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Investigating the Role of Nitrogen Fixation and Denitrification in Ameliorating Deteriorating Water Quality in a Highly Eutrophic Southern California Estuary

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Abstract

Estuaries are highly productive ecosystems that support many endangered and commercially important species. In most estuaries, nitrogen limits primary productivity. However, if present in excess, nitrogen leads to eutrophic conditions, which adversely affects water and habitat quality. Southern California’s estuaries have highly developed watersheds resulting in high loads of nitrogen from anthropogenic sources and eutrophication. Our overall goal was to understand the role of two biogeochemical processes, N-fixation and denitrification, that affect processing of N in estuaries and to investigate their response to increased N loads from the watershed. Nitrogen fixation transforms elemental nitrogen (N$_2$) into ammonium ions (NH$_4^+$) that can be used by primary producers, and therefore is a “new” source of nitrogen. Denitrification transforms nitrate (NO$_3^-$) into atmospheric nitrous oxide (N$_2$O) or nitrogen gas (N$_2$), and is therefore a loss of nitrogen from aquatic ecosystems. As these processes either contribute or remove nitrogen from the system, they have the potential to affect water quality and ecosystem health. However, little is known about nutrient dynamics in southern California estuarine environments.

We measured spatial and temporal variability of nitrogen fixation and denitrification and investigated relationships with abiotic/biotic factors in an eutrophic southern California estuary through field surveys and experiments in Upper Newport Bay Ecological Reserve, a large estuary in Orange County, California. Field surveys were conducted in two seasons (wet and dry) over two years and included sampling in 10 sites. Nitrogen fixation and denitrification rates were measured, as well as water and sediment nitrogen and Phosphorus, and sediment grain size, organic content, and chlorophyll a. Rates of N-fixation varied greatly spatially and temporally, with spatial variability more prevalent than seasonality. N-fixation correlated negatively with salinity and positively with nutrient (NO$_3^-$) concentration, and did not relate to any sediment characteristics, suggesting that short-term responses to rain events were driving patterns. N-fixation was not related to chlorophyll a, suggesting that the majority of fixation was by bacteria rather than blue-green algae. Overall, denitrification was low or immeasurable across all sites and seasons.

Three experiments identified biotic and abiotic factors affecting these rate processes. In a common garden field experiment, N-fixation found to be driven by the characteristic of the experimental common site rather than the transplanted sediment, also suggesting that short-term water column factors are controlling fixation. In a laboratory mesocosm experiment, we determined that water column nitrate greatly accelerated denitrification, but inhibited rates of N-fixation. However, even the enhanced rates of denitrification in our experiment remained far lower than the increases in nitrate supply typical of our highly eutrophic estuaries. Thus, denitrification, at best, removes a small portion of nitrogen supplied from the watershed. The third experiment found no effects of the prolific macroalgal mats that dominate upper Newport Bay on sediment nitrogen fixation in the field.

Our research has contributed to basic understanding of nitrogen cycling in these unique, understudied, and heavily impacted ecosystems. Unfortunately, we did not find that denitrification is an effective tool to ameliorate accelerating loads of N to our coastal ecosystems; rather reduction in loads appears to be the only viable solution.
Introduction and Problem Statement

Estuaries are among the most productive environments on earth (Lefevre et al. 2000), functioning as important habitat for several endangered and commercially important species. These ecosystems establish an interface between freshwater and coastal marine systems, and are characterized by intense physical-chemical gradients, including salinity, temperature, pH, oxygen, and nutrients. There is overwhelming evidence that nitrogen (N) limits estuarine primary productivity (e.g., Berner & Berner 1996); however, when in excess, N can lead to eutrophic conditions. Estuarine systems have the potential to contribute significant amounts of N to adjacent marine environments (Tibbles & Rawlings 1994) supporting coastal food webs, or may act as “filters” of excess N as water passes through to the ocean. Thus another important estuarine function is to support oceanic productivity as well as to protect and preserve nearshore oceanic water quality. In California and worldwide, the balance between these functions is threatened by increased anthropogenic sources of N from developed watersheds. Our overall goal is to further our understanding of the role of two biogeochemical processes, N-fixation and denitrification, that affect processing of N in estuaries and to investigate their response to increasing N loads from the watershed. The ultimate goal is to aid in protecting these important aquatic ecosystems by understanding the constraints and controls that function to balance these two processes and how these processes, especially denitrification, may act to ameliorate the negative effects of eutrophication.

