ANTHROPOGENIC EFFECTS ON ESTUARINE SHORELINE PRIMARY PRODUCTIVITY AND NUTRIENT CYCLING

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ABSTRACT

Theresa A. O'Meara: Anthropogenic Effects on Estuarine Shoreline Primary Productivity and Nutrient Cycling
(Under the direction of Dr. Michael Piehler)

Humans are extremely effective ecosystem engineers but the consequence of our actions warrant study. Multiple anthropogenic stressors including sea level rise, development, and nutrient loading currently threaten coastal systems. Significant alterations to coastal habitats affect their function and potentially causing irreparable damage. This study focused on understanding the function of the estuarine land-water interface, particularly the base of the food web, or primary producers and factors that may alter their distribution, abundance, speciation, and quality as a food source. These experiments were conducted along the coast of North Carolina, but are largely applicable to similar systems worldwide. Nitrogen is typically the limiting nutrient in estuarine systems, and eutrophication is a critical concern due to excessive supplies of nitrogen accelerating primary production to unsustainable levels. I have found that nitrogen processing in the coastal land-water interface can mitigate loading through denitrification, but quality is also important. Since different denitrifying microbes produce different end products, the distribution of these microbes is of critical concern, particularly because of the production of greenhouse gases. Data presented here show that different primary producers in estuarine marshes have staggered growing seasons as a functional means of resource partitioning. However, shifts in global temperatures are altering growing seasons and could potentially intensify competition between species. In some cases, facilitation between

primary producers may mitigate temperature effects. This research provides a baseline for future comparison of ecosystem health and function and offers projections of foreboding scenarios of changes to the land-water interface without a concerted effort to adapt our coastal development approaches and to acknowledge and plan for rising water levels and warming temperatures.

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PREFACE

This dissertation is formatted as a series of papers. Therefore, Chapters 2 through 5 were written to stand alone for publication. Together, some information may be repeated. Chapter 2 has been submitted for publication to Wetlands Ecology and Management and is currently in review.

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LIST OF ABBREVIATIONS AND SYMBOLS

Abbreviations

$^{15}N_2$	dinitrogen gas with ^{15}N stable isotope
$^{15}NH_4^+$	ammonium with ^{15}N stable isotope
$^{15}NO_3^-$	nitrate with ^{15}N stable isotope
^{63}Ni	radioisotope of nickel
A	surface area
ABT	acetylene block technique
AB	N_2O production from antibiotic treated cores
$ADEN_d$	anoxic denitrification potential
AE_d	area of exposed habitat
AF	N_2O production from antifungal treated cores
AFAB	N_2O production from antibiotic and antifungal treated cores
AI_d	area of inundated habitat
alt	*alterniflora*
annamox	anaerobic ammonium oxidation
ANOVA	analysis of variance
(aq)	aqueous
Ar	argon
B	bacteria
BDL	below detection limit
BH	bulkhead
^{12}C	carbon

^{14}C	radioisotope of carbon
C	element: carbon
	temperature: Celsius
	treatment: control
C_{in}	concentration of inflow
C_{out}	concentration of outflow
CBR	Currituck Banks Reserve
chl-a	chlorophyll-a
cyno	*cynosuroides*
d	water depth
DEN	denitrification
DIC	dissolved inorganic carbon
DPM_s	disintegrations per minute of sample
DPM_T	disintegrations per minute of radiolabelled seawater
exp	experiment
F	marsh width: fringing marsh/narrow
	microbe: fungi
	rate: flux
g	gram
GC	gas chromatograph
h	hour
ha	hectare
HM	high marsh

HM_a	average anoxic denitrification potential
HM_o	average oxic denitrification potential
I_k	irradiance at which photosynthesis is no longer light dependant
IMS	Institute of Marine Sciences
L	liter
LM	low marsh
LM_a	average anoxic denitrification in the low marsh
LM_o	average oxic denitrification in the low marsh
ln	natural log
M	concentration: molar (moles/liter)
	marsh width: medium marsh
MA	macroalgae
MF	maritime forest
m	meter
mol	mole
MPB	microphytobenthos
N	element: nitrogen
	marsh width: none/no marsh
	site: north/northern
N_2	dinitrogen gas
N_2O	nitrous oxide
NASA	National Aeronautic and Space Administration
NC	North Carolina

NE	northeast site
NERR	National Estuarine Research Reserve
NH_3	ammonia
NH_4^+	ammonium
NO_2^-	nitrite
NO_3^-	nitrate
NOAA	National Oceanic and Atmospheric Administration
NO_x	nitrate + nitrite
O_2	oxygen
$ODEN_d$	oxic denitrification potential
P	algae: photosynthetic rate
	sediment: porosity
p_i	proportion of area covered by species "i"
P_{max}	maximum photosynthetic rate
psu	practical salinity units
Q	conversion factor, constant
R	reference marsh, no bulkhead
RCR	Rachel Carson Reserve
s	seconds
S	south
S.	*Spartina*
SA:V	surface area to volume ratio
SE	southeast site

SOD	sediment oxygen demand
SOM	sediment organic matter
spp.	species
ST	subtidal
t	time
T_d	duration of inundation it a given depth
U.	*Ulva*
V	volume of wet sediment
Var	variance
V_{TG}	volume of dry sediment
W	marsh width: wide marsh
	site location: western site
X^2	Kruskal-Wallis chi-squared
y	year
z	core depth

Prefixes

µ	micro (10^{-6})
m	milli (10^{-3})
c	centi (10^{2})
k	kilo (10^{3})
G	Giga (10^{9})
µ	Tera (10^{12})

Symbols

°	degree
Δ	delta, change
>	greater than
<	less than
%	percent
α	photosynthetic efficiency
±	plus or minus
Σ	sum

CHAPTER 1
Introduction

Historically, our need for water and the resources it provides such as food, transportation, and trade have determined our distribution and population. Currently, advances in technology have allowed us to move further from food and water resources without adverse impacts. Therefore, population distributions are indicative of "want" rather than "need". According to NOAA's National Ocean Service, "coastal counties constitute only 17% of the total land area, but account for 53% of the total population". Coastal states receive about 85% of the tourist-related revenue in the US (World Almanac, 2001). In Carteret County, NC alone, tourism and travel revenue was approximately $278 million (CEDC, 2012). With beautiful views, fishing, hunting, and water sports, estuaries are popular coastal tourist attractions. The coastal environment can provide vital ecosystem services including storm protection, flood control, nutrient regulation and processing, waste treatment, pollution control/detoxification, and habitat for important fisheries. According to Costanza et al. 1997, the ecosystem services provided by the estuary are worth $22,832 ha^{-1} yr^{-1}. Although the value of this metric is hotly debated (Gatto and De Leo 2000), estuaries were the most valuable ecosystem assessed in the study, which included seagrass beds, coral reefs, and floodplains. Within the estuary, one of the primary habitats responsible for mitigating nutrient pollution, removing wastes, and shoreline stabilization is the salt marsh. Salt marshes have been studied around the world and noted as "hot spots" for nutrient processing for decades (Teal and Teal 1969). Currently, there are over 4 million acres of salt marsh in the US (Field et al. 1991) and 364 million acres worldwide, but

these habitats are taken for granted. While water resources have shaped our society, we in turn have altered these resources to suit our needs (Pastore et al. 2010). Anthropogenic stressors are significantly altering vital salt marsh habitat (Kearney et al. 1988; Kennish 2001). We directly change the hydrology and function of these environments through development (Bertness et al. 2002). Marshes are transient features. Development adjacent to the salt marsh, particularly shoreline armoring, can significantly decrease marshes' resilience to environmental change by blocking migration and/or sediment supply. Indirectly, significant damage is caused as a result of sea level rise, global warming, and pollution (nutrients, noise, and light). Sea level rise threatens to drown marshes and is a significant contributor to habitat loss when marshes are unable to migrate upland or when sediment supplies are too low to maintain accretion (Strahlberg et al. 2011; Reed 1995). Warming temperatures alter the rate of metabolic processes and can cause thermal stress for salt marsh inhabitants (Roessig et al. 2005). Noise and light pollution are important stressors to the environment, but are often overlooked because they are harder to quantify. Increased light pollution can disturb natural circadian rhythms, which can alter behavior and survival (Dwyer et al. 2012; Longcore and Rich 2004). Sound pollution has been known to cause tissue damage, alter behavior and decrease survival rates of fish and fish larvae (Banner and Hyatt 1973). Nutrient loading to the salt marsh as a result of agriculture, urban run-off, and sewage outflows can cause algal blooms, bottom water hypoxia/anoxia and shifts in primary producer community structure (Valiela and Bowen 2001; Mallin and Cahoon 2003). When these changes occur, it can cause fish kills and alter trophic structure (Paerl 1998). Watzin and Gosselink 1992, have estimated tidal salt marsh habitat loss caused by humans at more than 50%. As we continue to alter these coastal environments, it is important to 1.) develop

a baseline for comparing environmental change and 2.) understand how human actions alter the ecosystem function and services of salt marshes.

This dissertation focuses on the effects of anthropogenic stressors on the base of the food web, which includes 1.) assessing impacts of anthropogenic activities on nutrient processing and 2.) assessing interactions between primary producers as nutrient processing rates are altered. Nitrogen (N) is the primary limiting nutrient in salt marsh ecosystems (Howarth and Marino 2006). Therefore, N loading significantly contributes to eutrophication in coastal systems (Paerl 1995). Denitrification, which converts biologically active nitrates to relatively inert nitrogen forms, can help to mitigate nutrient enrichment in coastal systems (Kaplan et al 1979; Thompson et al. 1995; Koop-Jakobsen and Giblin 2010). However, denitrification pathways and end products are also important considerations. Both fungi and bacteria can conduct denitrification, but end products differ in proportion. Bacterial denitrification is considered high quality because it typically produces harmless atmospheric nitrogen (Herbert 1999). Fungal denitrification primarily results in the production of N_2O, which is a greenhouse gas (Shoun et al. 1992). Chapter 2 describes the distribution of fungi- and bacteria-mediated denitrification across salt marsh and associated habitats from the shallow subtidal to the maritime forest. Maritime forest and in some cases, marshes are lost as a direct result of development. Following coastal land development, hardened structures are often installed to protect shorelines from wave action and sea level rise. This 'squeezing' effect on the marshes, caught between upland development and increased wave reflection and sea level rise seaward of the structure, causes high levels of environmental stress and overall marsh loss (Silliman et al. 2009). Chapter 3 investigates changes in denitrification as a result of bulkheading (a method of hardening the shoreline with a permanent vertical structure) in estuarine habitats. As nutrient cycling changes and marsh grass

is lost in these overly stressed environments, the abundance, diversity, and distribution of primary producers is altered. Chapter 4 examines changes in primary producer distribution as a result of bulkheading estuarine shoreline. If the interacting effects of upland development and sea level rise drown salt marshes completely, we expect to see a conversion of marsh ecosystems to shallow subtidal habitats. In this case, algae will replace marsh grass as the dominant primary producer. However, not all algae are created equal and palatability, size, life cycle, nutrient requirements, and seasonality differ among algae types. Increases in temperature are expected to continue along with sea level rise and coastal development. It is important to determine shifts in the quality of food source for higher trophic levels as temperatures increase and the hydrology of marshes is altered. Chapter 5 discusses shifts in algal groups and production as a function of increasing temperature.

According to the IPCC, even if all greenhouse gas emissions stop tomorrow, the earth would continue to warm approximately 0.6°C over the next 50 years. We will not be able to stop climate change, but we can attempt to reverse some of the effects and restore ecosystems and the services they provide. Therefore, it is important to characterize current conditions along with the effects of change. These data can provide concrete goals for successful restoration/mitigation projects and help to reclaim services lost with habitat degradation.

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CHAPTER 2

Denitrifiers in the coastal gradient: Potential contributions to the N_2O budget

2.1 Introduction

Human modification of the nitrogen cycle has been extraordinary (Vitousek et al. 1997). Watershed development, agricultural waste, concentrated animal feeding operations, storm releases of raw sewage, and urban run-off are significant contributors of nitrogen loading to watersheds (Valiela and Bowen 2001; Mallin and Cahoon 2003). As nitrogen (N) loading to coastal zones continues to rise, N sinks and the processes that remove N from the system become increasingly important. Denitrification (DEN) is a microbially mediated process by which nitrate (NO_3^-, biologically active) is converted to N_2 (mostly biologically inactive; Herbert, 1999). Many studies have shown that DEN in estuarine marshes is an important process for the mitigation of N loading to coastal watersheds (Kaplan et al. 1979; Thompson et al. 1995; Koop-Jakobsen and Giblin 2010). For example, a study by Hammersley and Howes, 2003, has shown that DEN can account for 46% of ammonium loss through coupled nitrification/DEN and over half of algal N in estuarine marshes is remineralized and denitrified.

Denitrification in coastal systems was previously assumed to be predominately bacteria-mediated denitrification (B-DEN). However, recent studies have shown that fungi-mediated DEN (F-DEN) can be a significant contributor to nitrogen processing in wetland ecosystems (Seo and DeLaune 2010; Buesing and Gessner 2006; Shoun et al. 1992). The majority of F-DEN studies have bee conducted in freshwater and terrestrial systems (DeVries et al. 2011). In estuarine marshes, the majority of research on fungi focuses on decomposition of

marsh grass detritus (Benner et al. 1984; Buchan et al. 2003; Newell et al. 1987; Currin et al. 1995) and aerobic decomposition in the marsh grass canopy rather than benthic nutrient cycling. Few studies have investigated the process of F-DEN in estuarine or marine systems (Jebaraj and Raghukumar 2010; Sumathi and Raghukumar 2009) and none have directly linked F-DEN to N_2O production in intertidal zones. However, N_2O flux in estuarine systems has been measured in estuarine marshes (Smith and DeLaune 1983; Smith et al. 1983) and near shore marine sediments (Seitzinger et al. 1980) and modeled for global estuarine budgets (Kroeze et al. 2005). According to a recent study by Mohamed and Martiny (2011), fungal diversity and abundance in estuarine marshes is comparable to freshwater systems. Many species capable of DEN (Shoun et al. 1992) such as *Fusarium spp., Giberella spp, Chaetomium spp, Trichoderma viride, Botrytis cinerea, and Cylindroscarpon spp.* are found in coastal sediments (Pugh 1962; Gessner and Kohlmeyer 1976). Together, these pieces of the puzzle indicate that we are potentially missing a large part of the DEN story by neglecting F-DEN.

F-DEN and B-DEN are distinguished by the redox potential most conducive to the process and the rate of N_2O production (Figure 2.1; Seo and DeLaune 2010). B-DEN is an anaerobic process, which typically occurs when marsh sediments are inundated (creating a reduced environment). DEN requires nitrate (NO_3^-, an oxidized form of N), which can be supplied by overlying water, ground water discharge, or nitrification (Herbert 1999). In nutrient depleted systems, such as oligotrophic estuarine marshes, nitrification can be a significant source of NO_3^-, but the process requires aerobic conditions. Therefore, tidal inundation promotes B-DEN because it cycles between aerobic and anaerobic conditions. Low levels of NO_3^- limit B-DEN. However, abundant NO_3^- (as well as other biologically active forms of N) can make the process of B-DEN less efficient. This results in the increased production of intermediate

products, such as N_2O and reduced denitrification rates (Francis and Mankin 1977; Glass et al. 1997). In contrast, F-DEN readily occurs in aerobic conditions. Fungi are capable of concurrent respiration and DEN (Shoun et al. 1992). Because anaerobic conditions are not necessary for the process to occur, F- DEN may be favored over B-DEN in high marsh and maritime forest habitats. In addition, while some fungi are capable of complete DEN (end product, N_2), most F-DEN results in N_2O (Shoun et al. 1992) regardless of ambient NO_3^- levels. F-DEN can use other forms of N as substrate including organic-N, such as amino acids, aniline, or azides (Shoun et al. 1992). Therefore, unlike B-DEN which requires ambient NO_x supplied from the water column or by the rate limiting process of nitrification, F-DEN may be a significant contributor to DEN in systems which are N-limited, such as estuarine marshes.

Current estimates of DEN in estuarine marsh systems have not distinguished F-DEN from B-DEN. Studies in terrestrial systems have shown that shifts from fungal to bacterial-dominated communities results in decreased residence time of nutrients (Bardgett et al. 2006; Wardle et al. 2004a; DeVries et al. 2006; Gordon et al. 2008; De Vries et al. 2011). This indicates that fungal nutrient cycling is slower and more conservative in nutrient uptake (Van der Heijden et al. 2008; Wardle et al. 2004b). Changes in estuarine marsh characteristics, such as changes in nutrient availability or tidal range, could ultimately affect distributions of F- and B-DEN and impact rates of nutrient cycling. Knowing the distribution of DEN activity across the estuarine marsh elevation gradient is important as we directly impact these habitats with development and indirectly affect function with climate change and sea level rise.

My objectives were to assess DEN potentials in upland coastal habitats, which have been previously understudied, distinguish between fungal and bacterial denitrification across the coastal gradient, and determine the effects of tidal cycle on denitrification on a reserve-size scale.

In addition, I wanted to use data collected to estimate the potential of F-DEN to contribute to the global N_2O budget. I measured DEN from areas of high elevation, low inundation (maritime forest) to areas of low elevation, high inundation (shallow subtidal) to test the hypothesis that shifts in total DEN rate and production of N_2O coincide with changes in the relative rates of F-DEN and B-DEN. I hypothesized that F-DEN and B-DEN potentials would be inversely related, with F-DEN dominating at higher elevations (maritime forest, high marsh) and decreasing in importance with decreasing elevation. Conversely, B-DEN would dominate at lower elevation (low marsh, subtidal) and decrease in importance with increasing elevation. These measured rates were then used to model denitrification across the coastal gradient and extrapolate to yearly DEN rates within each reserve.

2.2 Methods

2.2.1 Study Sites

Three transects were established within two distinct marsh sites: the Rachel Carson NCNERR and the Currituck Banks NCNERR. Since estuarine marshes encompass habitats across salinity gradients, I chose a low salinity and high salinity site for comparison. Four habitats were assessed along each transect: maritime forest (MF), high marsh (HM), low marsh (LM), and shallow subtidal (ST). Habitats were visually distinguished by vegetation with the exception of the ST, which was collected below the low tide line (i.e. constantly submerged).

The Rachel Carson Reserve (RCR) site is located on the south east tip of Carrot Island near Beaufort, NC (Figure 2.2a). The RCR experiences semidiurnal tides and is a historic dredge spoil island with an average salinity of ~35 psu due to the island's connectivity/close proximity to the Altantic Ocean. The primary marsh vegetation is *Spartina alterniflora* and the martime

forest is sparse. Horses are common on the island and can significantly impact marsh vegetation density as well as local sediment compaction (Hay and Wells 1988; Axford et al. 2013). Additional information as well as habitat maps of the RCR can be found in Fear et al. (2008).

