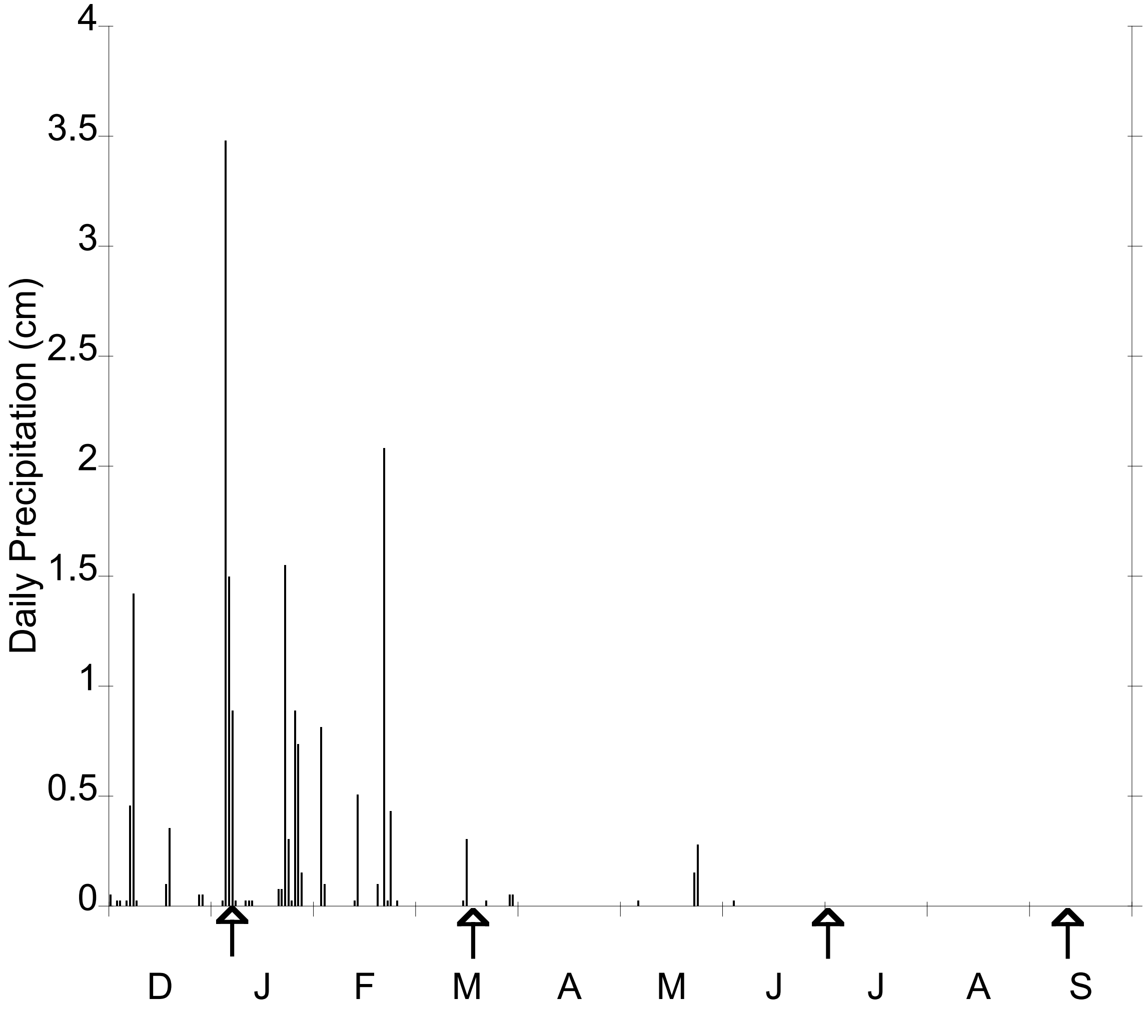

Figure 3-1. Daily precipitation in cm from December 2007 to September 2008. Arrows indicate sampling dates.