The importance of N fixation and denitrification as sources and losses of N in estuaries has been well established in temperate systems. For example, N fixation in one east coast U.S. intertidal saltmarsh was estimated to supply up to 83% of in situ plant nitrogen demand, while subtidal sediments were estimated to supply up to 0.43 Tg N/yr (1 teragram = 1 x 10^{12} g) to the oceanic environment (Capone 1988). Seitzinger (1988) concluded these two processes are in overall balance, as loss of N from denitrification was equal to or greater than N input from nitrogen fixation in coastal ecosystems. The role of denitrification in ameliorating N loading to estuaries is equivocal. In one UK estuary, denitrification was estimated to remove 25% of the inorganic nitrogen load to the estuary annually (Barnes & Owens 1999), while Nowicki (1994), in the northeastern US, found denitrification to remove less than 20% of N inputs, and concluded denitrification might not be significant in reducing eutrophication. We propose to study the processes of nitrogen fixation and denitrification in one southern California estuary to begin to understand their roles in processing nitrogen in this system. Only one study related to nitrogen fixation in an estuarine ecosystem has been performed in southern California. In this study, soil organic content, Spartina sp. detritus, and temperature affected sediment fixation rates in a San Diego Bay saltmarsh (Langis et al. 1991).

Nitrogen fixation, an energetically costly process, transforms elemental nitrogen (N2) into ammonium ions (NH4+) that can be used by primary producers; it is considered a “new” source of nitrogen as it adds to that imported from the watershed or recycled within the estuary (Figure 1). N fixation is a highly regulated process usually triggered by low or limiting ammonia concentrations, and therefore may be depressed in eutrophic systems subject to high concentrations of NH4+. On the other hand, N fixation is carried out by the nitrogenase enzyme, which is extremely sensitive to oxygen (Atlas & Bartha 1998), and therefore may be enhanced in eutrophic estuarine sediments characterized by low oxygen. Thus, it is essential to quantify the effects of eutrophic conditions on N
fixation rates as differences in rates of “new” N addition may either exacerbate or ameliorate impacts of increasing anthropogenic inputs.

Studies in temperate estuaries have related rates of N fixation to several environmental parameters that also vary widely in California estuaries subject to N enrichment. The presence of high concentrations of inorganic nitrogen may inhibit fixation. Nitrogen fixation takes place in areas with low NH$_4^+$ concentrations (Capone 1988), and can be inhibited at a concentration of 200µM NH$_4^+$ (Teal et al. 1979). In one experiment, sediment cores treated with water without NH$_4^+$ resulted in 7-8 times the fixation rate of the controls with *in situ* NH$_4^+$ levels of 500µM (Capone 1988). NO$_3^-$ in sediments of a variety of habitats showed inhibitory effects on nitrogenase at concentrations of 50-200µM, though the reason for inhibition is not clear and may be due to the conversion of nitrate to ammonium via dissimilatory reduction (Capone 1988). Eutrophic estuaries in southern CA have organic-rich sediments (Kennison et al. 2003). As nitrogen fixation is an energetically costly process (Atlas and Bartha 1998), organic content of sediments could enhance the activity of heterotrophic diazotrophs; organic C in the forms of glucose and sucrose, for example, have produced positive responses of nitrogenase activity (Capone 1988). Highly disturbed estuaries may also be poorly flushed, altering salinity and oxygenation of the water and sediments. Overall, salinity (deSouza & Yoch 1997, Pinckney et al. 1995) and oxygen (Pael & Pickney 1996, Day et al. 1998) have been negatively related to fixation rates. Since there are many different types of nitrogen fixers, oxygen may affect systems differently depending on which organisms are present and their adaptations to oxygen. Finally, fine grain sediments tend to have higher nitrogen fixation rates than course-grained sediments (Capone 1988), though it is not clear whether this is due to oxygen levels or nutrient conditions.

Denitrification is the process of transforming nitrate (NO$_3^-$) into nitrous oxide (N$_2$O) or nitrogen (N$_2$) (Figure 1). It is a net loss of biologically available nitrogen from aquatic ecosystems to unavailable gaseous forms in the atmosphere. Denitrification requires strictly anaerobic conditions and oxidizes organic material for energy (Atlas & Bartha 1998). The process is often limited by NO$_3^-$ supply; thus loss of N via denitrification may be greatly increased in eutrophic estuaries characterized by high NO$_3^-$, as is typical of southern CA (Boyle 2004, Kennison et al. 2003). Sediments in eutrophic estuaries are also characterized by abundant supplies of organic matter (Kennison et al. 2003), which may support high rates of denitrification. As denitrification requires both NO$_3^-$ and anaerobic conditions, it can only occur at the interface of anaerobic and aerobic zones of the sediment where it is coupled to nitrate supply from nitrification (Jenkins & Kemp 1984) or where water column nitrate has diffused into the porewater (Koike & Sørensen 1988). As denitrification is a loss of N, it is important to quantify rates of denitrification and the factors that enhance these rates, as there is potential for enhanced rates to moderate the impacts of increasing anthropogenic inputs.