The Currituck Banks Reserve (CBR) is located north of Corolla, NC on the soundside near the end of Highway 12 (Figure 2.2b). The CBR tidal cycles are predominantly wind driven, the average salinity is 3.5 psu (Caldwell 2001), and the sediment has not been significantly altered by dredge spoil. Natural vegetation cover has changed due to the invasion of *Phragmites* in this normally *Juncus gerardi* dominated marsh. Transects were established in areas dominated by *Juncus gerardi.* Horses can also be found at this site, but in contrast to the horses at the RCR, appear to spend most of their time in the vast maritime forest as shown by the lack of horse trails in the marsh site (*personal observation*). However, the marsh is more likely impacted by feral hogs. Additional information as well as habitat maps of the CBR can be found in Fear et al. (2008).

2.2.2 Elevation surveys

Three transects were established per site in the RCR (Figure 2.3a) and the CBR (Figure 2.3b). The transects marked on the reserve maps indicate the central transect. At each site, transects were varied in total length based on terrain and habitat changes. Each transect was surveyed using a laser level apparatus to determine transect elevations from the subtidal to the maritime forest. Transects were terminated in the maritime forest where the dense vegetation blocked the laser. Elevation was measured every 5 m. In addition, elevation was also measured on 2 transects 10 m from the central transect and these points were combined and mapped in GIS. The GIS map was used to calculate surface area of the site. Elevation of the site was related to a water level logger installed in the shallow subtidal. HOBO water level loggers

recorded water depth through tidal cycles at 20 minute intervals for 3 months. HOBOs were deployed at the end of site SE's center transect in the RCR and site C's center transect in the CBR. With surface area/elevation maps and water depth, area inundated or exposed was determined. Duration of inundation was determined by successive HOBO depth measurements.

2.2.3 Denitrification

DEN experiments were replicated 4 times from each reserve (8 total) during summer (2012 and 2013) when peak DEN rates are typically observed (Piehler and Smyth 2011; O'Meara et al. *in review*, Thompson et al. 1995). Acetylene block methods (ABT) adapted from Thompson et al. 1995 were used to measure control DEN (C-DEN), F-DEN, and B-DEN (Figure 2.4). Control DEN is calculated from unaltered sediment, i.e. contains active bacteria and fungi. Large sediment cores (6.4 cm in diameter) were collected along established elevation transects from each of four habitats: MF, HM, LM, and ST. Location of each collection point was referenced to autolevel data to determine elevation. The cores were returned to the Institute of Marine Sciences in Morehead City, NC and allowed to equilibrate to laboratory temperature (25°C) overnight. After ~18 hours, large cores were subcored (12mm diameter, 1cm depth) and placed into 60 mL scintillation vials. Nutrient solutions of 100μM NH_4NO_3 and 100μM glucose in filtered site (GF/F filters; Whatmann, 47mm) water were added to each vial to obtain potential rates in slurry incubations. ABT measurements are not 100% efficient at blocking the conversion of N_2O to N_2 and are ineffective below NO_3^- concentration of 10 μM (Oremland and Capone 1988). Additions of glucose and NH_4NO_3 to the slurry were used to help mitigate these effects. As a result, DEN measured is a potential rate rather than an actual rate. F-DEN slurry solutions were spiked in a single addition with 0.3 mg of streptomycin sulfate/L nutrient solution

(antibiotic solution) and B-DEN solutions were spiked with 0.2 mg of cycloheximide/L (antifungal solution) and shaken to homogenize the sample.

Serum vials were capped with rubber septa and either purged of oxygen (to induce anoxia) or left with atmospheric oxygen concentrations. DEN potential in the ST habitat was only assessed with anoxic incubations because we chose sites that were constantly inundated and rarely experienced aerobic conditions. Acetylene (3 mL/vial) was injected through the septa to block the reduction of N_2O to N_2 (i.e. force incomplete DEN; Buesing and Gessner 2006). Duplicate cores were sampled at 0, 3, and 6 hours and analyzed for N_2O concentration using a ^{63}Ni electron capture detector (ECD Shimadzu GC-2014; Seo and DeLaune 2010). Once sampled, a vial was not reused for successive measurement. Serum vials were shaken before each sampling. DEN potential rates were calculated as the slope of N_2O generated/incubation time (0, 3, or 6 hours). To account for N_2O production from non-DEN sources, samples without acetylene were incubated simultaneously and subtracted from calculated DEN potentials. Errors of slope were determined by standard error propagation.

Optimal pharmaceutical concentrations were measured in a preliminary incubation experiment. Methods were adapted from substrate-induced respiration inhibition (SIR) procedures outlined in Anderson and Domsch (1975) and adapted by Seo and DeLaune (2010) using N_2O production instead of CO_2 production. Using the additive ratio

$$1 = \frac{[(C-AF)+(C-AB)]}{(C-AFAB)} \tag{2.1}$$

where C is N_2O evolved from the control, AF is N_2O evolved from the sediment treated with cycloheximide, AB is the amount of N_2O evolved from the streptomycin treatments, and AFAB is the N_2O evolved when both are added to the sediment. Controls did not contain any pharmaceuticals. Three replicates were assessed for each habitat, pharmaceutical treatment, and

oxygen level. Serum vials were prepared and incubated as described above. While this pre-experiment optimized pharmaceutical concentration, it does not optimize temporal pharmaceutical additions, which was not addressed in this study.

2.2.4 Sediment characteristics

Sediments were characterized by measured porosity and sediment organic matter (SOM). Sediment samples for porosity and SOM content were collected from each habitat adjacent to denitrification cores. Porosity was measured using equation 2.2:

$$P = 100\left(\frac{V-V_{TG}}{V}\right) = 100\left(1-\frac{V_{TG}}{V}\right) \quad (2.2)$$

where P is porosity, V is volume of wet sediment, and V_{TG} is volume of dried sediment. Sediment samples were collected using a 60 mL syringe to 2.5 cm depth to keep a constant wet volume and dried at 105°C for 4 hours. Dried sediment volume was measured by water displacement in a graduated cylinder (Pettijohn 1938).

SOM percentages were measured using loss on ignition (Ball 1964). Samples were dried at 105°C for 4 hours and combusted at 525°C for 4 hours. SOM content was determined using equation 2.3:

$$SOM\% = 100\left(\frac{D-M}{D}\right) \quad (2.3)$$

2.2.5 Integrating inundation time, DEN rate, and oxygen presence

C-DEN potentials, inundation time and oxygen presence were integrated to model N removal potential from each site and total for each reserve. To accomplish this task, an estimate of inundation time for each habitat was calculated from water depth measured with the HOBO and elevation surveys. The elevation transect grid points were mapped in ArcGIS. Using GIS for each transect, surface area exposed for each cm of water depth was calculated. Depth data

(HOBO data) were blocked into 1cm increments and summed to determine total time water level remained at each 1cm depth. Water level and surface area were combined to determine total area inundated (anoxic) or exposed (oxic) for a given water depth as well as duration of inundation. Exposed does not always equal oxic and inundated does not always equal anoxic because of the diffusion of oxygen (Hofman et al. 1991), bioturbation (Gribsholt et al. 2003), and formation of anoxic/oxic microzones (Koop-Jakobsen and Giblin 2010; Helmer and Kunst 1998). The rates of these processes are highly heterogeneous throughout the marsh, difficult to assess on larger scales, and can cause fluctuations towards anoxic or oxic conditions. Therefore, to generalize across larger spatial scales, oxygen presence was assumed to be directly related to atmospheric exposure. The relationship between anoxic and oxic C-DEN potential and elevation for was used to determine total potential (oxic + anoxic) at each 1cm water depth. Total N processing for the duration of the experiment (mmol/exp) was determined for each site by equation 4:

$$\frac{mmol\ N}{exp} = \sum_{d=0}^{d=i}(AI_d)(T_d)(ADEN_d)(z) + \sum_{d=0}^{d=i}(AE_d)(T_d)(ODEN_d)(z) \quad (2.4)$$

where d is water depth (m), AI_d is area inundated at depth i (m^2), T_d is the amount of time water is at depth i (hr), $ADEN_d$ is the anoxic DEN potential at depth i (μmol N m^{-3} hr^{-1}), z is the core depth (0.01m), AE_d is area exposed (m^2), and $ODEN_d$ is oxic DEN potential at depth i (μmol N m^{-3} hr^{-1}). The first term is the total anoxic potential and the second is the oxic potential. The sum equals the total potential of each transect.

To extapolate to the whole reserve, GIS shape files for habitat coverage of MF, HM, and LM were obtained from the North Carolina Department of Environment and Natural Resources. Shallow subtidal area was estimated to be a 2m perimeter of the estuarine shorelines of each reserve. Shoreline distances were determined using Google Earth and included only sound-side and estuarine coastline, not marine (i.e. intertidal sands, beach dune, etc.) or palustrine

(freshwater wetlands) habitats because they were not assessed in this study. Shoreline distances were independent of tidal height because they were based on visual identification of emergent vegetation. Since inundation time was not measured for the entire reserve, it was not possible to use the model to determine whole reserve DEN potential. Instead, percent time inundated (for distribution of anoxic and oxic DEN) and C-DEN potentials for each habitat was averaged across sampling trips (4 total for each reserve). Estimates are based on a 12-hr day because rates were measured in the lab to represent dark conditions only. In addition, since rates were measured in the summer when potentials were greatest, annual rates were limited to a 6-month year to account for significant decreases in DEN rate with temperature based on previous studies in similar locations (Piehler and Smyth 2011; O'Meara et al. *in review*; and Thompson et al. 1995).

2.2.6 Measuring locally, extrapolating globally

With approximately 4 million acres of estuarine marsh in the US (Field et al. 1991) and 3.46E8 acres worldwide (Duarte et al. 2008), F-DEN has the potential to be a significant source of N_2O on a global scale. MF and ST contributions were excluded from calculations based on a lack of accurate estimates of US or global coverage. A 1:1 ratio was assumed between oxic and anoxic potential and estuarine marsh area included both HM and LM. Therefore:

$$US\ or\ Global\ Total = \left(\frac{HM_a + HM_o + LM_a + LM_o}{4}\right)(A)(C) \quad (2.5)$$

where HM_a is the average anoxic DEN potential in the HM (mmol m^{-2} day^{-1}), HM_o is the average oxic potential in the HM (mmol m^{-2} day^{-1}), LM_a is the average anoxic DEN potential in the LM (mmol m^{-2} day^{-1}), and LM_o is the average oxic potential in the LM (mmol m^{-2} day^{-1}), A is global or US estuarine marsh total area (m^2) and C is a conversion factor (2.52E-12 Tg day $mmol^{-1}$ yr^{-1}). Only C-DEN potentials were used and not the sum of B-DEN and F-DEN to reduce errors in

calculation. As in the total reserve DEN potential methodology explained above, a 12-hr day and a 6-month year were assumed.

2.2.7 Statistical Analyses

All statistics were run in R (Version 2.8.2008-12-19). Percentage data (SOM and porosity) were transformed using arcsin square root. Homogeneity of variance was determined using Levene's test and normality was determined with a Shapiro-Wilk test. ANOVAs were run for all data which fit the assumptions of the test and a Tukey post-hoc was used to assess significant differences between groups. If data required a non-parametic test, a Kruskal-Wallis was used. Data analyzed with an ANOVA is denoted with a F-value and Kruskal-Wallis data is shown with a chi-squared value (X^2).

2.3 Results

2.3.1 Site characterization

Marsh elevation slopes (from the ST to the MF) ranged from 0.003 to 0.016 in the RCR and 0.008 to 0.01 in the CBR. The overall elevation change for RCR was between 0.17m and 1.28m. Currituck Banks elevation change ranged from 0.31-0.39m (Figure 2.3).

Porosity was significantly different between habitats in both the RCR (Figure 2.5a; df=3, F= 3.33, p=0.03) and CBR (Figure 2.5b; df=3, F=23.83, p<0.01). Similarly, SOM differed between habitats in the RCR (Figure 2.5c, X^2=23.87, p<0.01) and CBR (Figure 2.5d; X^2=18.96, p<0.01). In the RCR, porosity (Figure 2.5a) and SOM (Figure 2.5d) were both lowest in the HM and increased with increasing and decreasing elevation. In the CBR, both porosity (Figure 2.5c) and SOM (Figure 2.5d) were highest in the HM and decreased towards both higher and lower elevations. When data from both sites was combined, SOM and porosity were significantly

correlated (R^2=0.3393, p<0.01). However, there was no significant correlation between SOM or porosity and DEN, F-DEN, or B-DEN potential.

2.3.2 Denitrification potentials

Generally, DEN potentials (both oxic and anoxic) were greater in the CBR than the RCR (Figure 2.6). However, ST DEN potentials were greater in the RCR than the CBR (df= 1, X^2=5.33, p=0.02). For both sites, anoxic DEN potentials tended to be greater than oxic DEN potentials. Specifically in the MF, both RCR (Figure 2.6a; df=1, X^2=14.55, p=<0.01) and CBR (Figure 2.6b; df=1, X^2=9.36, p<0.01) anoxic C-DEN potentials were significantly higher than oxic potentials. CBR B-DEN anoxic potentials were also greater than oxic potentials (Figure 2.6d). In the RCR, C-DEN (Figure 2.6a) and F-DEN (Figure 2.6e) potentials were not significantly affected by habitat (C: df=3, X^2=3.38, p=0.34; F: df=3, X^2=4.79, p=0.19). However, anoxic B-DEN (Figure 2.6c) in the RCR was significantly affected by habitat (df=3, X^2=7.83, p=0.05) and tended to decrease with decreasing elevation. Oxic B-DEN potential appeared to increase with decreasing elevation, but this trend was not significant (R^2=0.13, p=0.26). Oxic C-DEN and F-DEN potentials in the RCR were highest in the HM and decreased towards the HM and LM, but habitat was not not a significant factor (C: df=2, X^2=2.35, p=0.31; F: df=2, X^2=3.50, p=0.17). In the CBR, anoxic C-DEN and F-DEN potentials appeared to exhibit a logarithmic rather than linear trend. Anoxic C-DEN and F-DEN potentials in the CBR were not signficantly different in upland and intertidal habitats, but dropped drastically in the subtidal habitats (C: df=3, F=6.81, p=0.01; F: df=3, X^2=4.23, p=0.03). Anoxic B-DEN was also significantly impacted by habitat, but showed more of a gradual decrease from the MF to the ST (df=3, X^2=10.478, p=0.02). There was no relationship in the CBR between oxic C-DEN, B-DEN, or F-DEN potential and habitat (C: df=2, X^2=0.07, p=0.97; B: df=2, F=0.35, p=0.72; F:

df=2, F=.12, p=0.89). Total N removal rates were higher for the RCR than CBR both by transect and reserve totals (Table 2.1). The variation among transects was higher in the RCR then the CBR, but this is indicative of differences in transect area rather than DEN rates. Areal DEN potential for the RCR and CBR reserve were 1.50 g m^{-2} yr^{-1} and 2.67 g m^{-2} yr^{-1} respectively.

Combined F-DEN and B-DEN values were typically higher than C-DEN potentials. The slope of F-DEN +B-DEN vs C-DEN, should be equal 1 if the two potentials agreed perfectly. The slope of B-DEN + F-DEN vs C-DEN was 0.67 in the RCR and 2.26 in the CBR. Due to this discrepancy, percentage contributions reported are based on the sum of F-DEN and B-DEN rather than C-DEN potential. In the RCR, anoxic B-DEN contributions were greater than F-DEN contributions with the exception of the ST where F-DEN was dominant (Figure 2.7a). Anoxic B-DEN was greatest in the MF and LM and lowest in the HM and ST. F-DEN contributions were greater than B-DEN under oxic conditions except in the LM where B-DEN was greater.

DEN potentials in the CBR were dominated by fungi in both anoxic and oxic conditions with the exceptions of the anoxic ST and oxic LM where bacteria were the dominant denitrifiers (Figure 2.7b). Under oxic conditions, F-DEN contributions tended to decrease as elevation decreased and B-DEN increased with decreasing elevation. Under anoxic conditions, F-DEN was greatest in the HM and LM and decreased with both increasing and decreasing elevation.

2.4 Discussion

In oligotrophic systems, DEN potentials are based on a balance between redox potentials (oxic and anoxic conditions). Therefore, in the RCR and CBR, we would expect the opposite of current conditions to boost DEN potentials. Within the estuarine marsh, higher elevation areas

of low inundation were predicted to show elevated DEN potentials when inundated (especially when dominated by B-DEN) because it provided the anoxic conditions rarely seen in these environments. Since oxic conditions are dominant in these upland habitats, we expected to see a higher proportion of F-DEN since fungi can denitrify in oxic conditions. Conversely, the low elevation habitats of high inundation should have greater DEN potentials under oxic conditions because they are low in oxidized N sources and therefore limited by nitrification. B-DEN was expected to dominate in these reduced habitats because bacteria can out compete fungi in anoxic conditions (van der Valk 2012; Alexander 1977; Kendrick and Parkinson 1990; Gareth Jones and Pang 2012). Results from this study supported some of these patterns. In the RCR MF, high anoxic B-DEN potentials were measured as expected, but the relatively low oxic F-DEN potentials were not anticipated. In the CBR, higher oxic F-DEN potentials were observed in the HM, but B-DEN was not dominant in the LM. Therefore, oxygen presence may not have been the primary regulator of potential DEN. Neither porosity nor SOM correlated with F-DEN or B-DEN potentials. Sediment nutrient concentrations between habitats or reserves should not have affected DEN rates since samples were incubated in nutrient and carbon enriched site water. One variable not assessed was total concentration of microbial cells. Further study is necessary to determine how DEN rate is affected by microbial species, abundance, and activity. While potential may be a crude estimate of population size, it cannot assess the relative abundance of individual species.

Differences between DEN potentials could be caused by the characteristics of each reserve. Both the CBR and RCR have comparable porosity and SOM contents, but the CBR has lower salinity. Previous studies indicate an inverse relationship between DEN and salinity (Giblin et al. 2010; Rysgaard et al. 2005; Seo et al. 2008) or no relationship (Fear et al. 2005;

Maghalaes et al. 2005). My results also showed an inverse relationship between DEN and salinity, but are probably explained by Smith et al. 1983 who found N_2O flux increased as salinity decreased, which may be attributed to an increase in F-DEN rates. While B-DEN rates were similar between the CBR and the RCR, F-DEN rates differed between the two sites. It is possible that B-DEN potential was consistent across salinity, but F-DEN potentials increased overall DEN rates in the fresher CBR.

Percent contributions (Figure 2.7) of fungi and bacteria to potential denitrification were calculated independently of C-DEN potentials (Figure 2.6), but C-DEN potentials mimicked DEN potentials of the dominant microbe group. In the RCR, bacteria accounted for a greater proportion of combined DEN potential in anoxic conditions while fungi dominated under oxic conditions (Figure 2.7a). When compared to C-DEN in the RCR (Figure 2.6a), anoxic C-DEN potentials resembled anoxic B-DEN potentials (Figure 2.6c) and oxic C-DEN potentials were most similar to oxic F-DEN potentials (Figure 2.6e) in habitat pattern and proportion. In the CBR, where fungi dominated both oxic and anoxic DEN potentials (Figure 2.7b), C-DEN potentials shared similar patterns as F-DEN potentials (Figure 2.6f). These comparisons show that F-DEN can be distinguished from B-DEN in coastal systems using this experimental approach and that measuring N_2 production alone may not fully characterize DEN, particularly in less saline systems where fungi are dominant.