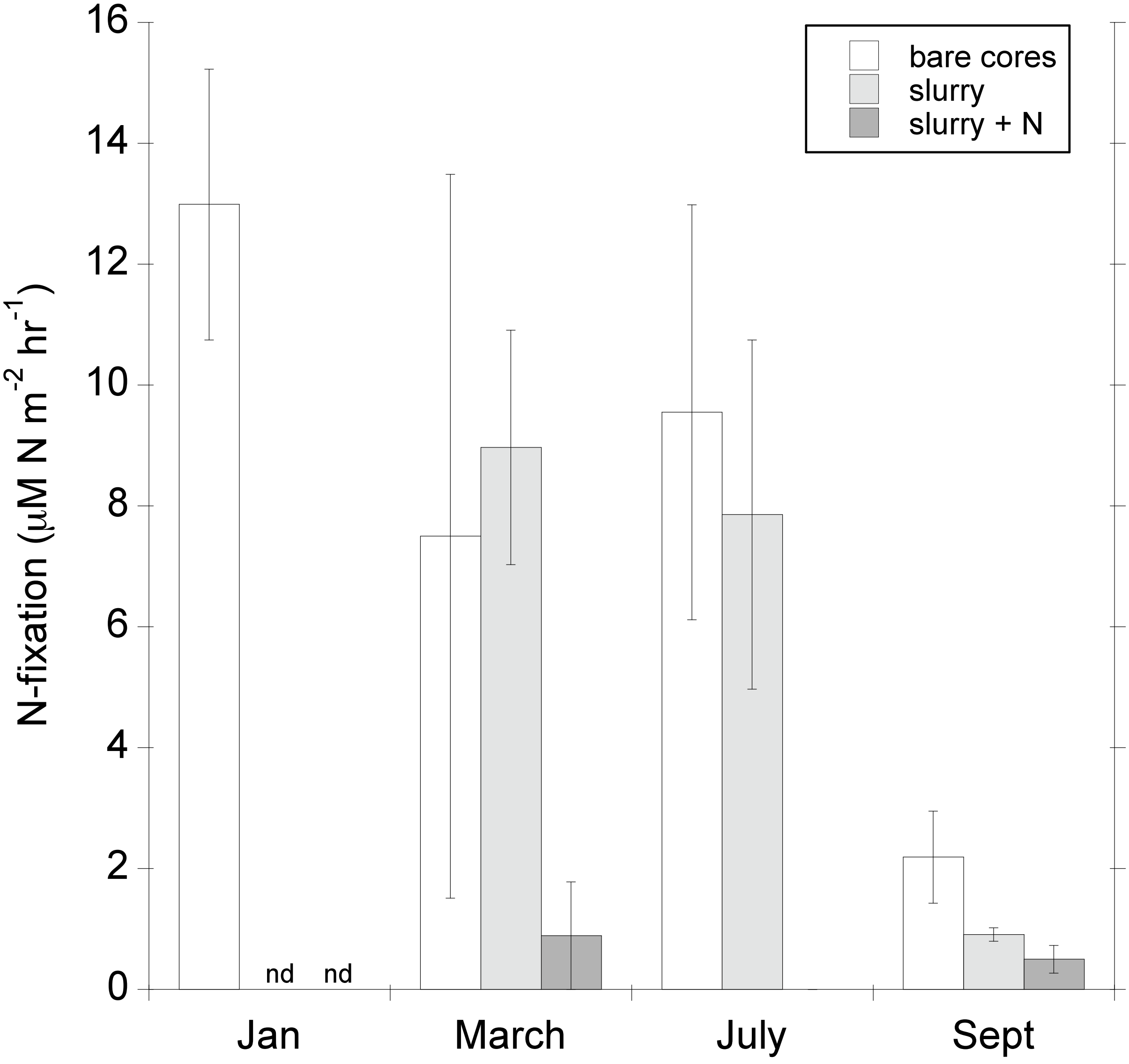

Figure 3-2. Mean (std err) of N-fixation rates for each treatment on all dates (n=3). nd = no data collected.