Rates of denitrification have been related to environmental conditions associated with eutrophication. Nowicki (1994) determined nutrient enrichment was important in controlling denitrification rates; in this study denitrification rates were up to eight times higher in enriched mesocosms. Denitrification activity tends to be greater with greater supplies of organic substrate and elevated NO$_3^-$ concentrations (Koike & Sørensen 1988) though above 500µM NO$_3^-$, denitrification rates can become saturated in many systems (Capone 1988). Oxygen levels can affect denitrification, as in some systems NO$_3^-$ is supplied by nitrification, an aerobic process. However, NO$_3^-$ is also supplied by the water column, and in areas of high nutrient loading would be expected to increase
denitrification rates. Kaplan et al. (1979) found *in situ* denitrification rates increased with sediment temperature (0°C to 35°C) in an intertidal area of Great Sippewissett marsh (Cape Cod, MA), and Barnes & Owens (1999) found the highest rates at the highest temperature in a UK estuary.

Microbial processing of N via N fixation and denitrification is virtually unstudied in Californian and other Mediterranean estuaries (but see Langis et al. 1991, Joye and Pearl 1994). Most research on these processes has been performed in temperate estuaries such as the Chesapeake, and may not be applicable to California estuaries. California is characterized by a Mediterranean climate, consisting of distinct wet and dry seasons and therefore estuaries function differently from those in other parts of the world (Zedler 1996, Williams and Zedler 1992). River flow is more seasonal with pulses of nutrient inputs to local estuaries usually associated with winter storms. With increased urbanization, nutrient input may extend into the dry season when input from land is historically low (Zedler 1996). Southern California estuaries have highly urbanized watersheds and experience increased total nutrient and freshwater loads, which alters nutrient levels and salinity (Zedler 1996). Shifts in land-use practices toward agriculture and urbanization result in greater nutrient loading (Valiela and Bowen 2002), as documented in Upper Newport Bay (Boyle et al 2004), and may alter rates of fixation and denitrification. It is imperative to understand these processes in California systems, especially since approximately 90% of estuarine and salt marsh systems have already been lost along California’s coast compared to pre-European settlement conditions (Zedler 1996). The remaining estuaries are critical habitat for several endangered species and are often severely degraded or eutrophic. Little is known about their basic functioning, especially nitrogen cycling, as studies are difficult due to the complexity of nitrogen cycling within estuarine environments (but see Page et al. 1995; Fong & Zedler 2000). As a result, it is difficult to ensure survival of the vital remaining habitat with our limited knowledge.

**Objectives**

Our objective was to investigate microbial processing of nitrogen via N fixation and denitrification among and within various habitats of an eutrophic Southern California estuary and the abiotic and biotic factors associated with eutrophication that affected these processes. This research addressed the following questions: 1) What were rates of nitrogen fixation and denitrification processes in different locations throughout an estuary?, 2) How did rates differ seasonally?, 3) What biotic and abiotic factors correlated with particular rates?, 4) What was the quantitative relationship between key abiotic factors and rates? By investigating the microbial processes of nitrogen fixation and denitrification in this system, we contributed to the basic understanding of nutrient cycling in estuarine environments, and provided much needed information to aid in managing southern California estuarine ecosystems impacted by increased N loading.

Ultimately, we hope to aid in protecting the health of these important aquatic ecosystems by understanding the factors that may enhance denitrification or reduce N fixation. Accelerating losses of N via denitrification may be a potential mechanism for reducing the impacts of increased anthropogenic loading of N. As southern California estuaries are highly impacted due to intense urbanization, understanding biogeochemical
processes that remove or add nitrogen allow us to make more informed decisions regarding management of watersheds and estuarine habitats.

**Procedures and Results**

**Field sites**

Our research site is Upper Newport Bay Estuary (UNB) in Newport Beach, California (Figure 2). Nutrient loading, eutrophication, and nuisance blooms of macroalgae in this estuary were quantified in a two-year study funded by the Water Resource Center during 1998-99. This research supported two dissertations (K. Boyle and K Kamer), resulted in 4 publications, and provided initial data for a 3 year $399,000 STAR grant from EPA to P. Fong. The research reported here used knowledge gained from this earlier project to determine field sites and approaches, supported another Ph.D student, and provided the data for a collaborative study with Southern California Coastal Water Research Project funded by the State Water Board ($188,000).

UNB is an ecological reserve that is densely surrounded by residential and commercial property with some street runoff; however, the main source of freshwater is San Diego Creek. The lower estuary has been physically modified by dredging for a large marina, keeping it permanently open to the ocean (Kamer et al. 2004). The remaining upper half is comprised of a wide and deep main channel with several small islands and a network of smaller tidal creeks along the edges that episodically support large opportunistic macroalgal blooms of Enteromorpha sp. and Ulva expansa (Kamer et al. 2001).