Efficiency of the ABT technique is a possible source of error in measured potentials. Acetylene does not always block the conversion of N_2O to N_2 (Slater and Capone 1989; Van Raalte and Patriquin 1979), which would impact B-DEN more than F-DEN rates because the primary product of B-DEN is N_2. Therefore, contributions of B-DEN may be underestimated and F-DEN contributions may be bolstered. In addition, pharmaceutical treatments may not be

100% efficient. Sorption, flocculation, and settling of pharmaceuticals can hinder their efficacy (Peterson et al. 2008). These effects were minimized though short incubation times (6 hours) and regular shaking of the slurries between samples, but assessing the activity of both fungi and bacteria will be important for the future. Different species of microbes vary in their response to streptomycin and cycloheximide and non-target effects can alter treatments (Badalucco 1994). Samples were collected from the same site and each treatment was replicated to reduce differences in the microbial population. The threshold for error between replicated was 10%. However, I did not need to remove any samples based on this criterion. In addition, non-target effects were addressed by using nutrient enriched water and a short incubation time. One of the main issues with the use of pharmaceuticals to measure relative contributions of microbes to soil processes is their degradation products, which can be used as nutrient rich substrate. Since these samples were already supplemented with nutrients, the increased supply in pharmaceutical treatments should be minimal in comparison. Badalucco et al. (1994) found that streptomycin and cycloheximide were effective at killing soil microorganisms for incubation times less than 2 days and became substrate for microbial processes for longer incubations. With an incubation time of 6 hours, these samples are within the assessed temporal range, but further temporal optimization of pharmaceutical addition is necessary to ensure the efficacy of these treatments.

DEN potential and DEN rates can be measured in many ways. Each method has its advantages and disadvantages. However, results in similar systems are comparable (Table 2.2). Studies by Piehler and Smyth 2011, O'Meara et al. *in review*, Thompson et al. 1995 were conducted in or near Bogue Sound, NC in estuarine marshes. DEN potentials from the current work are within the range of these previously reported values and fall within the low end of the range for estuarine marsh studies conducted in Massachusetts (Koop-Jakobsen and Giblin 2010;

Hamersley and Howes 2005; White and Howes 1994; Kaplan et al. 1979), Rhode Island (Davis et al. 2004), and Virginia (Anderson et al. 1997). This indicates that these results may be applicable to other North American estuarine marshes. In addition, these results are comparable to studies conducted in England (Koch et al. 1992; Abd Aziz and Nedwell 1986), Italy (Erikkson et al. 2003), and Denmark (Rysgaard et al. 2005), which shows wider geographical relevance.

The global N_2O budget is currently unbalanced (IPCC 2003). Thirty percent of N_2O sources are unaccounted for with unidentified mechanisms (Rubasinghege et al. 2011). With nearly 300 times the global warming potential of CO_2, even small sources of N_2O can have a significant impact (100 yr. horizon; IPCC 2007). Estimated production of N_2O from marshes in the US and the world based on F-DEN potentials measured in the RCR and CBR are shown in Table 2.3. These basic calculations indicate that the potential contribution of estuarine marshes to global N_2O is high. According to the IPCC (2003) and Syakila and Kroeze (2011), this range of marsh emissions could be similar to energy/industry or total indirect anthropogenic emissions. However, Smith et al. (1983), Kroeze et al. (2005), and Seitzinger et al. (1980) estimate that contributions of N_2O to the global budget are in the Gg vs Tg range and are more similar to biological N_2 fixation from agriculture (Syakila and Kroeze, 2011). Reported as a daily rate, Smith et al. (1983) estimates N_2O evolution to be 0.006 mmol m^{-2} day^{-1}, which is approximately two orders of magnitude lower than F-DEN potential rates measured here. However, Smith et al. (1983), measured N_2O flux and not DEN directly. *In-situ* rates are likely lower due to completion of DEN by bacteria or competition for nutrients and carbon between bacteria and fungi, which could reduce net N_2O flux. Bacteria may be able to complete denitrification for fungi, thereby reducing the N_2O production from the sediment. In addition, it is likely that bacteria out compete fungi for resources due to faster turn over rate, which would moderate N_2O emissions from

estuarine marshes. While these processes would be reflected in directly measured N_2O fluxes, they would not be observed in DEN potentials.

2.5 Conclusions

F-DEN and B-DEN both contributed to N removal from estuarine marsh sediments and these pathways are favored under different conditions. In oxic conditions, fungi were the primary contributor to DEN potential. The dominant microbe in anoxic conditions appeared to be a function of salinity with high salinities favoring denitrifying bacteria and low salinities favoring fungi. Through basic calculations, I have shown that F-DEN in estuarine marsh habitats can produce substantial quantities of N_2O, but studies have indicated that N_2O flux from marshes are minor contributors to the global N_2O budget. This suggests that processes that reduce N_2O emissions are occurring in estuarine habitats, such as competition between microbial groups or the continual processing by bacteria of N_2O produced by fungi. However, further study is necessary to determine if these or other mechanisms are responsible for the mitigation of N_2O production in coastal systems. This work demonstrated that an assessment of the entire marsh gradient is necessary for understanding nitrogen transformations. The maritime forest and high marsh habitats exhibited DEN rates similar to and, in some cases, higher than the low marsh and subtidal zones. These somewhat overlooked habitats are important for denitrification and warrant further study. Since upland habitats are "at risk" due to development, invasive species, and sea level rise, mitigation of nutrient loading by these environments would be lost. The effects of sea level rise on the marsh would include the conversion of LM (and possibly HM) to ST and an increase in salinity (salt water intrusion). While this would result in an overall decrease in DEN potential, it may increase the ratio of N_2O/N_2 because F-DEN is the dominant

process in the ST under more saline conditions (RCR). In addition, N-loading to estuarine marsh habitats is increasing, which can make B-DEN less efficient and produce more intermediates. Therefore, as we continue to alter coastal environments, we may see a decrease in DEN potential, but an increase in proportion of N_2O produced relative to N_2.

The information presented here is merely the beginning. To understanding denitrification across the entire coastal gradient and the relative contributions of fungi and bacteria, further study is warranted. Future project should include, but are not limited to, assessing microbial species distributions and abundance, measuring denitrification potentials at in-situ temperatures and ambient light conditions, determining the individual and interacting effects of increased temperature and nutrient pollution on the distribution between denitrifiers, and modeling actual F-DEN contributions to the global N_2O budget.

TABLE 2.1: Yearly DEN potential for each site and reserve totals

Reserve	Transect	Area (m^2)	N removal (kg/yr)
RCR	SE	2,258.45	5.29
	NE	1,522.29	2.63
	W	1,100.01	3.48
	Total	**2,215,687.41**	**3,317.49**
CBR	N	686.66	2.39
	C	701.22	2.35
	S	729.19	2.39
	Total	**640,934.66**	**1,710.79**

TABLE 2.2: DEN studies conducted in salt marshes in the US and the world. * indicates potential rather than directly measured rates

Source	DEN ($mmol\ N\ m^{-2}\ day^{-1}$)	Method
US----------		
Koop-Jakobsen and Giblin 2010	0.4-13.4	Isotope pairing
Hamersley and Howes 2005	0.4-11.9	$^{15}NH_4^+$ retention
White and Howes 1994	1.8	$^{15}NH_4^+$ retention mass balance
Kaplan et al. 1979	0.4-5.6	In-situ bell jar method
Present study	0.04-2.0*	ABT
O'Meara et al. *In review*	0.0-4.8	MIMS
Piehler and Smyth 2011	0.1-2.5	MIMS
Thompson et al. 1995	0.4-1.5 (natural)*	ABT
	0.0-0.04 (restored)*	ABT
Davis et al 2004	-9.0-10.1	$\Delta\ N_2$ overlying water
Anderson et al 1997	0.09-0.15	$^{15}NO_3^-$ isotope dilution
Global----------		
Rysgaard et al 1999	0-6.0	$^{15}N_2$ generated from $^{15}NO_3^-$
Koch et al 1992	0.1-2.8*	ABT
Abd Aziz and Nedwell 1986	0.1	$^{15}N_2$ generated from $^{15}NO_3^-$
Eriksson et al 2003	0.3-6.0	Isotope pairing

TABLE 2.3: Comparison of N_2O potential measured in NC to global salt marsh N_2O fluxes.

Source	F-DEN potential (mmol N m^{-2} day^{-1})	US total (Gg N/yr)	Global Total (Tg N/yr)
RCR	0.3 ± 0.16	12.9 ± 6.9	1.1 ± 0.6
CBR	0.8 ± 0.42	33.0 ± 17.2	2.9 ± 1.5
Kroeze et al. 2005	---	---	0.25
Smith et al. 1983	0.006-0.009	0.5-0.9	0.04-0.07
Seitzinger et al. 1980	0.005	0.4	0.04

FIGURE 2.1: Conceptual diagram of fungal and bacterial denitrification. To the left are substrates used for denitrification. The different boxes indicate need for oxic or anoxic conditions (note that fungi can denitrify under either scenario). To the right are the products of denitrification. Solid lines indicate dominant pathways. Dashed lines indicate secondary pathways.

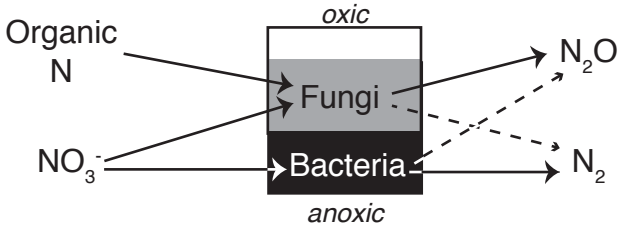


FIGURE 2.2: Location of study sites within the Rachel Carson Estuarine Research Reserve (a) and Currituck Banks Estuarine Research Reserve (b)

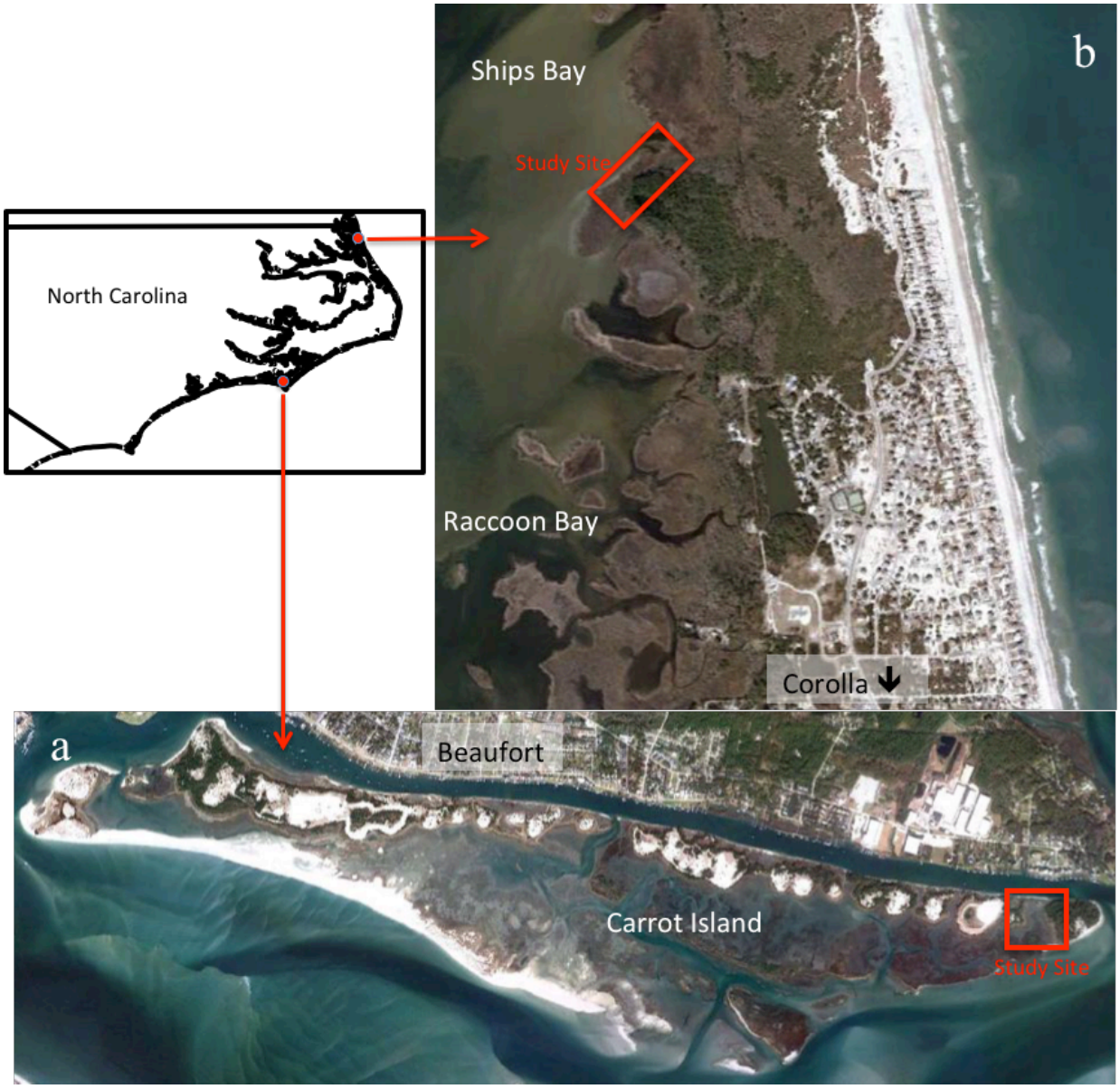


FIGURE 2.3: Transect data from the RCR (a) and CBR (B). Each GIS generated map edge as well as the center of the map represents a transect for a total of three transects per site.

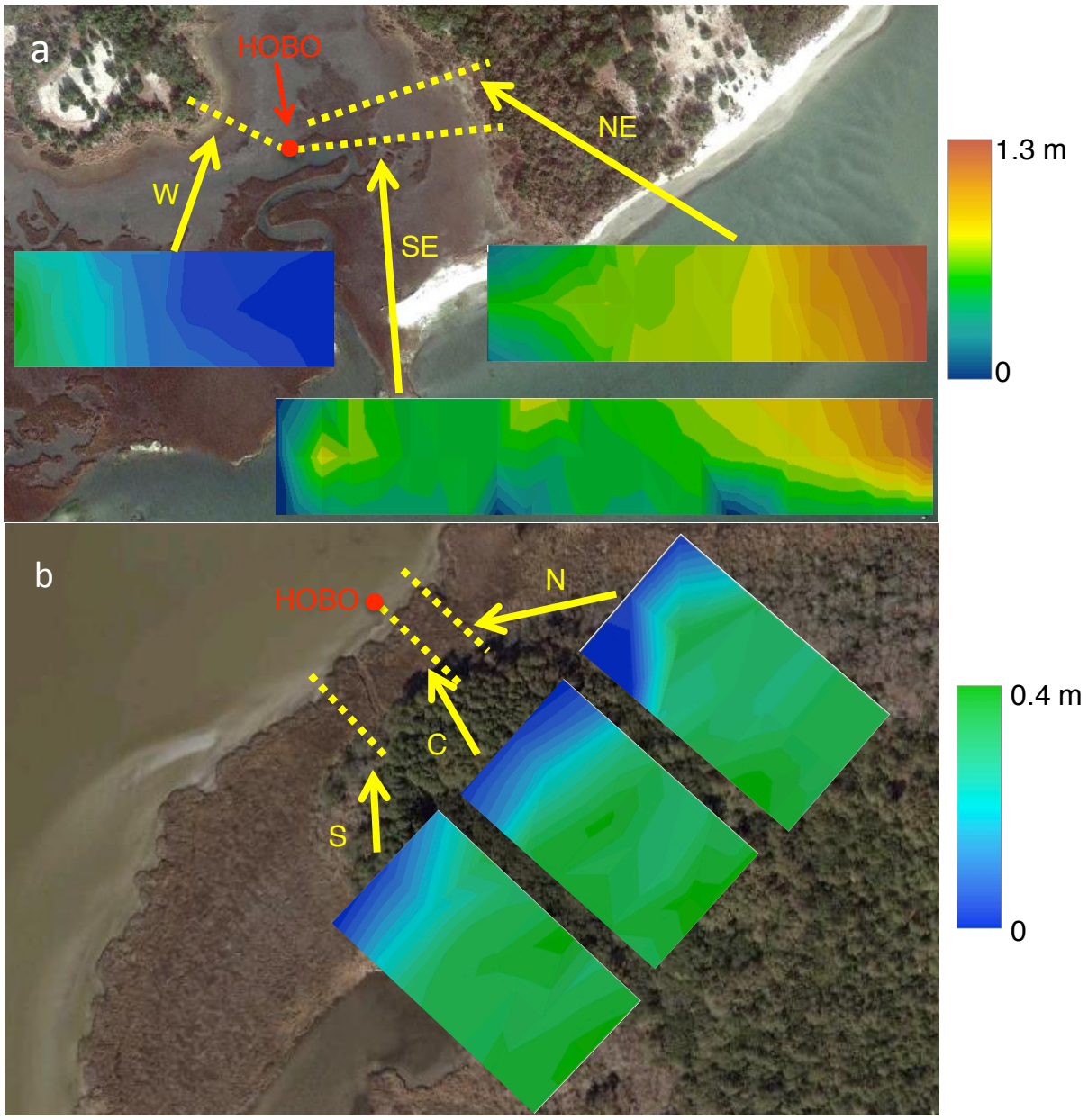


FIGURE 2.4: ABT core treatments. Different colored circles denote different pharmaceutical treatments to isolate bacteria, fungus, or neither (control, both present). Boxes indicate oxygen presence or absence in the samples. Finally, the number in each core refers to hours of incubation before the core is sacrificed to analyze for N_2O concentration.

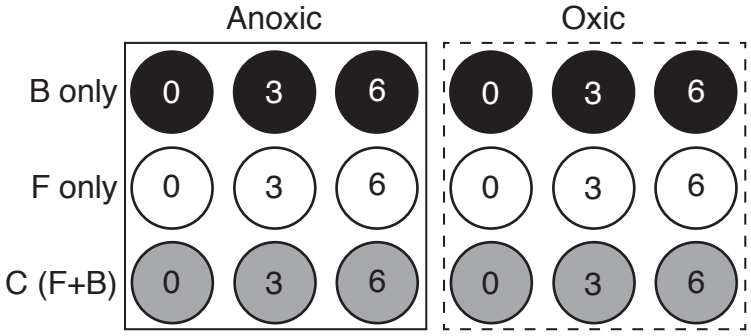


FIGURE 2.5: Soil characteristics of both sites. CBR characteristics are in gray (**a** and **b**) and RCR data is shown in white (**c** and **d**). Letter distinguishes statistically significant values.

