Dissertation title: Directional ecological change in the marine-terrestrial interface: Interactions of nutrient pollution and sea-level rise in estuary habitats of Elkhorn Slough, California.

A scholarship from the Garden Club of America would fund lab analysis of water and gas samples to determine rates of denitrification, central to my dissertation.

Introduction: A global perspective on estuaries

Estuaries are among Earth's most biologically productive systems (Teal 1962, Little 2000). At the same time, there are many anthropogenic stressors in estuarine ecosystems. Globally, 21% of the human population lives within 30 kilometers of the coast, and increases in population are disproportionately high in coastal areas, so nearly all estuaries are impacted by human activities (Zedler and Kercher 2005). Worldwide, the top two human stressors on estuarine systems, present and forecasted, are a) land use change and development; and b) eutrophication, particularly nutrient loading of N and P downstream of agricultural and urban lands (Emmett et al. 2000, Kennish 2002, Zedler and Kercher 2005). Superimposed on all of these threats is that of climate change. Global warming is expected to affect coastal wetlands due to eustatic sea level rise, warming sea surface temperatures, and greater outbreaks of toxic algal blooms (Scavia et al. 2002, Zedler & Kercher 2005).

Coastal estuaries are vulnerable to eutrophication and to climate change effects on sea-level rise. Nitrogen pollution has increased dramatically in recent decades, and poses one of the greatest threats to estuarine ecological function (Howarth and Marino 2006). Estuaries are vulnerable to sea-level rise because their continued existence depends on the interplay between sea level and sufficient sediment accumulation on the marsh plain: climate change predictions include both sea-level rise and increased variability in precipitation and sediment delivery (Meehl et al. 2007, Field et al. 1999). A recent semi-empirical study projects a global sea-level rise by 2100 of 0.5 to 1.4 meters above the 1990 level (Rahmstorf 2007), which exceeds IPCC estimates. Of particular concern, climate change is expected to exacerbate existing human impacts on the environment (Scavia et al. 2002).

A regional perspective: estuaries of the central California coast, starting with Elkhorn Slough National Estuarine Research Reserve

Coastal salt marsh ecosystems are among the most threatened in California, having lost 75 to 90 percent of their historic extent (Emmett et al. 2000, Zedler 1996). Elkhorn Slough, a central California estuary, is the second largest tract of salt marsh in the state and encompasses the Elkhorn Slough National Estuarine Research Reserve (NERR). Predicted sea-level rise due to climate change will affect the extent and function of salt marsh, living marine resources, and concomitant delivery of ecosystem services. The Slough contains distinctive habitat types of salt marshes, tidal brackish marshes, mudflats, tidal creeks, and subtidal channels.

Marsh plain elevation and sea-level rise: Elkhorn Slough is experiencing a trend of conversion from salt marsh to mudflat in an erosional environment (Van Dyke and Wasson 2005). Paleoecological records of the accretion of West Coast estuarine sediments can inform current concerns about sea-level rise (Watson 2004, Mudie and Byrne 1980), showing a range of sediment accretion from 1-3mm/yr in Elkhorn Slough.

Nitrogen pollution and the coastal marine nitrogen cycle: Nutrient pollution is the greatest pollution problem in estuaries and coastal waters of the United States (NRC 2001). Nitrogen is typically the limiting nutrient in estuarine and marine waters (Teal and Howes 2000), and at the same time, estuaries are vulnerable to nutrient loading leading to eutrophication (Little 2000). Nutrient concentrations in Elkhorn Slough have increased over the past 35 years with expansion and intensification of agriculture and increases in population size in the watershed (Caffrey et al. 2007a). Researchers observe algal growth in the estuary, but no severe events of anoxic "dead zones" (K. Wasson, pers.comm.). Dissolved inorganic nitrogen (DIN) concentrations in the mid-1990s were relatively low (10-70 μmol L^{-1}) in the dry season and high (20-160 μmol L^{-1}) during the rainy season (Caffrey et al. 2007a). Denitrification is the primary pathway by which nitrogen is lost from estuaries (Bianchi 2007: 320).