A year round salinity gradient is supported by freshwater inflow from agriculture, urban development, and treated wastewater, with lowest values nearest San Diego Creek increasing seaward. The gradient is more pronounced due to natural freshwater sources during the wet season (Kennison et al. 2003; Kamer et al. 2004). Average water temperature is lower during wet season months of December and February (16.5°C) and higher in dry season months of June and September (23°C) (Kennison et al. 2003). Studies of water nutrients demonstrate this estuary is highly eutrophic with a gradient of nutrients (NH₄⁺ and NO₃⁻) greatest where the river enters and lowest nearest the ocean inlet. NO₃⁻ is the dominant nitrogen available. (Boyle et al. 2004; Kamer et al. 2004; Kennison et al. 2003).

Boyle et al. (2004) and Kamer et al. (2004) found sediment nutrient concentrations to have no clear spatial pattern, though Kennison et al. (2003) reported TKN (Total Kjeldahl Nitrogen; includes all organic nitrogen and ammonia) was lowest at the mouth. Boyle et al. (2004) reported the widest range of sediment TKN values, 0.034-0.166 % dry weight, and Kennison et al. (2003) found percent organic content of the sediments was highest at the head and decreased toward the mouth. Organic content was between 1 to 3 % dry weight of the sediment (Kennison et al. 2003). Sediment grain size varied within the estuary, with sandier sediments (up to 69%) nearer to the ocean (Kamer et al. 2004; Kennison et al. 2003). Redox potential, a measure of oxygen availability, though highly variable demonstrated that oxygen was low with all sites less than +400mV, and most under +250mV (Kennison et al. 2003).

This estuary is subject to significant nitrogen loading from the watershed. Kennison et al. (2003) estimated loading rates from San Diego Creek for February and June 2002 using water flow data from the County of Orange. NO₃⁻, NH₄⁺, and TKN were
greater in the wet season verses the dry season. Total nitrogen loading for February was 3877.5 mol h\(^{-1}\), while in June it was 72.2 mol h\(^{-1}\). Variation in loading at different times of the year could affect microbial processes.

**Approach 1: Field Surveys**

Field surveys took place in UNB over two years, spanning both wet and dry seasons, to quantify seasonal changes in nitrogen fixation and denitrification rates. Large storm events were avoided as drastic fluctuations in salinity, turbidity, and scouring of sediments are not representative of the typical conditions during that time of the year. Surveys were performed along tidal creeks at two different areas of the estuary, the lower and middle reaches. Creeks, opposed to the main channel, were chosen for the likelihood of exhibiting maximum rates due to their calmer nature and deposition and retention of sediment nutrients. Results of Boyle et al. (2004) showed that creeks have higher sediment nutrient concentrations than the main channel of UNB, which has higher water column nutrients. It was concluded that the creeks function as “nutrient uptake and trapping environments” as opposed to the main channel which is mostly a location of nutrient transport.

We surveyed two areas that were chosen to incorporate measurements along the spatial gradient of the estuary. These areas include (1) “lower,” creeks near Shellmaker Beach, (2) “middle,” creeks near the mid-estuary (Figure 2.). In each area, five locations were selected in the intertidal mudflat. By measuring the same variables in each location it will be possible to elucidate differences in rates of nitrogen fixation and denitrification, and the relationships of these rates to physical and chemical conditions.

In each habitat, a 5m transect was deployed parallel to the water line. Nitrogen fixation and denitrification rates were measured on samples from random points along the transect using the acetylene reduction and the acetylene block techniques, respectively. Acetylene gas acts as a substitute for N\(_2\) in nitrogen fixation and by quantifying the production of ethylene gas from the reduction of acetylene gas, nitrogenase activity can be indirectly measured. Acetylene also blocks the final step of denitrification, where N\(_2\)O is reduced to N\(_2\) gas. By quantifying N\(_2\)O present in assay vessels, denitrification can be indirectly measured. By purging assay vessels with N\(_2\) gas, both acetylene reduction (nitrogen fixation) and acetylene block (denitrification) can be performed in the same incubation vessel. For these procedures, 10 cores along each transect were collected with a modified 30cc syringe corer to a depth of 5cm. Each core was returned to the laboratory in a cooler for incubation with acetylene gas. We used natural light regimes and incubated the cores at temperatures similar to natural conditions. The gas from the incubations was sampled over a period of time, at 0, 3, 6, 9, 12, 15, 18 and 24 hours. These times were chosen based on analysis of preliminary results and similar times have been used in comparable studies. All gas samples were stored in evacuated serum vials and processed within one month using a gas chromatograph in Dr. Doug Capone’s laboratory at the University of Southern California.