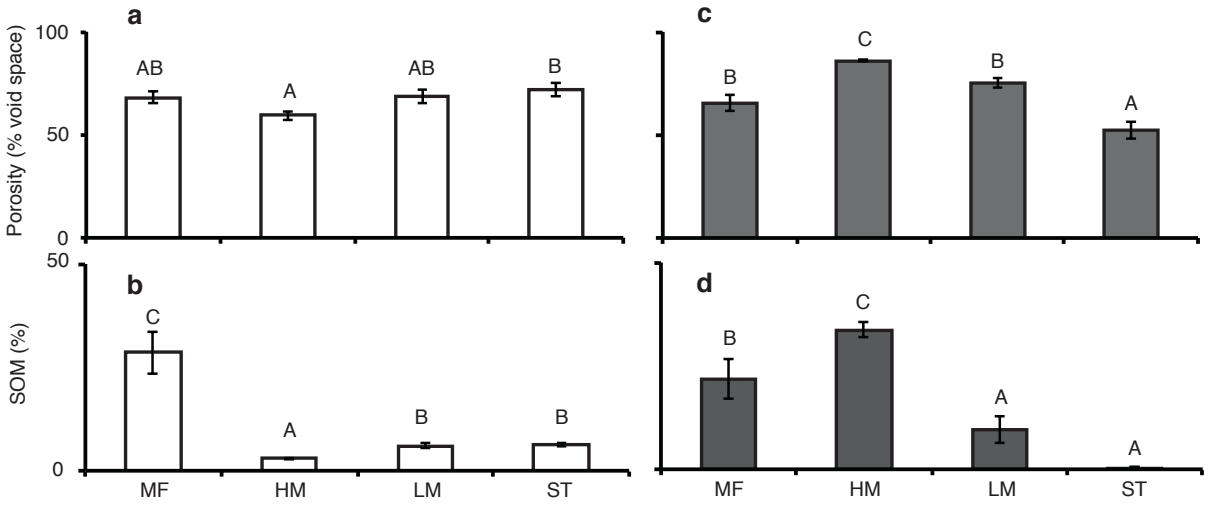


FIGURE 2.6 DEN potentials by habitat for C-DEN (a, b), B-DEN (c, d) and F-DEN (e, f) for both oxic and anoxic conditions. RCR data is shown in white and CBR data is shown in gray. Letters denote statistically significant groups. * indicates a significant difference between oxic and anoxic DEN potential.

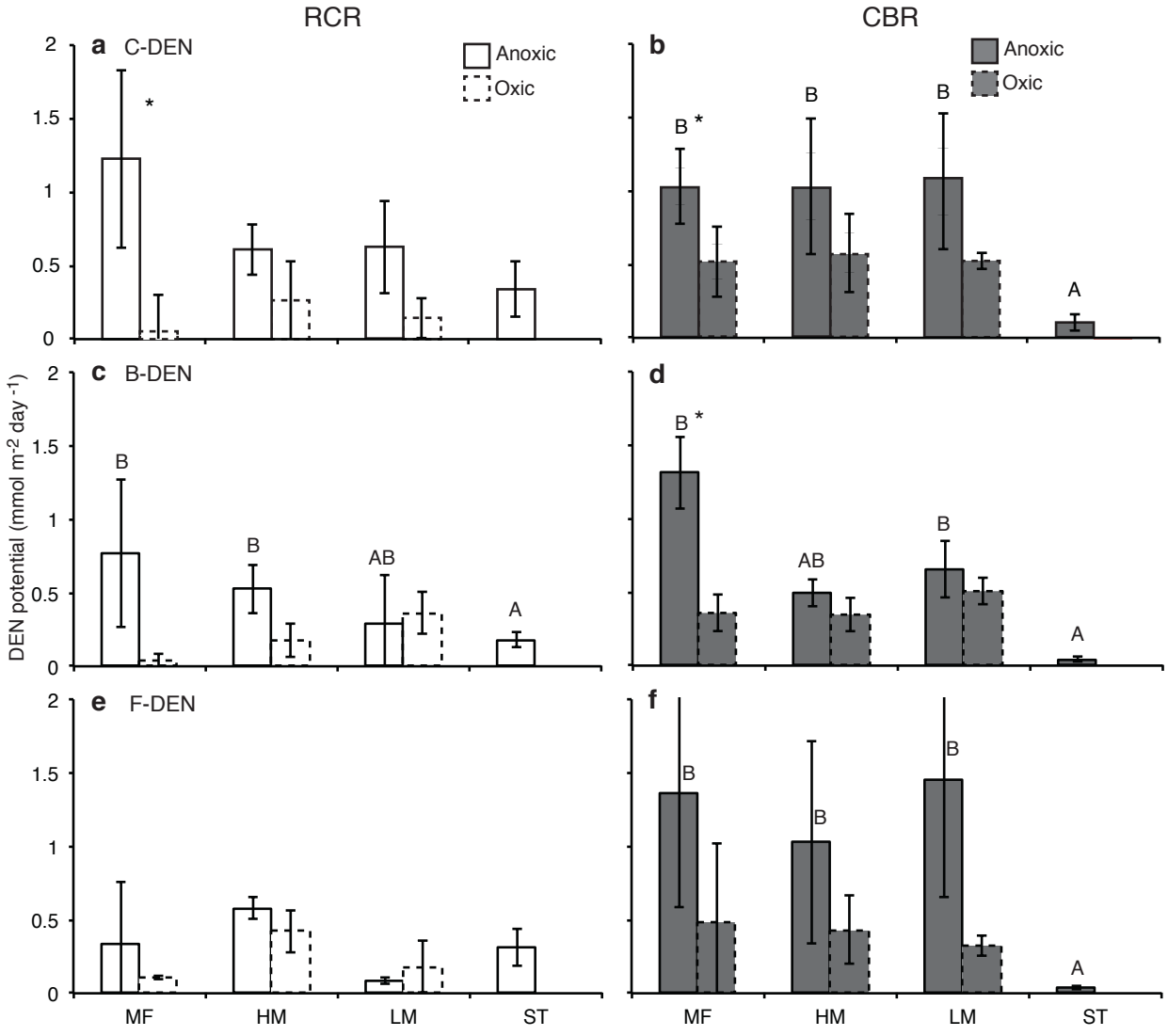
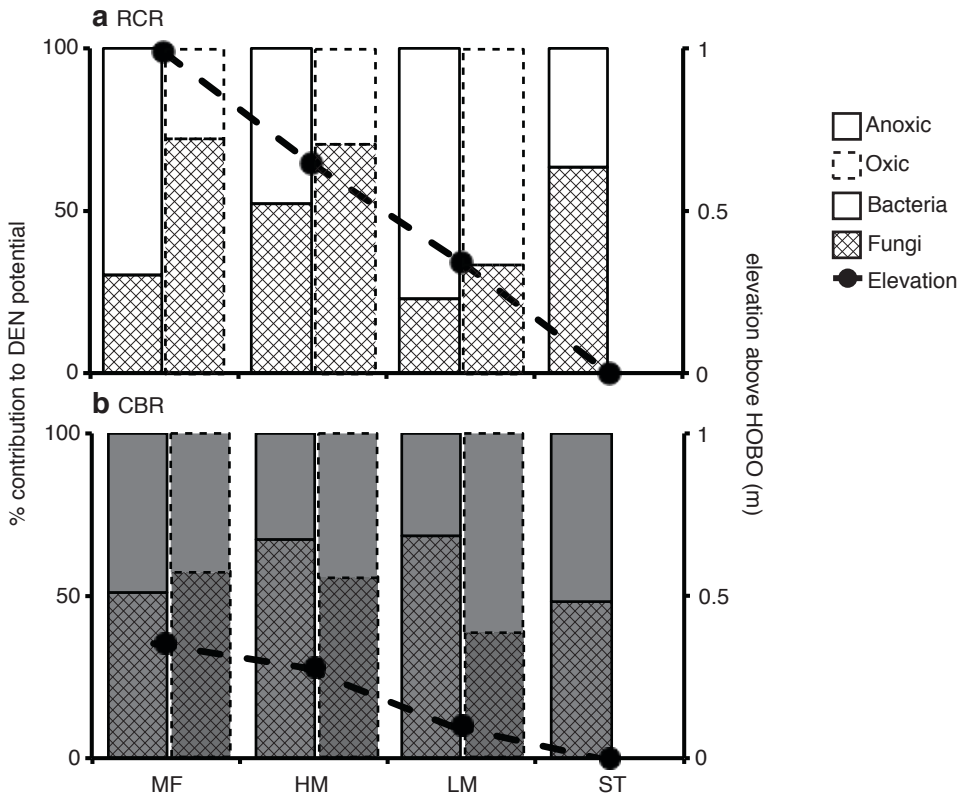


FIGURE 2.7: Relative contributions of F-DEN and B-DEN in anoxic and oxic conditions for the RCR (a) and CBR (b).



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CHAPTER 3

Effects of shoreline hardening on nitrogen processing in estuarine marshes of the U.S mid-Atlantic Coast

3.1 Introduction

Human modification of the nitrogen cycle has been extraordinary (Vitousek et al. 1997). Prominent effects of anthropogenically-enhanced nitrogen loading in estuarine systems are the increased occurrence and frequency of algal blooms (toxic and non-toxic), fish kills, hypoxia/anoxia, and habitat loss (Paerl et al. 2002; Cloern 2001). In coastal areas, excessive nitrogen loading has led to impairment of many of the world's estuaries (NRC 2000). Sixty-five percent of all estuaries in the United States are affected by excessive nitrogen loading (Bricker et al. 2003; Corbett et al. 2008). For example, since 1960, the Neuse River Estuary in North Carolina, USA has experienced a 45% increase in point-source nitrogen and 135% increase in nitrate (Paerl et al. 2004).

Shoreline hardening structures like bulkheads are an additional potential stressor affecting estuarine ecosystem function. Bulkheads are permanent vertical structures installed at or landward of the mean high water mark.. These structures impede dynamic processes in estuarine shorelines that naturally erode, accrete, and experience shifts in vegetation (Bozek and Burdick 2005; Currin et al. 2010). On natural shorelines, marsh vegetation adapts to erosion and/or sea level rise (SLR) by reestablishing inland to maintain its optimal elevation in the estuary. Shoreline hardening prevents landward migration (Figure 3.1; NRC 2007), which can result in the loss of marsh vegetation and the associated biogeochemical cycling that occurs in

these habitats (Schlesinger 1991; Long and Mason 1983), impairing estuarine ecosystem function (Bozek and Burdick 2005; Seitz et al. 2006). The combined effect of sea level rise and shoreline development has been deemed "the coastal squeeze" because of the negative forces on either side of marshes (Doody 2013).

Marshes contribute to estuarine function through shoreline stabilization, nutrient retention and removal, provision of habitat, and primary productivity (Millennium Ecosystem Assessment 2005). Anthropogenic hydromodification, or the alteration of natural flow pathways by development, such as bulkhead installation, likely affects all marsh functions (Bozek and Burdick 2005; Gergel 2005), but the impacts on nutrient cycling have not been quantified in coastal systems. Nutrient processing is a prominent feature of wetland function. As nitrate (NO_3^-) loading to estuaries continues to rise, any sinks and/or processes that remove NO_3^- from the system become increasingly important (Brush 2009). Denitrification (DEN) is the microbially-mediated process by which NO_3^- (biologically active) is converted to N gases (largely biologically inactive, Herbert 1999). Results of recent studies show that DEN can account for over 70% of NO_3^- removal from estuaries (Smyth et al. 2013; Koop-Jakobsen and Giblin 2010). Many papers have shown that DEN at groundwater seepage faces, commonly referred to as the subterranean estuary (Addy et al. 2005; Talbot et al. 2003; Spiteri et al. 2008; Santos et al. 2008), can significantly contribute to annual watershed scale N budgets (Addy et al, 2005). At the sediment surface, marsh habitat provides significant DEN in estuary ecosystems (Piehler and Smyth 2011; Dodla et al. 2008; Kaplan et al. 1979). Because DEN is an anaerobic process, it is favored when marsh sediments are inundated (creating a reduced environment). However, NO_3 is generated via nitrification under aerobic conditions is the primary source of NO_3^- for DEN in oligotrophic systems. Therefore, the tidal cycles of wet and dry are highly conducive to the

process of DEN. While low levels of NO_3^- limit DEN, excess NO_3^- can make the process of DEN less efficient, producing more intermediate products (N_2O, a harmful greenhouse gas) and reducing the rate of the process (Francis and Mankin 1977; Glass et al. 1997).

We hypothesized that the presence of a bulkhead and marsh width (Figure 3.1) would significantly affect denitrification rates. Bulkhead presence could directly alter nitrate or organic matter availability for denitrification, affecting the rates at which it occurs. Bulkheading of shoreline could cause a decrease in marsh width. With less surface area available, potential reaction sites for denitrification are lost, but smaller marshes may also have lower elevations and consist of fragmented habitat. Therefore, these changes to the marsh may alter nutrient cycling rates in these sediments. To test these hypotheses, we used flow through core incubations and analyzed dissolved gases in water using membrane inlet mass spectrometry to determine rates of DEN in systems with varying widths of fringing marsh and absence or presence of a bulkhead. In addition, to determine which factors would be most effective in predicting DEN rates, we characterized sediment oxygen demand, sediment organic matter content, and inorganic nitrogen fluxes.

3.2 Methods

3.2.1 Site Descriptions

Sites were selected along the North Carolina coast in each of three regions: southern, central, and northern (Figure 3.2). These regions were chosen based on differences in tidal range, salinity, and dominant vegetation to determine if these factors affected denitrification rates. Marsh widths for each site are shown in Table 3.1. Marsh width classifications for bulkhead sites were defined as none (no marsh present), narrow (width < 5m), medium (width 5-

15m), and wide (width >20m). These data were compared to a reference marsh (widths range from 14-20m), which did not have a bulkhead. Each site grouping contained 1 no marsh site, 1 narrow marsh site, 2 medium marsh sites, 1 wide marsh, and 1 reference marsh. Representative water was collected from each site at one or two sampling locations for use in continuous flow core incubations based on the proximity of sampling locations. If sites were <15 km apart, 1 water sample was used. Feed water attributes are shown in Table 3.2.

In the north, sampling locations were established at the convergence of Colling Creek and Kitty Hawk Bay (Figure 3.2a). The most common marsh vegetation included *Spartina cynosuroides* (33%) with an average stem density of ~160/m^2. The second most abundant species was *Juncus roemerianus* (19%). Water at the northern sites was lower salinity (1.05-8 psu) than the central and southern sites. One representative feed water was used for all incubations of northern sampling locations and collected from site 6. Tides in the northern sites are primarily wind-driven and do not exhibit diurnal cycling (Fear and Currin, 2012). Temperatures during sampling trips ranged from 10.8°C in the winter to 28.2°C in the summer.

Central sites were established in Bogue Sound along Pine Knoll Shores and Atlantic Beach (Figure 3.2b). The most common marsh vegetation included *Spartina alterniflora* (89%) with an average stem density of ~180/m^2. The next most abundant species was *Salicornia spp.* (4%). Cores from central sites were incubated with feed water collected from the Institute of Marine Sciences. Salinities ranged from 32-34 psu. Tides at the central sites are diurnal and are ~1m in range (Fear and Currin, 2012). Temperatures during sampling trips ranged from 6.3°C in the winter to 28°C in the summer.

Southern sites were located between Wrightsville Beach and Oak Island (Figure 3.2c). The most common marsh vegetation included *Spartina alterniflora* (85%) with an average stem

density of ~240/m^2. *Phragmities australis* was the second most abundant species (6%). Since the sites were located through a range of salinities (26-36 psu) feed water was collected from two locations (sites 2 and 4). All sites in the southern region were adjacent to the Atlantic Intracoastal Waterway. Tides in the southern regional are diurnal, but have a larger range than the central sites (1.5-2m; Fear and Currin, 2012). Temperatures during sampling trips ranged from 10°C in the winter to 28.5°C in the summer.

3.2.2 Core collection and incubation

Cores were collected in triplicate seasonally from each site at all locations. Cores were collected mid-marsh (perpendicular to shore). Protocols for core collection and incubation were adapted from Piehler and Smyth (2011). Sediment cores were 6.4 cm in diameter, 17 cm deep, and collected by hand in clear polycarbonate tubes. Plant shoots were avoided in the coring process to reduce disturbance to the sediment surface and to isolate sediment processes from plant or epiphyte processes. Cores were covered with ambient water and returned to the Institute of Marine Science, Morehead City, NC for incubation in an environmental chamber (Bally Inc.) at in-situ temperatures (measured with a Yellow Springs Instrument,YSI). Cores were capped with plexiglass tops equipped with two O-rings to attain air and water tight seals (Scott et al. 2008). Ports in each cap allowed a continuous flow of water collected from the field. Overlying water volume was maintained at approximately 400ml. Inflow water from the reservoir was passed over cores at a flow rate of 1ml per minute. Cores were pre-incubated for 18-24 hours prior to sampling to allow the sediment cores to reach steady-state (Erye et al. 2002; Scott et al. 2008).

3.2.3 Denitrification rates

Quantification of DEN is a difficult task due to the high background levels of N_2 (Groffman et al. 2006; Cornwell et al. 1999). A prevalent method of measuring denitrification is membrane inlet mass spectrometry (MIMS). Though no method is without drawbacks, unlike other methods of measuring DEN, MIMS allows for the direct measurement of N_2 flux on small samples with a high precision and without the addition of N to the system (Kana et al. 1994; Groffman et al. 2006).

Samples (5ml) were collected from the inflow and outflow in ground glass stoppered test tubes. MIMS was used to measure $N_{2(aq)}$ and $O_{2(aq)}$ in relation to $Ar_{(aq)}$. Argon is considered biologically inactive and serves as a conservative tracer. The use of Ar as a conservative tracer allows for the measurement of small changes in N_2 flux despite high background levels of N_2. Benthic fluxes were calculated using the following equation:

$$Benthic\ Flux = (C_{out} - C_{in})\frac{F}{A} \quad (1)$$

where C represents the concentration of an analyte, C_{in} and C_{out} are the inflow and outflow concentrations, respectively, F is the peristaltic pump flow rate (litres h^{-1}), and A is the surface area of the core (m^2; Miller-Way and Twilley 1996). The C_{in} was measured from reservoir water pumped through the flow-through system in a bypass (i.e. does not make contact with any sediment from cores) directly into sample vials to account for any changes in water chemistry through tubing and pump effects (Piehler and Smyth 2011). Dissolved gases in samples were measured against standards using DI water at 16°C and gas constants for the calculation of dissolved gases at incubation temperature and salinity. MIMS methodology was used to measure net DEN defined as the combined rates of traditional DEN (conversion of NO_3^- to N_2) and anaerobic ammonium oxidation (conversion of NH_4^+ to N_2) minus the rate of N_2 fixation

(conversion of N_2 to organic NH_4^+) but does not measure each N pathway individually. In addition, MIMS may underestimate DEN rate because it does not quantify N_2O emission, which is a possible product of incomplete DEN. Sediment oxygen demand (SOD) is the rate of oxygen consumption in the sediment from overlying water by biological activity and chemical oxidation of reduced species (Price et al. 1994). Ratios of N_2 to Ar were used to calculate denitrification rates. Ratios of O_2 to Ar were used to calculate SOD.