Denitrification is the microbial oxidation of organic matter in which nitrate or nitrite is the terminal electron acceptor. Denitrification occurs when three conditions are met: nitrate (NO_3^-) is available, oxygen concentrations are reduced, and electron donors are available (Chapin et al. 2002, Bianchi 2007).

Mechanisms for nitrogen removal in order to improve coastal water quality, and methods for assessing nitrogen removal: Since denitrification is the primary pathway of loss of inorganic nitrogen from an ecosystem, my study focuses on the role of microbial transformation of excess nitrates, in a comparison of vegetated marsh and mudflat habitats. Studies of nitrogen cycling in Elkhorn Slough sediments have been conducted (Caffrey 1996, Caffrey 2002, Caffrey et al. 2007a); however, denitrification activity has never been measured directly in the field.

Proposed research and Research methodology

My research objective is to investigate potential interactions between anthropogenic nitrogen loading and sea-level rise at multiple scales, starting in the central California coastal estuary of Elkhorn Slough NERR.

Questions:

1) **How might sea-level rise due to climate change, interacting with the anthropogenic stressor of nitrogen additions, affect salt marsh physiology and growth?**

2) **How do marsh and mudflat compare in terms of the ecosystem service of nitrogen (N) removal through denitrification?**

3) **How might climate change over the next century affect the ability of Elkhorn Slough to improve water quality, with the expectation of loss of salt marshes and increase in area of mudflats?**

***1*) Manipulative experiment of marsh plain elevation relative to sea level crossed with nitrogen addition.**
What are the effects of increased inundation and inorganic nitrogen on salt marsh plant biomass and plant tissue nitrogen?

Two-by-three factorial experiment: Relative sea level (RSL) and addition of nitrogen (N)

No change in RSL	no N +N
+ RSL (lower marsh plot) Two levels: lower 30 cm lower 10 cm	no N + N
– RSL (raise marsh plot)	no N + N

Hypothesis: With sea-level rise, salt marsh plants will experience increased inundation depths and times. The dominant plant, *Sarcocornia pacifica* (pickleweed), will decrease in growth due to "ecological drowning" and concomitant changes in the function of nutrient uptake. As marsh plain rises with sedimentation events, halophytes' ability to take up nutrients increases, within an elevation range of 0.4 - 1.4m above mean tide. I expect that nitrogen addition above background nutrient levels will increase plant growth, providing antagonistic effects to marsh drowning in the field (e.g., Boyer et al. 2001), but only up to a certain threshold: I expect that in plots lowered 30cm, all pickleweed will experience marsh drowning.

<u>Methods</u>: I initiated the manipulative experiment in Fall 2007. In my experimental design, I employed three blocks, each containing nine treatments (eight treatments shown in the table above, plus a control where I dug up the marsh vegetation and replaced it). *Elevation*: I conducted the raising and lowering of marsh plots (adapted from Fogel et al. 2004) by selecting a 1x1-m plot of marsh, removing it with intact roots, removing or adding sediment beneath the vegetation layer (depending on the treatment), and replacing the vegetation layer. A difference in marsh-plain elevation of 10-cm has been shown to have ecological effects (Fogel et al. 2004). *Nutrient addition*: I add ammonium nitrate (NH_4NO_3, an inorganic form of nitrogen) to designated plots, because that is the form used in conventional agriculture, in the amount of 15 gN m^{-2} every two weeks. *Statistical design*: A power of 60-70% chance of detection is robust for field manipulative studies (Quinn and Keough 2002) and power analysis determines that I have three replicates of each treatment. My response variables are plant growth (dry weight biomass) and plant tissue nitrogen (determined with a C:N elemental analyzer), analyzed statistically with a factorial ANOVA. I will also measure denitrification rates in my experimental plots – I piloted benthic chambers in the field in mid-January 2009.

Preliminary data analysis: Having run the manipulative experiment for a year, I find that the highest growth occurs in plots with no change in elevation and added nitrogen, followed by plots raised 10 cm – whether or not they were fertilized – and that the least growth, as expected, occurs in plots lowered 30 cm. For plant tissue nitrogen, there is a significant effect of elevation change, nitrogen addition, and the interaction of the two terms – I am still analyzing those data.