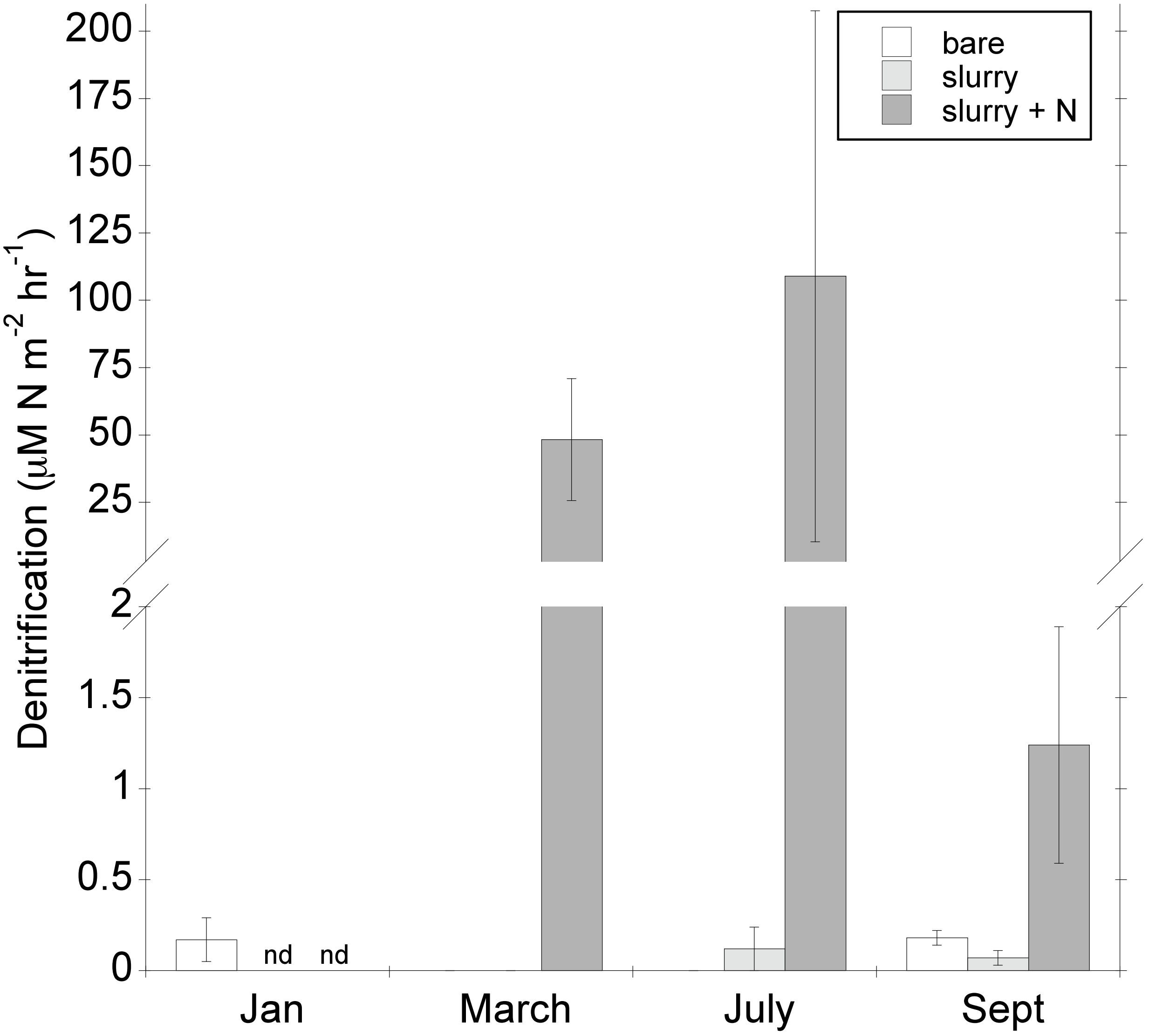

Figure 3-3. Mean (std err) of denitrification rates for each treatment on all dates (n=3). Note the break in the y-axis. nd = no data collected.

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CONCLUSION

In southern California estuaries, this research is the only study to measure denitrification rates under any conditions, and the first to provide N-fixation rates across a large estuarine area and over multiple wet and dry seasons. It is also the only research to investigate the response of N-fixation and denitrification to the extreme high levels of NO_3^- and PO_4^{3-} that can be experienced by these highly anthropogenically impacted estuarine systems. These estuaries are located in highly urbanized areas where modifications to hydrology and anthropogenic N inputs can be extreme. Studying these microbial N processes contributes to our understanding of biogeochemical cycles subjected to increased anthropogenic impacts in this and other highly modified mediterranean-type estuaries, and provides an important foundation for future N studies aimed at understanding eutrophication effects in this and similar systems. It is imperative that we continue to study the effects of N-enrichment on microbial N-processes that have the potential to improve or exacerbate eutrophic threats.

N-fixation appeared to play a minor role in supplying N to these estuaries. N-fixation rates, under both ambient and NO_3^- enriched conditions, were generally low relative to more oligotrophic systems, but comparable to rates in other highly and moderately eutrophic mediterranean-type systems. N-fixation was severely negatively affected by N enrichment, decreasing under both high and extremely high concentrations of NO_3^- (Ch. 2 & Ch. 3). While N-fixation did not provide a substantial source of N, it did contribute N to the system throughout the year; even under high inorganic N conditions, activity was never shut down completely. This supports the idea that N-fixation is adapted to occur, albeit at very low levels, even during high inorganic N inputs in chronically eutrophic systems. In Famosa Slough, rates for bare cores and ambient slurried cores, simulating emerged and submerged intertidal sediments, respectively, were similar to each other and comparable to rates in intertidal mudflats of Upper Newport Bay Estuary during emergent conditions (Ch. 1 & Ch. 3).