3.2.4 Site Characteristics

During each core incubation, 50ml of water were collected from the by-pass and core outflows for nutrient analysis. Samples were filtered using Whatman GF/F filters with a pore size of 0.7 μm. Nutrient samples were analyzed with a Lachat Quick-Chem 8000 automated ion analyzer for NO_3^- and NH_4^+. Nutrient fluxes were calculated using equation (1).

DEN efficiency is the total percentage of inorganic nitrogen that is released as N_2 from the sediment. It calculated using the following equation:

$$DEN\ \% = \left[\frac{DEN}{(DEN + NO_3^- + NH_4^+)}\right] * 100 \quad (2)$$

where DEN % is DEN percent efficiency, DEN is the flux rate of N_2, NO_3^- is nitrate flux from the sediment, and NH_4^+ is ammonium flux from the sediment (Owens 2009). All flux units are μmol m^{-2} h^{-1}. Negative fluxes indicate movement into the sediment and positive fluxes indicate release from the sediment. Because we are concerned with flux from the sediment, only positive fluxes were included in efficiency calculations.

Sediment samples for SOM content were collected from the surface sediment of each core at the end of MIMS experiments. Since our method of measuring DEN is limited to surface DEN, SOM samples were only collected from surface sediment. SOM content was measured using loss-on-ignition (LOI) methods adapted from Ball (1964).

3.2.5 Statistical Analyses

Data were prescreened with a Levene's test (homogeneity of variance) and Shapiro-Wilk test (normality). Since these data did not pass these tests of normality and homogeneity of variance, they were treated as non-parametric data and analyzed using Kruskal-Wallis tests and Mann-Whitney U post-hoc tests with a Bonferroni correction. Kruksal-Wallis analyses were used to compare DEN by site, season, vegetation type, bulkhead presence, and marsh presence. Linear regressions were used to assess the relationships between temperature, nutrient flux, SOD, SOM, DEN, and DEN efficiency. P-values less than or equal to 0.05 were considered statistically significant. All statistical analyses were completed in R (Version 2.8.2008-12-19). Errors shown throughout this paper are standard errors.

3.3 Results

3.3.1 Membrane Inlet Mass Spectrometry

The average DEN rate for all sites was 93±7 μmol m^{-2} h^{-1} and ranged from 0-482 μmol m^{-2} h^{-1}. The DEN rates were significantly affected by season (X^2=129.08, df=3, $p<0.01$) and site (X^2=27.25, df=2, $p<0.01$). However, site differences in DEN rate were primarily driven by salinity and dominant vegetation (X^2=26.28, df=1, $p<0.01$). Therefore, data were pooled by vegetation/salinity differences rather than region. DEN rates were positively correlated with temperature (R^2=0.44, $p<0.01$) and were generally higher during the summer and fall and lower in the winter and spring (Figure 3.3). With all data pooled, bulkhead (X^2=0.13, df=1, p=0.71) and marsh presence (X^2=2.20, df=1, p=0.14) did not significantly impact DEN rate. The lack of effect of marsh presence was driven by overall low rates in the winter and spring. When seasonal data was combined to calculate yearly DEN rates, marsh presence was a significant

factor (Figure 3.4; X^2=4.92, df=1, p=0.03). Marsh width was not a significant predictor of DEN rate in low salinity/*S. cynosuroides* dominated marsh (R^2<0.01, p=0.98), but did have an effect in high salinity/*S. alterniflora* dominated sites (R^2=0.03, p=0.04). With all data pooled, NO_x flux did not impact DEN rate (R^2=0.01, p=0.13), but NH_4^+ trended positively with DEN rate (R^2=0.07, p<0.01).

Efficiency of DEN was only calculated when DEN occurred (i.e. rates were greater than zero). Average DEN efficiency was 62 ± 3% and ranged from 0 to 100%. DEN efficiency was affected by season (X^2=22.11, df=3, p<0.01) and site (X^2=16.01, df=2, p<0.01). However, site differences were attributed to vegetation/salinity profiles (X^2=15.84, df=1, p<0.01) with lower efficiencies in the northern region (45±5%) than the central and southern combined (70±3%). For all sites, efficiency was lowest in the winter (C+S: 33±7%; N:15±11%), but efficiencies were highest in the spring for the central and southern regions (C+S: 87±5%) and the fall in the north (N:85±14%). DEN efficiency was not affected by bulkhead (C+S: X^2=0.81, df=1, p=0.37; N: X^2=0.03, df=1, p=0.87) or marsh presence (C+S: X^2=1.92, df=1, p=0.17; N: X^2=0.08, df=1, p=0.77). In addition, width (C+S: R^2<0.01, p=0.71; N: R^2=0.01, p=0.64), DEN rate (C+S: R^2=0.02, p=0.10; N: R^2=0.04, p=0.27), and SOD (C+S: R^2<0.01, p=0.89; N: R^2<0.01, p=0.72) did not significantly impact efficiency.

Average SOD was 1,562 ± 67 μmol m^{-2} h^{-1} and values ranged from 95 to 3,966 μmol m^{-2} h^{-1}. DEN and SOD were positively related (R^2=0.43, p<0.01). SOD was affected by season (X^2=107.13, df=3, p<0.01) and site (X^2=22.59, df=2, p<0.01). However site differences were not related to dominant vegetation/salinity. SOD at northern and southern sites were not significantly different from each other, but both were significantly different from central sites (N: Wilcoxon p<0.01, S: Wilcoxon p<0.01). SOD was generally lower at the northern and southern

than central sites (Figure 3.5). Although SOD was higher in vegetated sites, marsh presence (X^2=5.44, df=1, p=0.02) and marsh width (R^2=0.08, p=0.01) were only significant factors for central sites. SOD was not significantly impacted by bulkhead presence (X^2=0.771, df=1, p=0.38) and did not trend with NO_x flux (R^2=0.01, p=0.09), but NH_4^+ flux tended to increase as SOD increased (R^2=0.05, p<0.01).

3.3.2 Nitrogen Fluxes

N fluxes were significantly impacted by site and season (Figure 3.6). NO_x fluxes ranged from -29 to 81 μmol m^{-2} h^{-1}. NO_x flux trended negatively with temperature (R^2=0.05, p<0.01), but seasonal trends were not consistent between sites (Figure 3.6a). In the north, NO_x flux was lowest in the spring and highest in the winter and summer. In the central sites, NO_x flux was only positive in the fall and negative in all other seasons. In the south, NO_x flux was lowest in the fall and summer and highest in the winter. NO_x flux site differences (X^2=9.21, df=2, p<0.01) were not driven by dominant vegetation/salinity differences since the central and southern sites were significantly different (X^2=6.69, df=1, p<0.01). Marsh width did not affect NO_x flux in *S. alterniflora* dominated/high salinity marshes (C: R^2=0.01, p=0.44, S: R^2=0.01, p=0.56), but it was negatively correlated with marsh width in *S. cynosuroides* dominated/ low salinity marshes (R^2=0.06, p=0.05). Overall, bulkhead (X^2=2.29, df=1, p=0.13) and marsh presence (X^2=0.73, df=1, p=0.39) did not significantly impact flux rates.

NH_4^+ fluxes (Figure 3.6b) ranged from -705 to 4,679 μmol m^{-2} h^{-1}. NH_4^+ flux was affected by temperature (R^2=0.03, p<0.01), but seasonal trends were not consistent between sites (X^2=15.14, df=1, p<0.01). Differences between sites were driven by differences in salinity/dominant vegetation (X^2=13.73, df=1, p<0.01). In marshes dominated by *S. cynosuroides*/low salinity, NH_4^+ fluxes were lowest in the fall compared to all other seasons. In

marshes dominated by *S. alterniflora*/high salinity, NH4 fluxes were lowest in the spring and highest in the fall and winter. Summer NH_4^+ fluxes in the southern and central sites were not significantly different from any season. NH_4^+ fluxes (all sites pooled) were not significantly impacted by bulkhead presence (X^2=0.05, p=0.82) or marsh width (R^2=0.01, p=0.08). However, marsh presence was a significant factor (X^2=4.86, df=1, p=0.03), but this was only observed in *S. alterniflora* dominated/ high salinity marshes in the summer months (X^2=7.06, df=1, p=0.01).

3.3.3 Organic matter content

Average sediment organic matter content was 2.2 ± 0.2% and ranged from 0.1 to 12.9% (Figure 3.7). SOM content differed between sites (X^2=5.51, df=2, p<0.01, but this trend was driven by differences in salinity/dominant vegetation (X^2=25.23, df=1, p<0.01). In *S. cynosuroides* dominated/low salinity environments, SOM content was not affected by marsh presence (X^2=0.01, df=1, p=0.93). In *S. alterniflora* dominated/high salinity environments, SOM content was significantly higher in sites with marsh vegetation (X^2=40.31, df=1, p<0.01). SOM content did not affected by NO_x (R^2<0.01, p=0.75) or NH_4^+ flux (R^2<0.01, p=0.70). However, it did trend positively with DEN (R^2=0.05, p<0.01).

3.4 Discussion

Estuarine shorelines are dynamic systems that provide disproportionately high contributions to ecosystem function relative to their area. These valuable systems are also subject to significant natural and anthropogenic disturbances. Rising water levels and frequent coastal storms have been the impetus for coastal property owners to stabilize shorelines. Ecological effects of shoreline stabilization occur on many time scales, from immediate to decadal. We examined the effects of vertical bulkheads on nitrogen processing in adjacent intertidal habitats

by measuring DEN rates in salt marshes of varying size. We hypothesized that wider marshes would have significantly higher rates of DEN. Though this hypothesis was rejected, we found that the presence of any size marsh seaward of bulkheads was associated with higher rates of DEN.

Dominant vegetation and salinity appeared to significantly impact nitrogen cycling. Studies have shown that type of vegetative cover can impact DEN (Hernandez and Mitsch 2007; Lance et al. 1978). However, the literature shows an inconsistent relationship between salinity and DEN along estuarine gradients. Rysgaard et al. (1999) and Giblin et al. (2010) found that salinity and DEN to be negatively correlated. However Magalhaes et al. (2005) and Fear et al. (2005) found that salinity had no significant effect on DEN. In contrast to these findings, our results showed significantly lower DEN at less saline sites (northern site) when compared to more saline sites (southern and central sites). Since only N_2 production was quantified, DEN rates may have been underestimated. Because the production of N_2O is typically higher in less saline systems (Smith et al. 1983), we would expect N_2O production to be greater in the northern sites than the central and southern sites. In addition, these lower DEN rates in the northern region should exhibit decreased DEN efficiency due to the increased production of N_2O. Because we did find that the northern site has significantly lower efficiencies than the central and southern sites, it may be important to quantify N_2O production in addition to N_2 in the future.

Carbon is required in addition to NO_3^- for DEN. Carbon content in the sediment as well as carbon quality (or lability) has been shown to significantly impact DEN rates (Francis and Mankin 1977; Glass et al. 1997; Kim et al. 2002; Gardner and McCarthy 2009; McMillan et al. 2010; Ferguson et al. 2003; Narkis et al. 1979; Beauchamp et al. 1989; Davidsson and Stahl 2000). Our measurements of carbon content in the sediment (SOM) and carbon lability (SOD)

indicate that DEN is more closely correlated with carbon quality (lability) than quantity (SOM %). DEN and SOD were positively related and exhibited similar trends with N fluxes and temperature. The lack of correlation between DEN and NO_x flux provides evidence of coupled nitrification/denitrification. An alternate supply of NO_3^- produced via nitrification could result in DEN rates that are independent of NO_x flux.

Coastal wetland loss has accelerated. According to Dahl and Stedman (2013) annual loss of coastal wetlands has increased 25% from 1998 to 2009. While it is important to note that certain wetland types did increase in size, 95,000 acres/year is the net loss rate. At this increasing rate of loss, salt marshes (and all other saltwater wetlands) may be gone by 2060. Therefore, the need to retain and restore these habitats has increased. In the US, salt marshes are protected by Section 404 of the Clean Water Act, but salt marshes remain subject to threats such as sea level rise (Scavia et al. 2002; Morris et al. 2002) and nutrient loading (Vitousek et al. 1997; Valiela and Bowen 2001; Deegan et al. 2007). Therefore, multiple approaches toward restoration (Zedler, 2000) are critical to help sustain marshes and the services they provide.

One effective approach to restoring marshes or mitigating loss is the use of living shorelines over bulkheads (Currin et al. 2010). Because bulkheads are perceived as effective at protecting property from shoreline erosion they are seen as the default option for coastal landowners. However, they have been shown to reduce marsh width (Bozek and Burdick 2005; Broome and Craft 2000). Bulkhead presence was not found to directly impact N flux. Indirectly, bulkheads decrease DEN through marsh loss. In high salinity/*S. alterniflora* dominated sites, DEN rates did increase with marsh width, but, with an R^2 value of 0.04, width was an ineffective predictor. However, it is clear that increased marsh width results in increased surface area for DEN. Therefore, wider marshes process more N. In North Carolina, there are approximately

212,800 acres of marsh (Field et al. 1991). According to our data, total elimination of marsh would result in the reduction in N processing by approximately 5.0 ± 1.9 Gg y^{-1}. For comparison, yearly N-NO_3^- loading to the Neuse River Estuary (1 of 6 estuarine drainage areas in North Carolina; Field et al. 1991) is estimated to be 1.9 Gg y^{-1} (Paerl et al. 1998). This demonstrates that marsh loss has and will significantly reduce the resilience of coastal systems to nutrient loading. Therefore, the protection of marshlands should be a priority for coastal managers.

3.5 Conclusions

We detected a significant increase in DEN rate with marsh presence seaward of a bulkhead, but DEN rates were, generally, independent of marsh width. This indicated that marsh habitat, of any size, provided similar ecosystem function in terms of areal DEN. Our data illustrate that even a narrow marsh provides significant benefit to the estuary and conserving or restoring any area of marsh seaward of shoreline stabilization structures provides ecosystem benefits. It is also important to note that increasing marsh area increases available space for DEN, which, in turn, increases the overall amount of N removed from the biologically active pool of nitrogen (Smyth et al. 2013). Furthermore, marshes with or without bulkheads demonstrated the capacity to denitrify at significant rates. Human development of coastal areas is likely to continue to affect ecosystem function as long as population continues to concentrate at the coasts. Understanding the value of natural systems and ways in which those values can be retained in developed areas is critical to retaining coastal environmental quality.

TABLE 3.1: Sampling location classifications by site. Sampling location number corresponds to the numbers seen in **FIGURE 3.2** and BH = bulkhead present.

Site	Northern	Central	Southern
1	BH, narrow marsh (4m)	BH , no marsh	BH , medium marsh (16m)
2	BH, no marsh	BH , medium marsh (12m)	BH , medium marsh (16m)
3	BH, wide marsh (20m)	BH , narrow marsh (2m)	BH , narrow marsh (4m)
4	BH, medium marsh (12m)	BH , medium marsh (9m)	BH , no marsh
5	BH, medium marsh (12m)	Natural marsh, (14m)	Natural marsh (15m)
6	Natural marsh (20m)	BH , wide marsh (23m)	BH , wide marsh (18m)

TABLE 3.2. Site characteristics for each incubation, BDL =below detection limit

Location	Season	Incubation Temperature	Salinity (psu)	$[NO_x]$ (μM)	$[NH_3]$ (μM)
Northern	Fall	14.0°C	8	0.37	4.87
	Winter	10.8°C	4	0.03	1.00
	Spring	14.0°C	1.1	1.41	0.56
	Summer	28.2°C	5.3	0.34	3.56
Central	Fall	25.4°C	32.3	BDL	7.36
	Winter	6.3°C	32.5	BDL	0.72
	Spring	20.2°C	33	BDL	2.53
	Summer	28.0°C	32	0.03	0.96
Southern (1-3)	Fall	25.0°C	27	0.66	12.00
	Winter	10.0°C	28.2	2.86	4.12
	Spring	16.4°C	26	BDL	5.30
	Summer	28.5°C	31	1.51	47.10
Southern (4-6)	Fall	25.0°C	36	16.79	16.79
	Winter	10.0°C	30	1.35	1.35
	Spring	16.4°C	34	1.55	1.55
	Summer	28.5°C	33	27.40	27.40

FIGURE 3.1 Marsh crossection for bulkheaded (a) and reference (b) sites. Note that the bulkhead is a physcal barrier to marsh migration

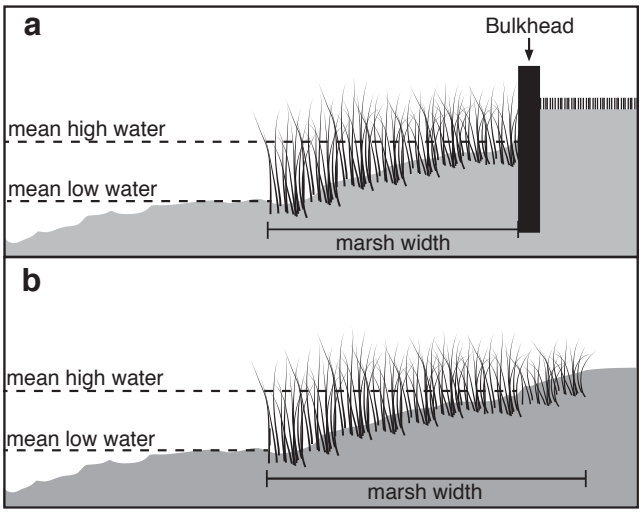


FIGURE 3.2 Site locations. Sites were located along the coast of North Carolina in the Northern (a), Central (b), and Southern (c) portions of the state. UNC IMS (b) was included to show where collected samples were analyzed

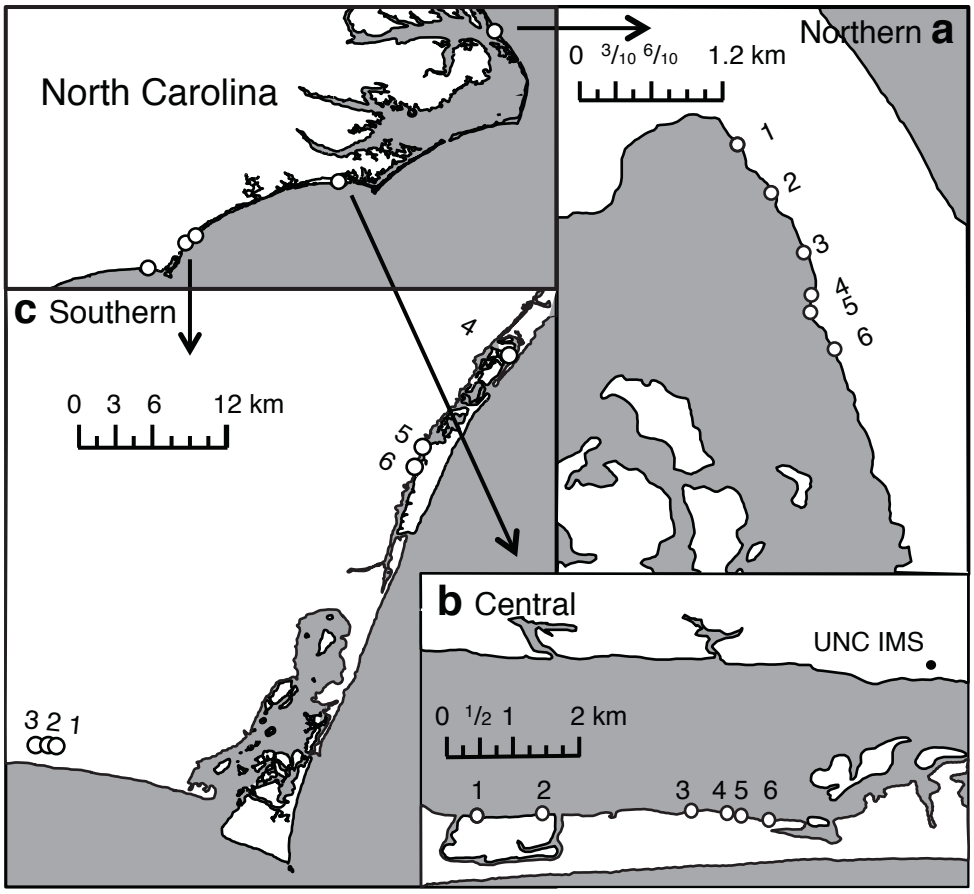


FIGURE 3.3 Denitrification rates. Letters indicate differences between seasons and * indicate differences between marsh types