Question 2: **How do marsh and mudflat compare in terms of the ecosystem service of nitrogen (N) removal through denitrification?**

***2*) An observational experiment, with at least six marsh sites around Elkhorn Slough.**

Hypothesis: Correlated with the pattern of vegetation change is a pattern of nitrate uptake and denitrification. Microbial communities in densely vegetated salt marsh will demonstrate more denitrification activity than in either patchy salt marsh or mudflat. This hypothesis is supported by a sparse literature of biogeochemical studies comparing mudflat and any subset of subtidal, intertidal, or high-marsh vegetation (e.g., Caffrey and Kemp 1990, Caffrey and Kemp 1992, Lillebo et al. 2006). Uptake of nitrates into plant tissue will be an additional source of removal of nitrogen from terrestrial-water runoff and the water column. If I find a significant difference in denitrification activity, I could design a secondary experiment to examine mechanisms, focusing on microbial abundance, genetic identity, or patterns of anoxia and oxic zones in the rhizosphere, through collaborative research. I am already collaborating with Dr. Chris Francis of Stanford University, and I will collect cores for genetic identification of denitrifiers.

Methods: Denitrification is challenging to measure directly (Groffman et al. 2006), but methods appropriate to estuarine sediments include measuring di-nitrogen to argon ratios (N_2:Ar) with membrane inlet mass spectrometry (Kana et al. 1994; Cornwell et al. 1999, Groffman et al. 2006) when sediments are inundated. I will also use a labeled tracer method, with a stable isotope of nitrogen (^{15}N), following methods developed in terrestrial ecology (Holtgrieve et al. 2006, Panek et al. 2000, Hart et al. 1994). In the terrestrial method, one injects the sediment with a labeled tracer, encloses that soil volume in a chamber with headspace, and samples the headspace at frequent intervals, for evolved gases containing the label. I will assess denitrification seasonally using the benthic chambers (suggested by Caffrey et al. 2007b), and measure factors which are correlated with denitrification: sediment temperature, microbial biomass, and soil nitrogen pools (Bianchi 2007, Kaplan et al. 1979). I will measure denitrification monthly using the terrestrial labeled tracer method, since the marsh plain is more often sub-aerial than underwater. To assess nutrient pools as I calculate fluxes, I will measure plant-available nitrogen in sediments and sediment porewater .

Data Analysis: I will analyze data using ANOVA (transformed when necessary to meet assumptions), with the treatment groups of dense vegetation, patchy vegetation, and mudflat. Power analysis using estimates of denitrification from the Slough (Caffrey 1996, Caffrey 2002) suggests that I select 2-3 sites within each treatment group for a 70% power to detect a difference (SYSTAT v.10). I have selected four sites around the Slough and will select two more.

Expected results: I expect to see a significant difference in denitrification rates between vegetated salt marsh, patchy salt marsh, and unvegetated mudflats. I hypothesize that microbes in vegetated sediments will show the highest denitrification activity, and that uptake of nitrates by plant tissues may provide additional sequestration of nitrate, but not in winter when surface water runoff is greatest.

Question 3**: How might climate change over the next century affect the ability of Elkhorn Slough to improve water quality, with the expectation of loss of salt marshes and increase in area of mudflats?**

***3*) Incorporating results of field work into a landscape-level model.**

Hypothesis: Salt marsh distribution is affected by variables that climate change will alter. I hypothesize that *i)* a simple model based on the factors of salinity, marsh plain elevation, soil moisture and soil redox has robust explanatory power for the current distribution of salt marsh habitat in the Slough; *ii)* I can generate plausible future scenarios of salt marsh distribution according to depictions of 0.5m and 1.0m rise in sea level; *iii)* a future scenario, over the next century, will provide a first-cut estimate of expected changes in the ecosystem service of nitrogen transformation and buffering anthropogenic nutrient loads.

Methods: Using a geographic information system (GIS), I will identify and map areas that meet the criteria for salt marsh habitat and mudflat in terms of elevation and salinity in future tidal regimes. I will initially conduct this exercise with no barriers to salt marsh "migration," and then repeat it with the present-day structures of tide-gates, culverts, railroad tracks, roads, and other obstacles to habitat migration. I will then create an estimate of changes in the ecosystem service of water quality improvement provided by microbial denitrification over the next century in Elkhorn Slough.

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