Seasonal precipitation dynamics, typical of mediterranean-type climates, played an important role in regulating abiotic factors that affected N-fixation (Ch. 1). Episodic rain events during the wet season brought pulses of both freshwater and nutrients. The changes in salinity and inorganic N concentrations related to these precipitation events influenced N-fixation rates, though they acted in opposing ways to influence N-fixation and may have complicated the response of N-fixers to storm runoff. There was a relationship between lower porewater salinity and higher N-fixation during wet weather, when highest N-fixation rates occurred despite high NO_3^- inputs. During dry conditions, porewater NH_4^+ and NO_3^- were negatively related to N-fixation. While highly significant, relationships with abiotic characteristics were weak and did not explain a large proportion of the variability in N-fixation, which suggests N-fixation rates were not strongly driven by individual abiotic factors, but were likely regulated by co-variation of positive and negative relationships between driving variables. We found minimal evidence of N-fixation patterns related to seasonal precipitation in Famosa Slough.

These studies demonstrated anthropogenic nutrient enrichment had strong positive effects on denitrification in sediments of eutrophic mediterranean-type estuaries (Ch. 2 & Ch. 3). Denitrification rates were limited by inorganic N supply, though the strength of limitation varied across wet and dry seasons (Ch. 3). Without NO_3^- enrichment, rates were barely detected. Our bare core and ambient slurry rates in Famosa Slough were extremely low or not detected, likely due to the small volume of available low-NO_3^- porewater in the bare treatment and the very low ambient NO_3^- concentration of slurry treatment water even in the wet season. Extensive modification to Famosa Slough and the small size of the watershed kept water column N concentrations low on all sampling dates. With NO_3^- enrichment, denitrification activity increased dramatically, making it clear that a responsive denitrifier community existed year-round in the sediments. Enriched rates were within the range of reported values for other mediterranean-type systems, regardless of eutrophication status, even when our NO_3^- concentrations were considerably higher than other estuaries.

Despite the dramatic increase in denitrification rates with NO_3^- enrichment, it was clear that denitrification is not a solution for the extreme high levels of eutrophication found in some mediterranean-type estuaries. Denitrification rates increased dramatically in the extremely high NO_3^- experimental treatment (Ch. 2). However, our rates were lower than were expected based on the high concentration of NO_3^- to which our cores were exposed, especially when comparing our study to other systems in mediterranean-type climates. The greatest increase in denitrification rates occurred in the high NO_3^- concentrations in the slurry treatments (Ch. 3), rather than the extremely high NO_3^- conditions of the bare incubations of the N & P experiment (Ch. 2). This difference in activity suggests NO_3^- accessibility in bare vs. slurry incubations was important, and suggests the possibly that there could be an inhibitory effect of denitrification with extremely high NO_3^- concentrations. Under the highest NO_3^- concentrations, denitrification as the percentage of total dissolved inorganic N was <1%. Therefore, denitrification did not occur at a level high enough to compensate for the large dose of N. Other nutrient management strategies are necessary to reduce anthropogenic eutrophication in these systems.

This research provides much needed insight into how N is processed at the microbial level via N-fixation and denitrification in mediterranean-type estuaries. As the addition and removal of N is important in eutrophic estuaries, future research should continue to investigate seasonal patterns of N-fixation and denitrification under a range of nutrient regimes. However, much research is still needed to understand N-cycling in these estuaries. Additional processes at the sediment–water interface, which we did not measure, are likely influencing N-fixation and denitrification rates. For example, internal nutrient recycling processes in the sediment that generate NH_4^+, which could include remineralization of organic matter and dissimilatory nitrate reduction to ammonium (DNRA), may be important in the regulation of N-fixation. While our assays accounted for denitrification based only on water column and porewater sources, changes in water nutrients incorporate all nutrient transformation processes that occur, which may include uptake by macroalgae, nitrification, and DNRA. DNRA may be an especially important to consider in eutrophic systems, as it recycles NO_3^- to NH_4^+ within the system, rather than removing N as in denitrification. Investigating the role of N-fixation and denitrification, as well as additional microbial N processes, in a range of estuarine conditions over time and the response to alterations in nutrient regimes is vital to further our knowledge of N-cycling and is imperative to the overall understanding of eutrophication in these anthropogenically impacted mediterranean-type systems. Additional studies are needed in both eutrophic and non-eutrophic estuaries in mediterranean-type climates in order to understand how N-fixation and denitrification contribute to N dynamics in these systems and how these processes might respond to future changes, especially in the context of anthropogenic influences that impact nutrient dynamics and hydrology, and potential alterations to precipitation related to climate change.