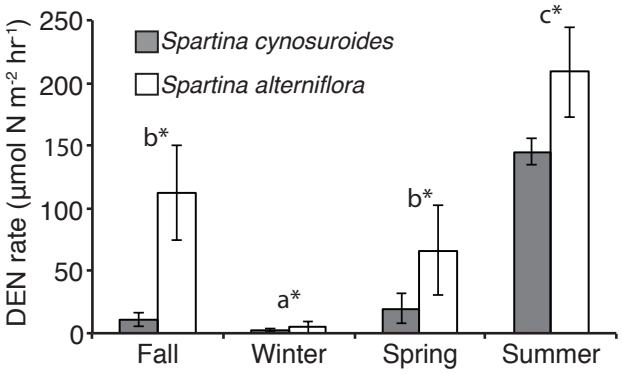


FIGURE 3.4: Yearly denitrification rates. * denotes a significant difference between sites with and without marsh vegetation

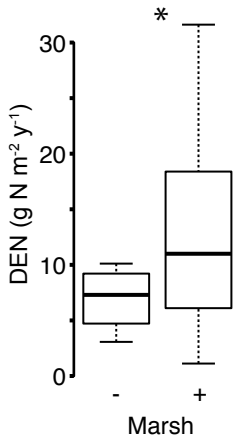


FIGURE 3.5: Sediment oxygen demand by site. * denotes a significance between sites with and without marsh vegetation. N=northern sites, S=southern sites, and C=central sites

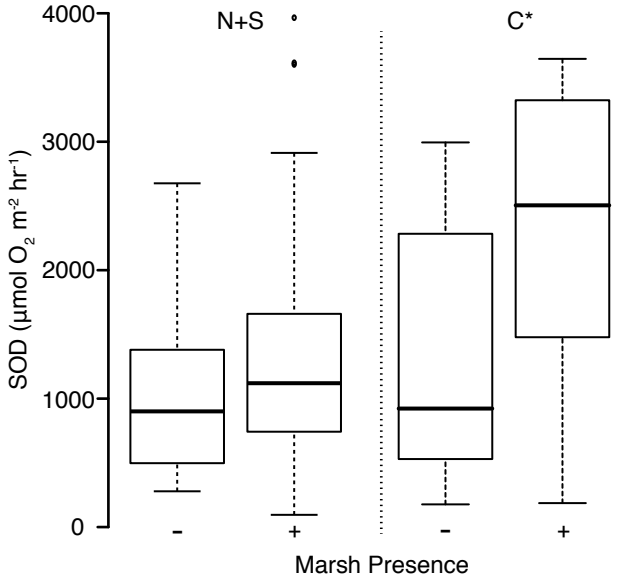


FIGURE 3.6: Nitrogen fluxes. (a) NO_x fluxes. Lower case letters show differences between seasons for a single site. Upper case letters in parentheses indicate differences between sites within a season. (b) NH_3 fluxes. Letters indicate seasonal differences within a site. * indicate differenced between marsh types within a season

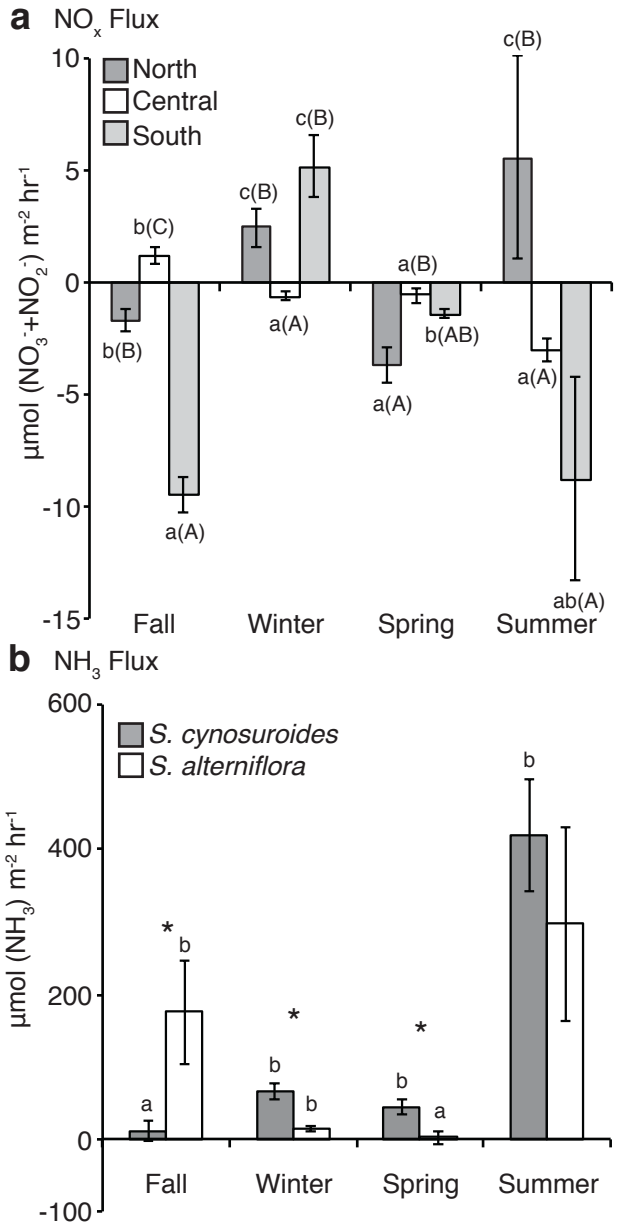
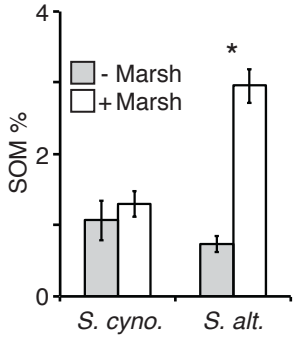


FIGURE 3.7 Sediment organic matter content. * indicate significant difference between sites with and without marsh vegetation



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CHAPTER 4

Impacts of shoreline hardening on salt marsh primary producer distribution, diversity, and richness

4.1 Introduction

Primary production is the base the estuarine food web and supports life for higher trophic levels. However, anthropogenic changes to the estuary can significantly alter distributions of primary producers and impact both resource quality and quantity. Since each primary producer type is associated with a specific subset of ecosystem services, any shift in their distribution could significantly alter the habitat. For example, if the primary species in an intertidal flat shifted from microphytobenthos to macroalgae, the overall quality of the carbon source for consumers would decrease. In salt marshes, a primary concern is the potential for a shift from marsh grass to macroalgae. Marsh vegetation provides many ecosystem services including water filtration, wave energy dissipation/shoreline protection, carbon sequestration, production of raw materials, and habitat for many commercially important species (Costanza et al. 1997; Barbier et al. 2011). When marshes are lost, so are their services. Therefore it is important to understand how anthropogenic effects directly or indirectly impact marsh size and primary production.

Coastal development has significantly altered nutrient loading to estuarine systems (Howarth et al. 1993; Vitousek et al. 1997; Paerl 2002; Cloern 2001; NRC 2000). Where marsh vegetation is directly adjacent to developed properties, the loss of a natural riparian buffer can increase nutrient loading directly to the marsh (Bertness et al. 2002; Lowrance et al. 1997; Glode

2008; Hubbard and Lowrance 1994) and can lead to eutrophication. Macroalgae blooms (MABs) caused by anthropogenic eutrophication are an emerging problem in coastal systems (Valiela et al. 1997; Newton 2011). Macroalgae compete with marsh plants for light and nutrients and can smother swaths of vegetation when washed over these environments (Bertness and Ellison 1987; Valiela and Rietsma 1995). As algae invade seaward of the marsh, the presence of shoreline hardening structures landward, particularly bulkheads, can also exacerbate the effects of MABs on marsh vegetation. Since bulkheads are physical barriers to sediment transport and marsh migration, their presence can negatively impact marshes through decreased sediment accretion and marsh drowning as sea level rises (Titus et al. 2009; Currin et al. 2010). Scouring at the base of the bulkhead caused by wave reflection can increase the rate at which marshes elevation decreases (Sumer and Fredsøe 1997). At lower elevations, macroalgae can be washed further inland. High marsh zones will be more likely to accumulate macroalgal wrack during storms or high tides as elevation decreases. In both cases, marsh loss is possible.

The focus of our study was to determine the direct and indirect impacts of shoreline development on the abundance and distribution of primary producers in estuarine marshes. We focused on the marsh grass and algal abundance within fringing salt marshes adjacent to bulkheaded shorelines and compared them to a reference marsh that did not have a bulkhead. In addition, we wanted to determine if speciation, abundance, and distribution varied on a seasonal basis.

4.2 Methods

4.2.1 Study Site

To determine if shoreline development and land use significantly impact distributions of marsh macroalgae (MA), surveys were conducted in Bogue Sound, NC, seasonally from fall 2009 through summer 2010 for MA. Surveys of marsh grasses were conducted once in the summer, when production was highest, instead of seasonally. Species distribution and abundance were determined in marshes of a range of sizes (landward to seaward) along bulkheaded shorelines. Microphytobenthic (MPB) community abundance was assayed with sediment chl-a concentrations. These data were compared to a reference marsh site. Sites were located in Pine Knoll Shores, NC (Figure 4.1). Upland development at each site was residential Marsh slope, elevation, and bulkhead types are shown in Table 4.1.

4.2.2 Vegetation Surveys

Vegetation monitoring quadrats (1 m^2) were surveyed along four transects extending perpendicular to the shoreline at each site. For the narrow marsh sites (<10m and including no marsh site), quadrats were spaced at 1m intervals along each transect. For all other sampling locations quadrats were spaced at 5m intervals with the exception of the marsh/water interface where two quadrats were located at consecutive 1m intervals to characterize the transition zone. Within each quadrat vegetation percent cover by species using visual methods (Peet et al. 1998) and stem counts of the dominant species present were recorded. Marshes were surveyed once during the period of peak biomass (July – September 2009; Fear and Currin 2012)

4.2.3 Algae Surveys

Algal transects were established perpendicular to shore. One transect was established within each 10m alongshore section of marsh. MA percent cover was surveyed within 0.25 m^2

quadrats at regular intervals so that 5 quadrats were measured along each transect within the marsh and one on the marsh edge (Figure 4.2). MA were identified to the lowest taxonomic level possible (typically genus) based on morphology and color.

4.2.4 Plotting primary producer distribution

Dot density figures were created in the statistical program R based on the percent cover recorded. The quadrat data collected were extrapolated to the area between quadrat samples over each 10 m swath of marsh. For example, if quadrat data were taken every meter, each 1m x 10m area of marsh is plotted with the percent data from its corresponding quadrat. The density of points in any given area represents the percent cover of each alga measured in the corresponding quadrats (1% macroalgal cover = 2 dots/50 m^2). For each site and season, species richness (R, total number of species) was recorded and evenness (E) was calculated using the following equation:

$$E = \left(\frac{-\sum p_i \ln p_i}{\ln R}\right) \quad (4.1)$$

where p_i is the area of coverage of the "i" species divided by the total area covered in algae or vegetation and R is the total number of species present of either algae or vegetation (Pielou, 1975).

4.2.5 Benthic chlorophyll-a

Chlorophyll-*a* concentrations in the sediment were used as a proxy for microphytobenthic abundance. Small sediment cores (surface area: 0.5027 cm^2) were collected to a depth of 5 mm in triplicate mid-marsh (both perpendicular and parallel to shore) at each site. Chl-a was extraced from sediments for approximately 18 hours in a solvent mixture of 45:45:10 % methanol: acetone: water, sonicated and measured using a Turner Designs Trilogy Fluorometer (Welschmeyer et al. 1991; Pinckney and Zingmark 1993).

4.2.6 Statistical Analyses

Linear regressions were used to assess relationships between richness, evenness, marsh morphology, and stem density. Kruskal-Wallis non-parametric tests were used to assess the direct impacts of bulkheads on both marsh vegetation and algae because these data did not pass test of normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test). All statistical analyses were completed in R (Version 2.8.2008-12-19). Error bars shown throughout this paper are standard error.

4.3 Results

4.3.1 Bulkheads and algal richness, evenness, and seasonal distributions

Algae were most abundant in the winter and spring (Figure 4.3 and 4.4) and least abundant in the summer and fall (Figure 4.5 and 4.6). Total species richness was greatest winter (6 taxa) and the lowest in the fall and summer (1 taxa). During the winter, the dominant algal species was *Ectocarpus spp.,* and non-wrack algae were found higher in the marsh than any other season. In the spring, algal coverage was concentrated at the marsh edge except at site N, where no marsh is present. The primary species during the spring were *Ulva spp*. and *Gracilaria verrucosa*. The dominant summer species was cyanobacteria, which appeared to be epiphytic rather than a wrack species. Finally, in the fall, the only species found was *Gracilaria verrucoso,* which occurred primarily as wrack. Algal species richness and evenness were calculated only when more than 1 species was present. Therefore, values were only reported for the spring and winter (Table 4.2). Algal richness and evenness were not affected by elevation (richness: R^2=0.28, p=0.09; evenness: R^2<0.01, p=0.96) or bulkhead presence (richness:

X^2=3.06, df=1, p=0.08; evenness: X^2=0.70, df=1, p=0.40). Slope did affect evenness (R^2=0.40, p=0.04), but not richness (R^2<0.01, p>0.99).

Marsh slope/elevation and bulkhead presence did not significantly impact percent cover or benthic chlorophyll-a concentrations. Average benthic chl-a concentration was 181.39 ± 13.57 mg/m^2 and ranged from 0 to 2,320.13 mg/m^2 (Figure 4.7). There was no significant response in benthic chl-*a* concentration as a function of marsh size (X^2=4.97, df=5, p=0.42), average percent algae cover (R^2<0.01, p=0.30), stem density (R^2=0.13, p=0.12), or season (X^2=2.65, df=3, p=0.45).

4.3.2 Bulkheads and vegetation richness and evenness

The dominant vegetation for all sites was *Spartina alterniflora* (90%) followed by *Distichlis spicata* (3%) and *Salicornia spp.* (2.8%). Lower abundances of *Borrchia fuctescens, Juncus roemerianus, Limonium carolinium,* and *Spartina patens* were found in our sites. The average live stem density for *Spartina alterniflora* was ~180/m^2. Marsh vegetation richness was not directly impacted by the presence of the bulkhead (X^2=2.12, df=1, p=0.14), but evenness was affected (X^2=9.36, df=1, p<0.01). As an indirect result of bulkheading, marsh vegetation richness and evenness were affected by marsh width (Table 4.3; richness: R^2=0.82, p<0.01; evenness: R^2=0.65, p<0.01). Two factors impacted by marsh width are elevation and slope. Richness was more affected by elevation (slope: R^2=0.18, p=0.07; elevation: R^2=0.38, p<0.01), but evenness was more strong associated with slope (slope: R^2=0.23, p=0.04; elevation: R^2=0.17, p=0.08). When mid-marsh quadrats were compared, there was a positive trend between stem density (both live and dead stem counts) and marsh size (alive: R^2=0.22, p=0.04; dead: R^2=0.52, p<0.01). Elevation at the mid-marsh quadrat was a significant factor for live (positive trend,

R^2=0.27, p=0.02), but not dead stem counts (R^2=0.06, p=0.34). Overall slope of the marsh was not a significant factor for live or dead stem counts.

4.4 Discussion

The purpose of this study was to determine the direct and indirect impacts of bulkheads of the distribution and abundance of primary producers in fringing estuarine marshes. We found that species richness and distribution are affected by marsh elevation and slope rather than the direct impact of bulkhead presence. This indicates that bulkheads do not directly impact primary producer abundance, but indirectly change their distribution by altering marsh topography.

Vegetation distributions at all marsh sites were primarily dominated by single taxa. On the other hand, the dominant species of algae changed seasonally. This trend is similar to algal surveys conducted by Pomeroy and Weigert (1981) and O'Connor et al. (2011). O'Connor et al. (2011) found that MA percent cover was highest in the winter and lowest in the summer and measured little seasonal change of benthic chl-a in Bogue Sound, which is similar to our observed trends. However, in contrast to our results, O'Connor et al. (2011) found that the presence of shoreline stabilization (sills) did have an impact on algal abundance. The morphology of each shoreline stabilization structure can most likely account for differences in the results between our study and O'Connor et al. (2011). Sills armor the marsh edge while bulkheads are located at the upland edge of the marsh. Since algal cover is naturally concentrated at the marsh edge, sill presence could significantly alter MA and MPB abundance, but bulkheads do not have the same potential. According to our studies, marsh slope and elevation are more important predictors of marsh plant diversity, live stem density, and algae coverage. Marsh function was not significantly affected by the presence of hardened structure,

indicating marshes and the associated primary producers can be successful when a bulkhead is present. However, once a bulkhead affects a marsh through increased wave reflection, scour, and prevention of marsh migration, negative impacts will occur.

The impacts of algae on salt marshes can be limited based on the seasonality of these primary producers. Salt marsh vegetation does not appear to be significantly impacted by algal cover in our surveys or in research conducted by O'Connor et al. 2011. Where marsh grass was present, algae were concentrated at the marsh edge. Vegetation prevents the movement of algae landward and light is more readily available at the marsh edge. However, as the marsh grass dies back in the winter and before it returns in late spring, algae can be washed further inshore. With reduced marsh grass cover, algae have reduced competition for nutrients and light allowing it to persist higher in the marsh instead of concentrating at the marsh edge. Survey data showed that marsh plant richness was related to marsh morphology, but algal richness was not. Because macroalgae is primarily concentrated at the marsh edge, marsh width is not predicted to affect richness or evenness. Therefore, algal diversity is more directly related to seasonal growth patterns and marsh elevation/slope rather than speciation of marsh vegetation. In contrast, marsh plant diversity is significantly impacted by marsh size, similar to a species-area curve (Hopkins 1955; Coleman et al. 1982). Larger areas can support more organisms and larger populations. Since larger populations are more diverse, diversity increases as a function of habitat size. Though marshes have low plant diversity, they are stratified. As marsh size increases, change in elevation increases. This allows for plants with varying levels of salt tolerance to persist in the same site.