At each point along each transect, biotic and abiotic characteristics were measured and means for each transect used to characterize the site. Along each transect, macroalgal and macroalgal density was measured. Biomass of benthic microalgae was measured as chlorophyll a concentration. Macroalgae was determined by measuring the wet weight of all algae collected from a known area. Sediment temperature was be measured by insertion of a thermometer in the top 3-4cm of sediment. Redox potential, a quantitative measure of the ability of the sediments to oxidize or reduce substances (Mitsch &
Gosselink 1993), was measured by insertion into the sediment of six brightened platinum electrodes and one calomel reference electrode connected to a redox meter (Kennison et al. 2003). For other characteristics, three sediment cores (5cm depth) were collected from each of the ten transect points, composited, and returned to the lab. All sediments were dried, ground, and sieved to less than 2 mm. A portion of the sediment was sent to DANR Analytical Laboratory at UC Davis for quantification of Total N and Total C using the combustion gas analyzer method (APHA 1998). Another portion of the sediment was analyzed for organic content, determined by loss on ignition using a muffle furnace set at 400°C in our laboratory. On the remaining sediment, grain size was measured for % sand, % silt and % clay using the hydrometer method (Bouyoucos 1962).

We currently have no information on porewater characteristics in UNB, though they are expected to be important factors in biogeochemical cycling. Porewater that pools in the holes remaining after collection of cores for sediment characteristics (10 per transect) were measured for salinity and temperature. Water was collected using a large syringe and transferred to a collection bottle. Triplicate samples of overlying creek water were also measured for salinity and temperature. All water samples were returned to the lab on ice, filtered (Whatman GF/C), and frozen. Water samples were sent to the Marine Science Institute Laboratory at UC Santa Barbara where they were analyzed for NO$_3^-$ (NO$_3^-$ + NO$_2^-$), NH$_4^+$ and PO$_4$ using flow injection analysis (Switala 1999, Wendt 1999).

Two factor ANOVA, with the factors season (wet, dry) and area (upper, middle, lower), was used to determine if there were significant differences in the mean values of the response variables (nitrogen fixation, denitrification) that can be attributed to each factor. Correlation between response and abiotic variables was also determined.

**Results 1: Field Surveys**

There was a significant effect of site and season, with a significant interaction (2 Factor ANOVA, p<0.0001 for factors and interaction), on rates of nitrogen fixation in Upper Newport Bay demonstrating that the effect of site was not consistent across seasons (Figure 3). For example, the mean maximum fixation rate occurred in site Mid 5 in the 2005 wet season, while maximum rates were lower and co-occurred in Mid 5 and Low 4 in the 2006 wet season. Further, spatial patterns in the dry season were very different in the 2005 and 2006 seasons.

Overall, the highest nitrogen fixation rates occurred in March 2005, followed by September 2006. This positively corresponded to the pattern of rainfall in these months. March 2005 was the rainiest month, September 2005 had trace amounts, while neither February nor September 2006 experienced any rainfall, despite February being within the wet season. Correlation analysis supported this finding. Nitrogen fixation was negatively related to water salinity (R = -0.3459, p < 0.0001), yet positively related to water nitrate concentration (R = 0.2213, p < 0.0001). Both relationships, however, were relatively weak. Our experimental results, however (see below), suggest that the latter correlation may simply be due to covariance rather than mechanistic, as large amounts of nitrate are always washed into the estuary from the watershed with freshwater inflow.

Rates of denitrification were only measurable in the Mid 2 site; all other sites displayed no activity in any season. Rates different significantly by date (p = 0.0012) and were consistently higher in 2006 compared to 2005.
Overall, maximum denitrification rates were an order of magnitude lower than nitrogen fixation rates, suggesting no net removal of nitrogen via these two biogeochemical processes from this estuarine ecosystem.

**Approach 2: Field experiments**

**Experiment #1. Field Experiment to Determine Effects of Sediment Characteristics**

**Introduction.** We investigated the effects of different sediment types and organic contents on nitrogen fixation and denitrification rates in the sediments with a common garden transplant experiment. We hypothesized that both nitrogen fixation and denitrification rates were greater in finer sediments with higher organic matter content.

Eutrophic estuaries are generally composed of fine sediments enriched in organic matter. In southern California, Langis et al. (1991) found nitrogen fixation and microbial remineralization were lower in a restored (coarse grained) versus a natural marsh (fine grained). Currin et al. (1996), however, found the opposite in North Carolina; nitrogen fixation was 5-10 times higher in transplanted marsh sediments than in a natural marsh. This might suggest that grain size alone does not determine activity. However, fine grain sediments do tend to have higher organic content and lower O\textsubscript{2} penetration, and generally exhibit higher nitrogen fixation rates than course grained sediments (Capone 1988). It is expected that these conditions would affect denitrification in a similar way. Trimmer et al. (2000a) found much higher rates of denitrification in muddy sites of the River Thames estuary than was found in the sandier sites. The relationships between grain size, nutrient availability and biogeochemical nitrogen transformations are complex and not completely understood. Investigating sediment characteristics with regard to nitrogen fixation and denitrification rates will provide a clearer picture of how these processes are being affected by sediment characteristics.