Benthic chl-a concentrations did not trend with any of our measured parameters, but there are several possible explanations for this lack of correlation. Because MPB are a high quality

food source, marsh-dwelling organisms could be significantly altering chl-a concentrations through grazing. According to Cebrian and Duarte (1994), top-down control of plant biomass (i.e. herbivory) is independent of primary production. This indicates that herbivores consume a maximum percentage of plant biomass regardless of total plant abundance. However, their plant consumption is not spread evenly across all species. Herbivores tend to graze on more palatable substrate. According to Cebrian (1999), microalgal communities are more highly grazed than macroscopic primary producers. Therefore, the grazing may be a more significant control of microphytobenthic biomass than macroalgal or marsh grass biomass. In addition, epiphytes were not quantified in this study. Several studies have shown that epiphytic algae can be an important source of primary production (Jones 1980; Currin and Paerl 1998) and should be included in future studies.

Bogue Sound is an oligotrophic system (Paerl 2000). The diversity and percent cover measured in our transect surveys reflect a system low in nutrients. According to STORET data from the EPA (2009), nutrient concentrations in Bogue Sound are increasing. Our studies, as well as other research groups, have documented species capable of creating a green tide in North Carolina (Cahoon et al. 1998). Most green tide species have been known to increase productivity as nutrient availability increases (Fletcher 1996; Taylor et al. 2009). Therefore, as nutrient levels continue to rise, the risk of a species outbreak in Bogue Sound rises as well. A green tide in stabilized habitats could exacerbate marsh loss by smothering vegetation (Lyons et al. 2012). In addition, these effects would only intensify as nutrient concentrations and sea level continue to rise.

4.5 Conclusions

Anthropogenic stress on salt marshes can cause both readily observable impacts such as sediment scour and vegetation loss as well as less obvious impacts on marsh function. We found that direct impacts of bulkhead presence were minimal for marsh vegetation, macroalgae, and microphytobenthos. However, indirect effects resulted from changes in marsh morphology caused by the addition of a bulkhead. The increase in slope and decreased elevation associated with bulkheads cause a decrease in the richness and evenness of marsh vegetation. Macroalgal richness was independent of marsh morphology because it is concentrated at the marsh edge. However, distribution of algae (reflected in evenness) was affected by elevation. In addition, different primary producer types appeared to be dominant during different seasons, similar to niche partitioning. However, if marsh characteristics were to change drastically through land development, warming, or rising water levels it could result in a shift of growing season. While competition can increase diversity, it may alter the quality, or overall palatability, of the carbon source. Changes to the base of the estuarine food web could have negative implications for higher trophic levels as well as organisms that utilize the habitat structure provided by marsh vegetation. This study has shown that bulkheads indirectly alter primary producer distribution and abundance. Alternatives to bulkheads, such as living shorelines, should be used in the future to protect marsh habitat.

TABLE 4.1: Site descriptions. Note that marsh size refers to distance from the bulkhead or where marsh vegetation begins to the marsh edge. Abbreviations are as follows: N= no marsh, F=fringing marsh, M=medium width marsh, W=wide marsh, and R= reference site (no bulkhead present). *data taken from Fear and Currin 2012

Site ID	Marsh size (m)	Structure	Slope*	Elevation* (m)
N	0	PVC bulkhead	0.01	-0.5
F	1.4	PVC bulkhead	0.15	0.22
M_1	9.2	PVC bulkhead	0.06	0.29
M_2	12	Concrete bulkhead	0.05	0.23
W	23	Wood bulkhead	0.04	0.60
R	14.3	None (reference)	0.03	0.27

TABLE 4.2: Richness and evenness calculated for algae cover in the winter and spring

	Winter		Spring	
Site	*R*	*E*	*R*	*E*
N	1	---	2	0.92
F	4	0.59	4	0.68
M_1	3	0.74	2	0.92
M_2	3	0.49	2	0.92
W	3	0.23	2	0.65
R	4	0.68	4	0.43

TABLE 4.3: Richness and evenness for marsh grasses in summer during peak growth.

Site	R	E
N	---	---
F	1	---
M_1	2	0.12
M_2	2	0.37
R	3	0.43
W	5	0.39

FIGURE 4.1: Map of sampling sites in Bogue Sound, NC. UNC-IMS was included as a point of reference.

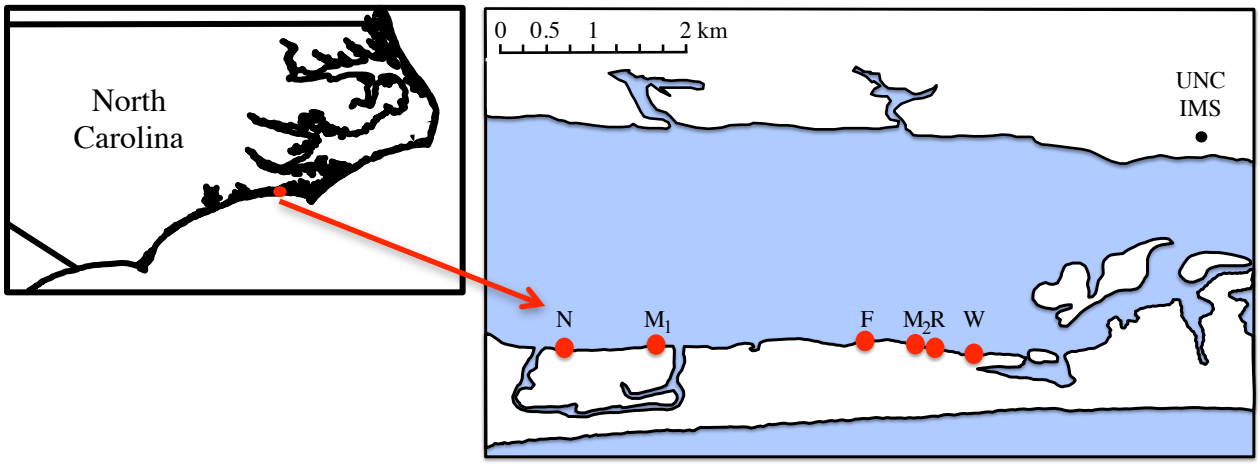


FIGURE 4.2: Example of transect establishment and quadrat placement for the collection of algal percent cover data. Note that each transect can be a different length based on the width of the shoreline.

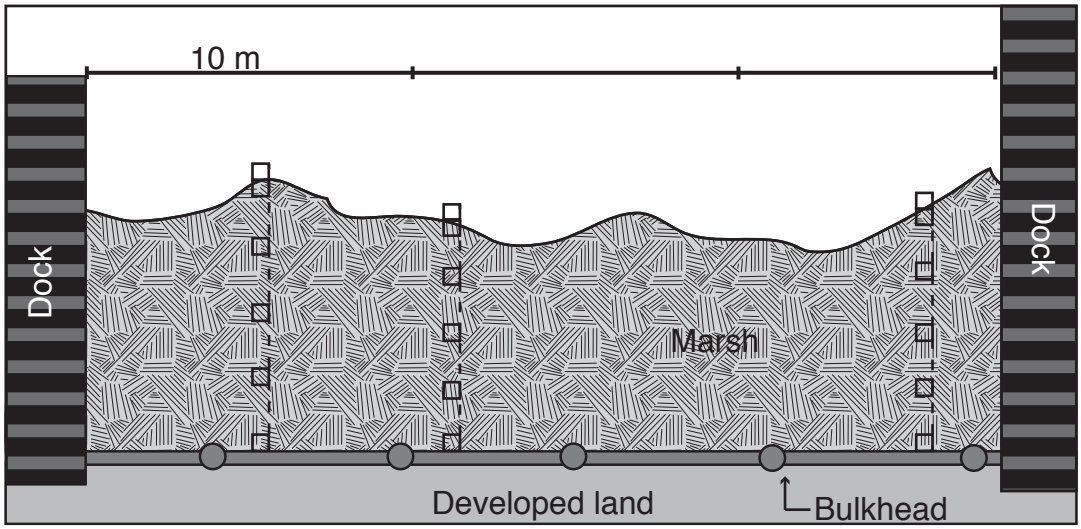


FIGURE 4.3: Winter distributions of algae. Site are organized by ascending marsh size. Note that the dashed brown line refers to the marsh edge. The final plot refers to the reference site (R).

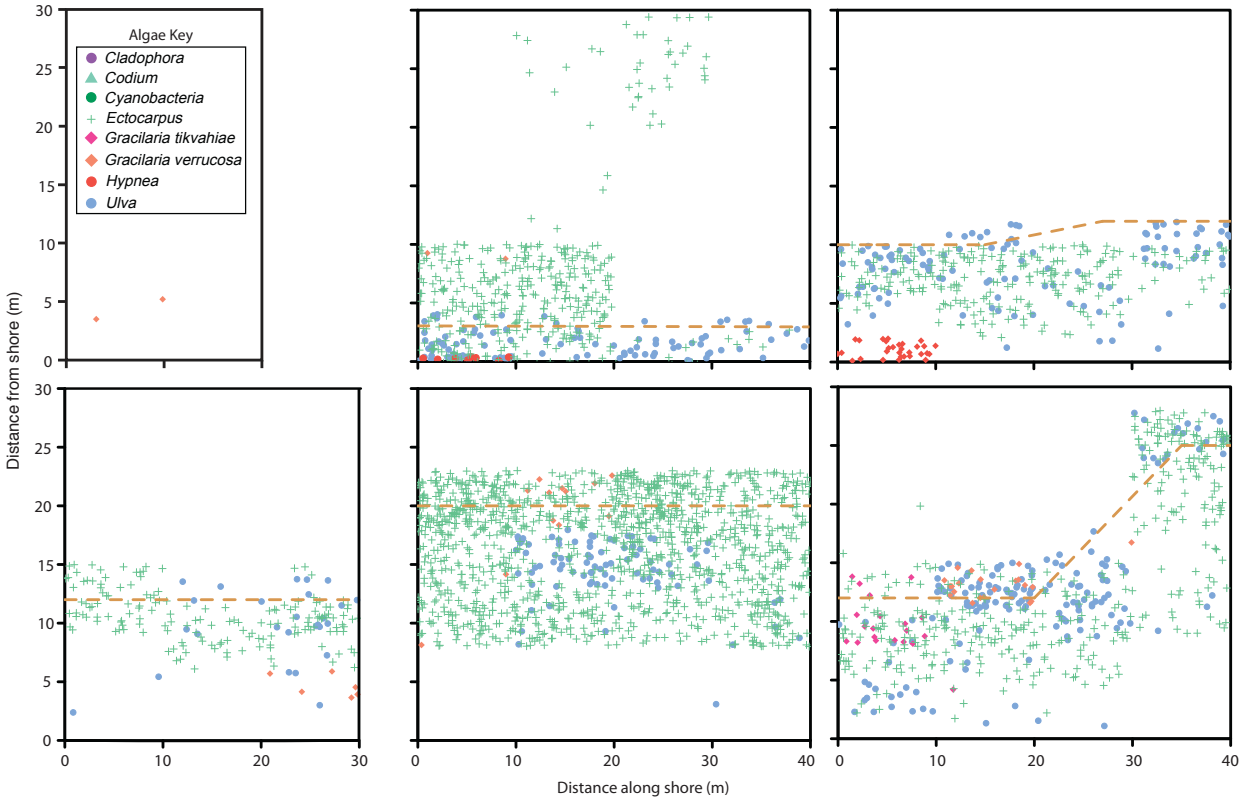


FIGURE 4.4: Spring distributions of algae. Site are organized by ascending marsh size. Note that the dashed brown line refers to the marsh edge. The final plot refers to the reference site (R).

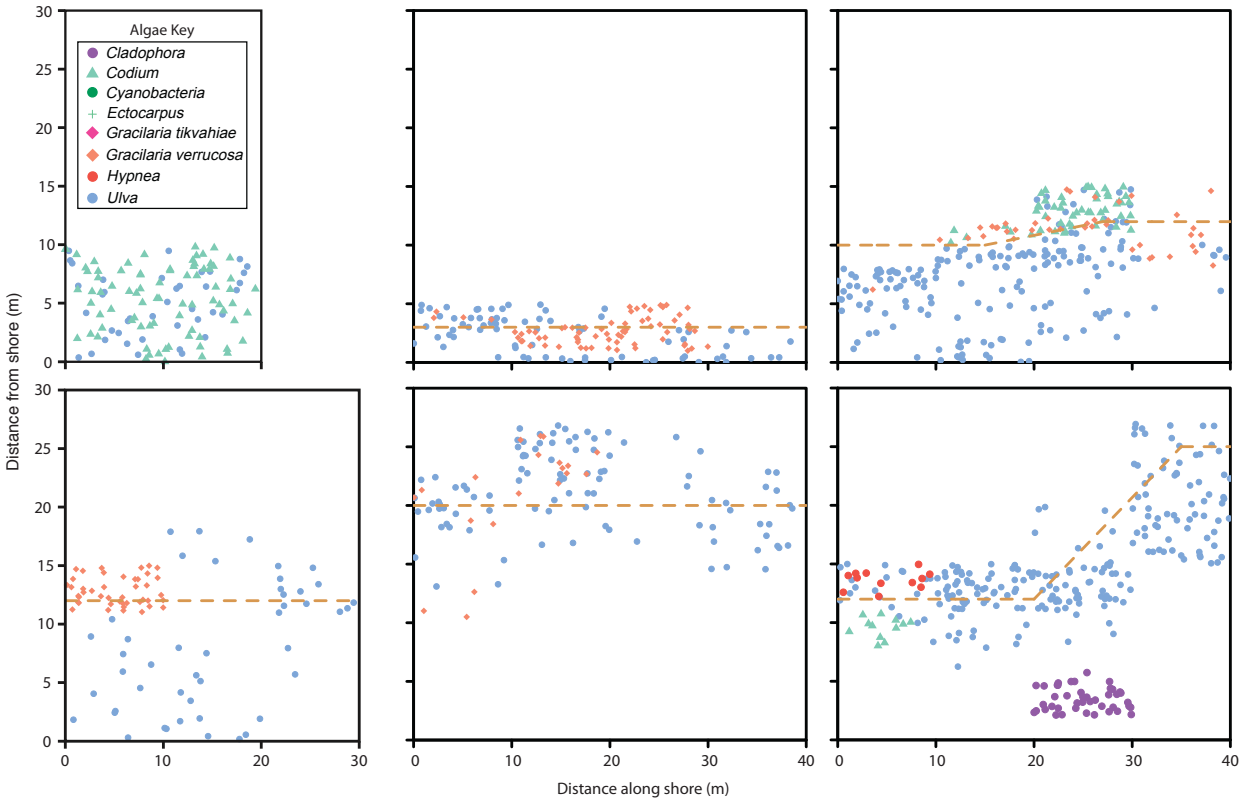


FIGURE 4.5: Summer distributions of algae and marsh grasses. Site are organized by ascending marsh size. Note that the dashed brown line refers to the marsh edge. The final plot refers to the reference site (R).

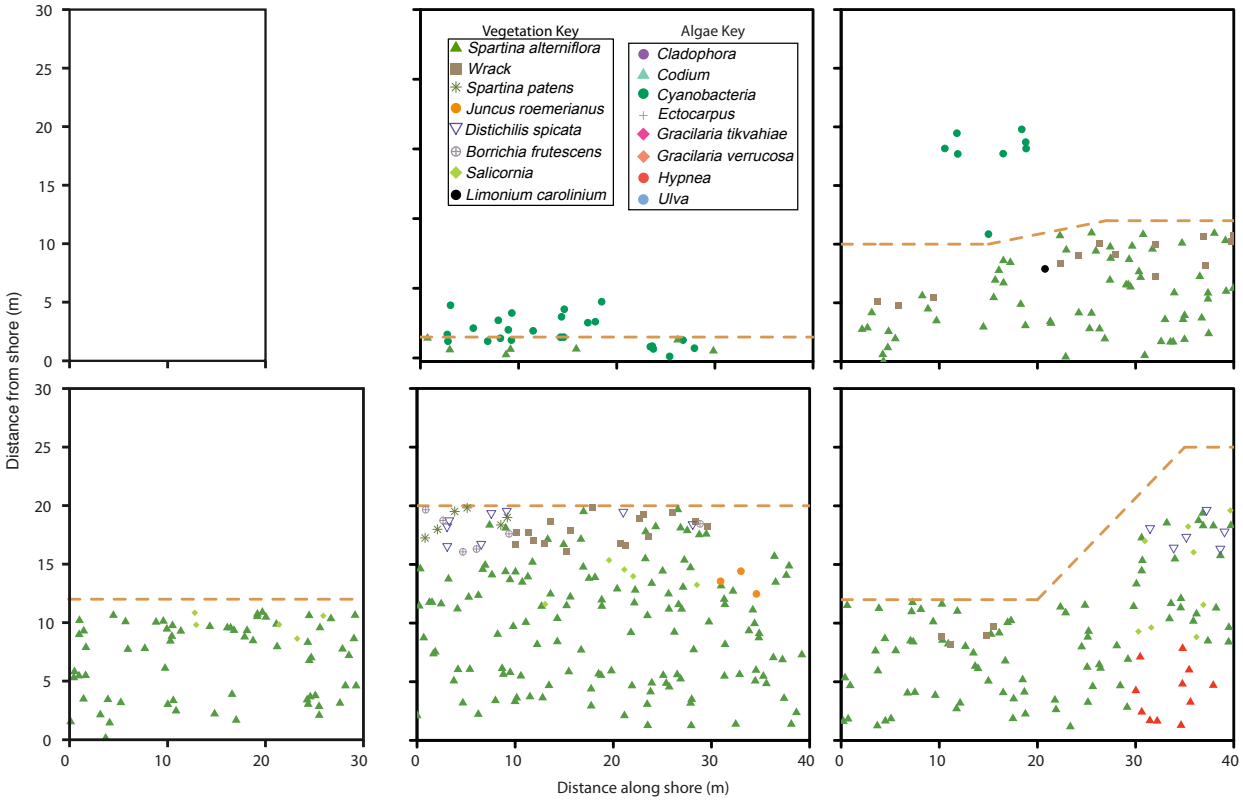


FIGURE 4.6: Fall distributions of algae. Site are organized by ascending marsh size. Note that the dashed brown line referes to the marsh edge. The final plot refers to the reference site (R).