**Methods.** Using a common garden design, we collected 5 sediment cores (size: 10cm diameter by 12cm depth) from each of 3 different sites with contrasting sediment types and transplanted them to one location. This location was a broad and relatively flat mudflat in the Mid estuary location (from the field surveys), close to Mid 3. We also cored and replanted 5 cores within the common garden and chose 5 sites that were not disturbed as an additional set of controls. At each collection location we took “pre-transplant” measurements of nitrogen fixation, denitrification and abiotic parameters. The surfaces of the transplanted cores were set flush with the sediment surface. Cores remained in the field for 25 days. At the end of the experiment, 3 cores (10cc, 5cm depth) from each experimental unit were collected and composited for nitrogen fixation and denitrification measurements, and sediment grain size, organic content, and Total N, and porewater salinity, NO\textsubscript{3} and NH\textsubscript{4}. Denitrification rates were low or immeasurable across all locations and treatments, so are not reported. One factor ANOVA (location) was used to determine if there was a significant difference in the mean values of the nitrogen fixation that can be attributed to location/sediment type. T-tests were used to compare means between fixation in the original location versus the transplanted location for each treatment.

**Results.** Rates of in situ nitrogen fixation varied significantly across the locations sampled prior to transplanting (1 factor ANOVA, p = 0.0036), with maximum mean values from the Low 1 site (Figure 5). After 25 days in the common garden, there were no significant differences among treatments (1 factor ANOVA, p = 0.4414). Rather,
rates began to converge at a relatively high fixation rate, by either remaining the same, or increasing in the common garden compared to initial values. This suggests that the physical, chemical, or biotic conditions associated with a site may be more important in controlling nitrogen fixation than sediment type. This supports the results of the field surveys that suggested shorter term processes rather than longer term sediment characteristics control rates of nitrogen fixation.

**Experiment #2. Laboratory Microcosm Experiment Examining Nutrient Effects**

**Introduction.** To investigate the effect of water column nutrients on sediment nitrogen fixation and denitrification rates we conducted a 2 factor experiment varying nitrogen and phosphorus concentration of overlying and sediment porewater in laboratory mesocosm experiments. We hypothesized that nitrogen enrichment, in the form of nitrate, will decrease nitrogen fixation and increase denitrification. Phosphorus may enhance fixation rates, as some diazotrophs could become phosphorus limited.

As $\text{NO}_3^-$ can be transformed to $\text{NH}_4^+$ in the sediments via assimilatory and dissimilatory nitrate reduction, high $\text{NO}_3^-$ concentrations might be expected to slow nitrogen fixation activity. Conversely, nitrate availability can stimulate denitrification as the process requires a supply of $\text{NO}_3^-$ to produce $\text{N}_2\text{O}$ or $\text{N}_2$ gas. Dong et al. (2000) found denitrification rates to be highest in areas with greater nitrate concentrations and increased organic nutrients, and in this case water column $\text{NO}_3^-$ was not found to be saturating to denitrification at concentrations up to 600µM. The effects of phosphorus are not well studied; we have found no literature on phosphate limitation of denitrifiers. Phosphorus additions have been shown to increase fixation in lake ecosystems (Day et al. 1989) and in pure cultures of planktonic cyanobacteria in lakes and estuaries (Howarth et al. 1988b). As phosphorus can be a secondary limiting nutrient in estuarine systems (Fong et al. 1993) we expect that it may have an effect on growth of photosynthetic microbes and thus could affect nitrogen fixation rates.

**Methods.** We investigated the effects of added nitrogen and phosphorus on sediment nitrogen fixation and denitrification in laboratory microcosms. The four treatments, with 5-fold replication were +3000µM $\text{NO}_3^-$ and +30µM $\text{PO}_4^{3-}$ (+N,+P), +3000µM $\text{NO}_3^-$ only (+N-P), +30µM $\text{PO}_4^{3-}$ only (-N+P), and no nutrient addition (-N, -P). Though very high, nitrate enrichment values were in the range found in southern California estuaries. We collected 25 sediment cores (10cm diameter by 12cm depth) using clear plexiglass tubes from the intertidal mudflat in a single location of typically low $\text{NO}_3^-$ concentration (near Mid 3 site from field surveys). Sediment cores remained in the tubes for the entire experiment. Cores were immediately transported to UCLA and allowed to drain during transport. Once in the lab, the bottom of each core was wrapped in 60micron mesh secured with a hose clamp to assure sediment would not be washed out of the unit during treatments. Five core were harvested at the beginning of the experiment to use as comparisons of ‘initial’ site conditions. The remaining 20 experimental units were flushed with treatment water for 24 hours, with the intent of simulating conditions during a nutrient pulse. At the end of experiment, we collected 3 small (10cc, 5cm depth) cores from each unit and composited them for nitrogen fixation and denitrification measurements. We used the remaining sediment from each larger core unit to measure grain size, organic content, and Total N, and collected water from each unit to determine salinity and $\text{NO}_3^-$, $\text{NH}_4^+$ and $\text{PO}_4^{3-}$ concentrations of water that passed through the cores. We used a two-way ANOVA with the factors being nitrogen (+, -) and phosphorus (+, -).
to determine if there are significant differences in the mean values of the response variables that can be attributed to each factor.

**Results.** Addition of N to cores affected both nitrogen fixation and denitrification, though the effects were opposite. Nitrogen fixation rates were up to 30% lower with the addition of NO\textsubscript{3}\textsuperscript{-} compared to cores with no NO\textsubscript{3}\textsuperscript{-} addition (Figure 5a), suggesting increased NO\textsubscript{3}\textsuperscript{-} inhibited nitrogen fixation. This response was significant (2 factor ANOVA, p < 0.05 for N addition) for NO\textsubscript{3}\textsuperscript{-} addition and showed no response to the addition of PO\textsubscript{4} alone or in combination with NO\textsubscript{3}\textsuperscript{-}. Rates of nitrogen fixation in both treatments were within the range found in the field surveys (see Figure 2), but in the lower end.

Denitrification increased with NO\textsubscript{3}\textsuperscript{-} addition by more than 250 times as compared to the no addition treatment (Figure 5b). The dramatic response to NO\textsubscript{3}\textsuperscript{-} was significant (2 factor ANOVA, p < 0.05 for N addition) independently of PO\textsubscript{4} addition, though denitrification rates were highly variable. Initial rates measured at the site were comparable to those of the treatments without NO\textsubscript{3}\textsuperscript{-} addition. This suggests the cores behaved as they would in the natural environment. In initial cores and in cores with no nutrient addition, denitrification rates were lower than nitrogen fixation rates by more than an order of magnitude. This relationship was reversed with the addition of NO\textsubscript{3}\textsuperscript{-}; denitrification rates stimulated by NO\textsubscript{3}\textsuperscript{-} addition were up to 8x the highest nitrogen fixation rates we measured for all cores.

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**Experiment #3. Field Experiment to Determine Macroalgal Effects**

**Introduction.** To investigate the effects of various densities of mats of *Enteromorpha* sp. and/or *Ulva expansa* on nitrogen fixation and denitrification rates in the sediments, we conducted a field experiment caging macroalgae in and out of plots. We hypothesized that macroalgal mats will decrease rates of nitrogen fixation; it is unclear whether denitrification rates will be enhanced or inhibited.

Southern California estuaries, including Upper Newport Bay, are dominated by macroalgae with *Ulva expansa* and *Enteromorpha* sp. being the most abundant (Kamer et al. 2001). They form excessive blooms, which can smother sediments, changing sediment redox potential and creating an anaerobic environment (Sfriso 1987). Macroalgal blooms may also affect nutrient availability and supply of organic matter to surrounding sediment and water (Tyler et al. 2001). Nutrient fluxes in and out of the sediments and altered oxygen and light levels could affect microbial processes. Trimmer et al. (2000b) found high N recycling (mineralization) under mats of *Enteromorpha* sp. and *Ulva expansa*, but low rates of denitrification. Valiela et al. (1997) said sediments are an important nutrient source to macroalgal blooms, and Zimmerman and Montgomery (1984) found a drift algal mat in Florida greatly increased sediment NH\textsubscript{4}\textsuperscript{+} concentrations. We hypothesize NH\textsubscript{4}\textsuperscript{+} released by mats of macroalgae may decrease nitrogen fixation. Though some work has been done on flux of nutrients between sediments and macroalgal mats, it is not clear what effect added algal material may have on the rate of denitrification in the sediments.

**Methods.** To investigate the effects of macroalgae, we manipulated macroalgal density on the sediment surface in the field and compared nitrogen fixation and denitrification in the underlying sediments. Along a single elevation in one location in the estuary (mudflat near mid 3), we established 30 plots (size: 0.25 m\textsuperscript{2}) randomly arranged on the intertidal mudflat. Each plot was divided into 3 subsections that received equal amounts of algae and served as the temporal sequence for sampling). We manipulated the
density of macroalgae on the sediment surface, creating experimental units of high and medium densities, and no macroalgal cover (250g, 150g, and 0g wet wt per 0.25m$^2$ plot), with ten-fold replication. Treatment density determinations were based on biomass measurements from Kamer et al. (2001). The experiment remained in the field for 40 days, during which time algae began decomposition. In Mugu Lagoon (southern California), there was a 44\% loss in mass of macroalgal mats on the sediment after three weeks in a similar experiment (L. Green, pers. com.). We expected macroalgal mats in UNB to behave similarly. We took initial and 3 time series of measurements. From each unit 3 cores (10cc, 5cm depth) of underlying sediment were collected and composited for nitrogen fixation and denitrification measurements. Sediment grain size, organic content, and Total N, and porewater salinity, NO$_3^-$, NH$_4^+$ and PO$_4^{3-}$ was measured for each experimental unit. Since the same plots were sampled over time, we used a one factor repeated measures ANOVA, with the factor being algal density treatment (zero, medium, high) to determine if there are significant differences in the mean values of the response variables that can be attributed to treatment. Denitrification rates were unmeasurable in most experimental units, so results are not reported.

**Results.** Although variable over time and among treatments, N$_2$ fixation was not significantly affected by algal density, nor did it vary significantly by date. In addition, no other physical, chemical, or biotic variables showed any significant correlations with N$_2$ fixation activity.

**Conclusions**

Overall, nitrogen fixation rates (range from 0 - 80 µmol N \cdot m^{-2} \cdot hr^{-1}) greatly exceeded denitrification rates (range from 0 - 8 µmol N \cdot m^{-2} \cdot hr^{-1}) in highly eutrophic Upper Newport Bay. Thus, the balance between these two biogeochemical processes most likely does not result in net nitrogen removal at the scale of the estuary, even in this highly eutrophic estuary. Rather, high rates of nitrogen supply to this estuary coupled with the high relative rates of nitrogen fixation, both suggest that this estuary will need to be aggressively managed for excessive nutrient impacts in the future. Reduction of external loading rather than increases in internal biogeochemical processing seems to be the only viable option for future estuarine management.

In the field surveys, we found that nitrogen fixation was highly spatially and temporally variable, and not explained by simple seasonality or strongly related to longer term site characteristics such as sediment grain size, organic content, or nutrients. Rather rates of fixation were related to shorter term events that varied on the scale of individual rainfall events within a rainy season, as measured by water salinity and inorganic nutrient content.

In contrast, denitrification was consistently below detection limits in most locations under low tide conditions that we sampled. In the one site that denitrification was measured, it was consistently occurring across both wet and dry seasons. Our field surveys were unable to detect any differences in physical, chemical, or biotic factors in this site, suggesting that we did not measure the controlling factor. Clearly there is something about this site that supports consistent populations of denitrifying bacteria that did not occur in any of our other sites in Upper Newport Bay.
Experiments provided some mechanistic understanding of the tremendous spatial and temporal variability found in the field. Results of the first experiment support findings from the surveys that shorter term processes rather than longer term sediment characteristics control rates of nitrogen fixation. Thus, the physical, chemical, or biotic conditions associated with the water of a particular site may be more important in controlling nitrogen fixation than sediment type. This experiment also supported that denitrification rates were negligible in Upper Newport Bay. In laboratory microcosms modeling Upper Newport Bay, denitrification increased dramatically with NO$_3^-$ enrichment. This demonstrates that populations of denitrifying bacteria are present and the system is capable of responding to increased nitrogen loads. However, even at the highest rates we measured, denitrification is only removing a small portion of N in the treatment water, which were levels realistic of this highly eutrophic system. Therefore, during high pulses of nutrients, even the highest denitrification rates will not keep up with high nitrogen inputs. While denitrification does help to alleviate high N-input, in this system it will not provide a solution for eutrophication.

Literature Cited


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Figure 1. Biogeochemical cycling of N in estuaries including nitrogen fixation and denitrification.
Figure 2. Sites for the field surveys. Five sites each in the lower and middle portions of the estuary were surveyed in wet and dry seasons for 2 years.
Figure 3. Spatial and temporal patterns in nitrogen fixation rates in Upper Newport Bay. Means ± SE.
Figure 4. Spatial and temporal patterns in denitrification rates for the ‘Mid 2’ location over all sampling dates in Upper Newport Bay. Means ± SE.
Figure 5. Nitrogen fixation rates from each location from which cores were transplanted (Initial) and final rates after incubation in the common garden for 25 days (Final). Means ± SE. The star indicates significant differences between means using a t-test.
Figure 6. Rates of a) nitrogen fixation and b) denitrification in mesocosm experiments with cores from Upper Newport Bay varying water column and porewater nitrogen (NO$_3$) and phosphorus (PO$_4$) concentrations. Means ± SE.
Figure 7. Rates of nitrogen fixation under differing macroalgal treatments in Upper Newport Bay. Means ± SE.