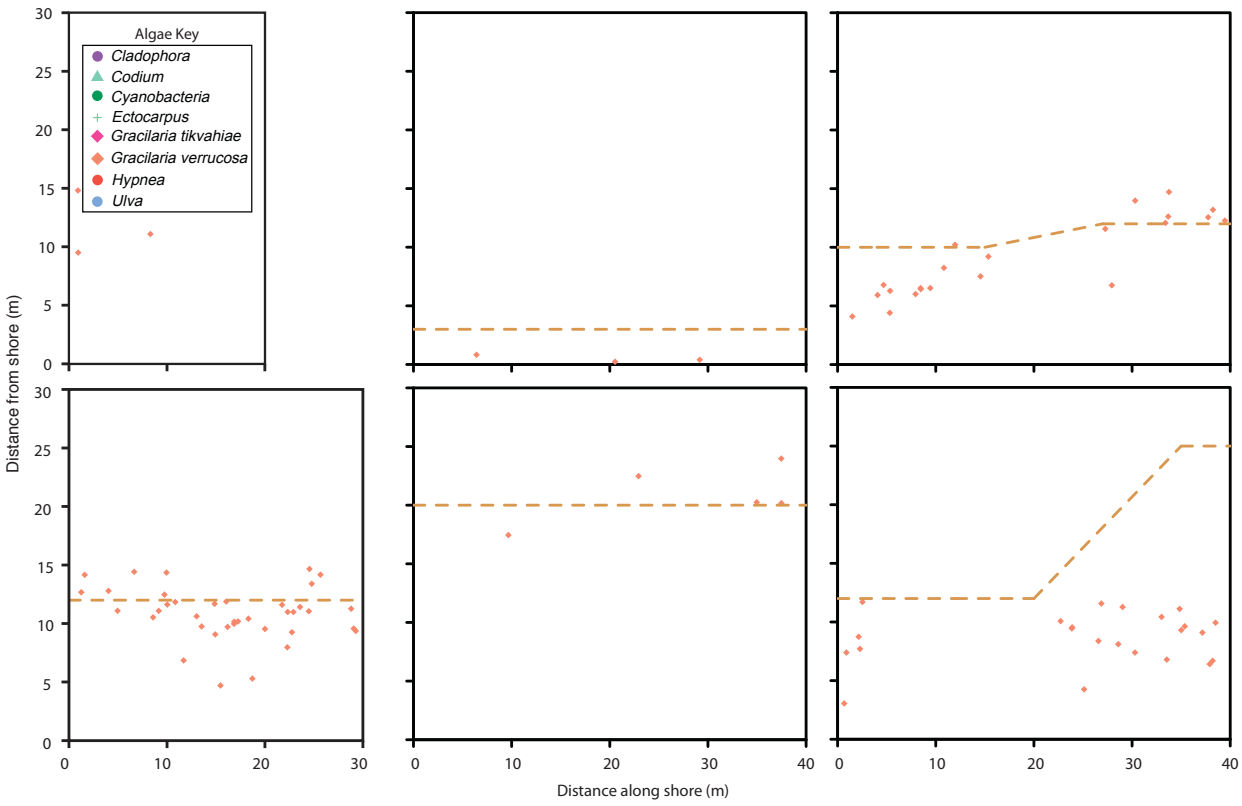
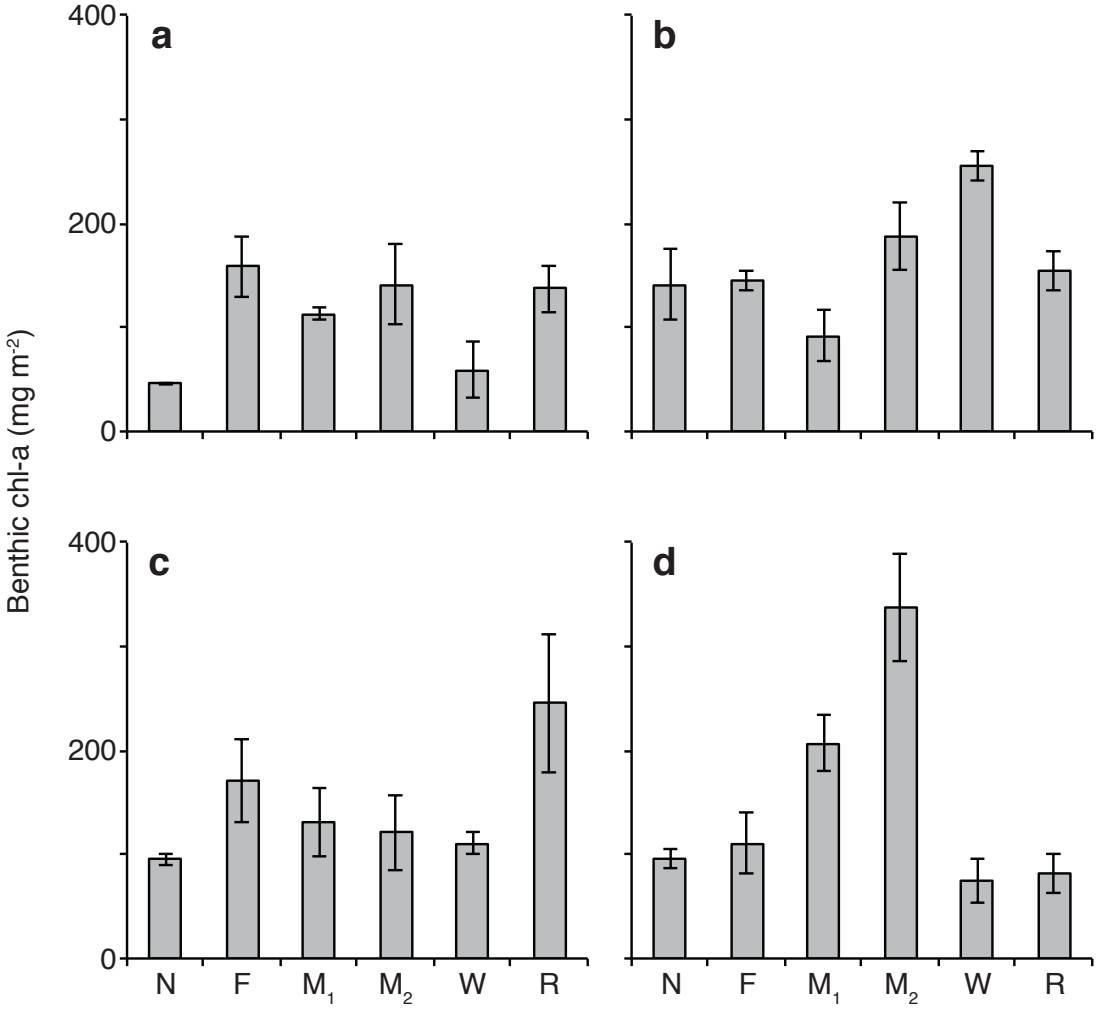


FIGURE 4.7 Benthic chl-a concentrations for the (a) winter 2009-2010, (b) spring 2009, (c) summer 2010, and (d) fall 2009. N= no marsh, F=fringing marsh, M=medium width marsh, W=wide marsh, and R= reference site (no bulkhead present).



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CHAPTER 5

Warming affects macroalgal and microphytobenthic abundance

5.1 Introduction

Community dynamics are thought to be controlled by adaptations to physical stressors, such as thermal and insolation stress, in high stress environments and adaptation to biological stressors, such as competition and predation, in low stress environments (Sanders 1968; Menge and Sutherland 1976; Connell 1975). These trends are typically observed across trophic levels, however within a trophic level, these principles still apply. Two examples include algal zonation in the rocky intertidal and salt marsh elevation gradients. In areas of high stress, the dominant species is determined by adaptation to the stressor such as salt tolerance of *Spartina alterniflora* in salt marshes (Bertness 1991) or desiccation tolerance of *Fucus spp.* in the rocky intertidal (Lubchenco 1980). However, in areas of low stress, the stronger competitors are the dominant species such as *Spartina patens* in salt marshes (Bertness 1991) or *Chondrus crispus* in rocky intertidal environments (Lubchenco 1980). Primary producers compete for light (Pasternak et al. 2009; Hautier et al. 2009), nutrients (Fong and Zedler 1993, Fong et al. 1996; Bintz 2003) and space (Bertness 1991; Lubchenco 1980). Survival is based on an energetic balance. Competitors typically devote most of their energy to rapid growth and/or competitively exclude other species in resource rich habitats. Stress-tolerant species devote more of their energy to adaptations that allow them to survive in extreme conditions. However, the impacts of stressors can be mitigated by facilitation (Bruno et al. 2003).

Non-trophic facilitation, or positive species interaction, have been shown to significantly alter competitive interactions and increase tolerance to environmental stress (Bruno et al. 2003; Altieri et al. 2007; Altieri et al. 2010). Most studied facilitative interactions occur between organisms at the same scale (i.e., micro, meso, or macro). Facilitation between organisms of different size classes has been studied in a few systems such as coral and their symbiotic algae (Bo et al. 2011), mycorrhizal fungi and plants (Bever 2002; Daleo et al. 2007), and chemoautrophic bacteria and hydrothermal vent organisms (Cavanaugh et al. 1981). Often, these interactions are mutualistic, in which both organisms benefit, rather than one organisms benefitting and the other being unaffected. In addition, the coupled organisms in these cases have evolved to rely heavily on each other for survival. We wanted to determine if adaptation to biological stressors (competition and facilitation) would significantly alter abundance of two non-mutualistic algal types in a high thermal stress environment without the presence of predators.

Temperature affects organisms differently based on physiological factors such as size, metabolic rate, and desiccation tolerance (McGlathery et al. 2004; Baluch et al. 2005; Dell et al. 2013). Temperature has also been shown to differentially affect components of pelagic estuarine food webs (O'Connor et al. 2009, O'Connor 2009). A few studies have assessed the effects of temperature on near-shore macroalgal communities (Bintz et al. 2003; Fong and Zedler 1993; Piñón-Gimate et al. 2008). These studies have focused primarily on competition between macroalgae and phytoplankton or different species of macroalgae. However, comparisons between macroalgae and MPB competition under thermal stress are lacking. Comparing nitrogen assimilation data from McGlathery et al. 2004 and climate data from NOAA, it appears that differences in nitrogen assimilation rates between macroalgae and microphytobenthos

(MPB) vary with temperature (Figure 5.1). These data show that MPB have higher rates of nitrogen assimilation than macroalgae at lower temperatures, but this relationship is inverted as temperature rises. Since nitrogen assimilation can be a proxy for growth (Allen and Arnon 1955; Pregitzer et al. 1998), these results suggest a shift in algal growth with thermal stress and support the possibility of shifts in algal dominance as global temperatures rise. However, algae can be affected by the presence and activities of other algae (Fong et al. 1993, Fong and Zedler 1993). Algae compete not only for nutrients, but also for light. Because increased temperatures support growth of larger algae, macroalgae may be able to outcompete MPB by decreasing available light through shading (Gillooly et al. 2001; Smith and Horne 1988; Hauxwell et al. 2003). We investigated the interactions between macroalgae and MPB as thermal stress increases. We hypothesized that: 1) macroalgae will respond more favorably to increased temperature than MPB; 2) When grown together, macroalgae will outcompete MPB through shading and thermal tolerance at all temperature treatments.

5.2 Methods

5.2.1 Sample collection

We collected all samples from a Bogue Sound beach in Morehead City, NC (34°43'20.64"N, 76°45'7.94"W). *Ulva lactuca* was selected as a representative macroalgae species. Macroalgae were rinsed in filtered seawater and amphipods were removed with tweezers. MPB is ubiquitous in marsh sediments exposed to sunlight (Sullivan and Currin 2000). Therefore, sediment samples were collected to obtain MPB communities. Sediment samples were collected by core at low tide to reduce disturbance to the sediment surface.

Sediment cores were 10 cm in diameter and 5 cm deep for a total sediment volume of 0.393 L. Cores were taken adjacent to collected macroalgae mats.

5.2.2 Mesocosm preparation

Mesocosms were established in the following combinations: MPB, macroalgae, and macroalgae+MPB (Figure 5.2). Macroalgae mesocosms contained 6 grams of *U. lactuca*. "MPB" mesocosms were filled with one sediment core. Macroalgae+MPB was the combination of both treatments. Mesocosms were 5L plastic tubs with approximately the same surface area of the sediment core to reduce disturbance to the sediment surface. Mesocosms were filled with 2L of unfiltered seawater from Bogue Sound, NC and kept as semi-closed systems i.e., they were open to the environment, but were not in flow through chambers to reduce loss of algal biomass. Evaporative loss was compensated with DI water additions to maintain salinity. Mesocosms were checked daily for visible herbivore presence. Seawater only controls containing 2 L of unfiltered seawater were incubated simultaneously with algal mesocosms to address changes in water column chlorophyll-*a* (chl-a) concentrations. Mesocosms (60 total) were incubated for two weeks during the fall of 2011. Incubations of 2 weeks were ideal. Longer incubations were subject to biofouling. Shorter incubations showed no measurable change.

Mesocosms were incubated outside in water baths to stabilize temperatures. Temperature increases were achieved using a network of heaters (Baluch et al. 2003) and monitored using HOBO temperature loggers. Average ambient temperature throughout the experiment was 18°C. The two additional temperature treatments were ambient +2°C (actual +2.60 ±0.02) and ambient +4°C (actual +4.20 ±0.03).

5.2.3 Assessing Algal Biomass

Macroalgal wet weights were measured before and after the experiment to determine growth rates. Macroalgae was spun in a salad spinner to reduce excess water following methods from O'Connor, 2009, and then weighed. Benthic chl-a was used as a proxy for MPB biomass. Surface benthic chl-a samples were collected using 3mL syringes (surface area: 0.50 cm^2) to a depth of 5 mm. Chl-a was then extraced from sediments for approximately 24 hours in a solvent mixture of 45:45:10 % methanol: acetone: water, sonicated, and analyzed using a Shimadzu spectrophotometer (Welschmeyer et al. 1991; Pinckney and Zingmark 1993). Samples were acidified to account for phaeophytin concentrations. Water column chl-a concentrations were also measured as a proxy for phytoplankton abundance. Since we were using unfiltered sea water, phytoplankton were present in the samples and could possibly affect macroalgae and MPB growth. Therefore, we wanted to account for any changes in water column chl-a. Water was filtered through GF/F filters (Whatmann, 47mm) and chl-a was extracted from filters for approximately 18 hours in 90:10 % acetone:water, sonicated, and analyzed with a Turner Designs Trilogy Fluorometer (non acidification module). Sediment organic matter (SOM) was collected initially from the surface sediment at the field collection site and from each mesocosm at the end of the experiment using sediment corers (1.5 cm depth, v=3.7 cm^3) to determine if changes in organic matter would affect algal biomass. In addition, SOM was used to parametrize varibility in sediment characterisitcs between replicates within a treatment. The method for analysis of SOM was loss-on-ignition (LOI) adapted from Ball (1964).

5.2.4 Photosynthetic Parameters

Algal productivity at varying light intensities was measured by photosynthetron incubation using a ^{14}C tracer. Incubations were conducted at average mesocosm temperatures

(18°C, 22°C, and 26°C). macroalgae photosynthetron samples were prepared by collecting algae from mesocosms, gently spinning in a salad spinner to reduce excess water (O'Connor 2009), and packing the algae into a small column (0.5 ml syringe with the tip removed). Macroalgae were dispensed in small aliquots (0.03 cm^3), cut from the column with a razor blade and dropped into scintillation vials. MPB photosynthetron samples were prepared by collecting surface sediment (1mm) from MPB-only mesocosms and homogenizing the samples by mixing the sediment. Two 0.1 cm^3 aliquots of sediment were dispensed into each scintillation vial. Scintillation vials were filled with seawater spiked with radiolabelled (^{14}C) sodium bicarbonate. Samples were incubated in the photosynthetron for 30 minutes. The activity was terminated with formalin and acidified with 1 mL of 10% HCl. Light intensity in the photosynthetron was measured using a 4π scalar irradiance meter (Biospherical Instruments, LLC QSL2101). Activity of ^{14}C was measured in a scintillation counter used to calculate photosynthesis based on equation 1:

$$P = \frac{D_S * 1.05 * DIC}{D_t * t} \quad (5.1)$$

where P is the photosynthetic rate (mgC m^{-3} hr^{-1}), D_s is the activity of the sample in Bq, 1.05 is the preferential uptake of ^{12}C over ^{14}C, DIC is the dissolved inorganic carbon content of the seawater, DPM_t is the activity of the radiolabelled seawater added to the sample in Bq, and t is the time of incubation in hours (Johnson and Sheldon, 2007). A representative DIC constant was set as 22,420.31 mgC m^{-3}. P-E curves generated from photosynthetron data were used to determine maximum chl-a peak photosynthetic rate at saturation (P_{max}), maximum photosynthetic efficiency (α), and the transition light intensity between light dependent and light saturated photosynthesis (I_k; Lewis and Smith, 1983). Initial slopes and light saturated photosynthetic rates were determined using a quadratic P-E model (Johnson and Barber 2003)

and optimized using the Microsoft Excel Solver function (Lobo et al, 2013). The initial slope of the P-E curve, or where P still increases as a function of I, is the α value. P_{max}, was determined by averaging photosynthetic rates measured under light saturated conditions (i.e. all points not included in the calculation of α). The light intensity at which the light dependent photosynthesis shifts to light saturated photosynthesis (I_k) was determined by the intersection of P_{max} and α.

5.2.5 Statistical Analyses

Our experiment has a factorial design with two factors (Underwood, 1997), which are temperature of incubation and presence of competing algae. A two-way multivariate analysis of variance (MANOVA) was used to analyze the effect of each factor on algal growth rate. Normality and homogeneity of variance was tested with the Shapiro-Wilk test and Levene's F-test respectively. Percent SOM data were transformed using a basic arcsin square root transformation. Both SOM and water column chl-a did not meet the assumptions of an ANOVA and were analyzed using a Kruskal-Wallis non-parametric test (Kruskal and Wallis 1952). All statistical analyses were conducted in R (Version 2.8.2008-12-19). All errors reported are standard errors.

5.3 Results

5.3.1 Macroalgae Growth

Growth rates, calculated from macroalgae wet weight, were highly variable ranging from 0 to 247 mg/day for macroalgae+MPB treatments and 0 to 293 mg/day for macroalgae only treatments (Figure 5.3a). Macroalgae growth was higher at +2°C without the presence of MPB ($F=11.52$, $df=1$, $p<0.01$). However, this was the only significant difference. Temperature did not significantly affect growth rate within algal treatment. Overall trends in growth (although

not significant due to high error) showed that macroalgae growth peaked at +2°C and decreased at both higher and lower temperature treatments. However, macroalgae growth rates in macroalgae + MPB mesocosms appeared to remain the same at ambient and +2°C conditions with a slight (although statistically insignificant) increase in growth rate at +4°C.

5.3.2 Microphytobenthic Algae Biomass

MPB biomass, as assessed through benthic chl-a concentrations was less than or equal to initial levels and ranged from 51.5-108.9 mg/m^2 in macroalgae+MPB mesocosms and 53.1-115.8 mg/m^2 in MPB only mesocosms. Therefore, the percent change in benthic chl-a concentrations was negative for all treatments (Figure 5.3b). Sediment chl-a concentrations were affected differently by presence of macroalgae and temperature. MPB biomass in macroalgae+MPB mesocosms was not impacted by temperature ($F=5.72$, $df=2$, $p=0.21$). In MPB mesocosms, benthic chl-a concentrations were significantly affected by temperature ($F=1.71$, $df=2$, $p=0.01$) with the lowest decline of chl-a found at ambient temperature with a greater decline at elevated temperature treatments. In addition, at ambient temperatures, benthic chl-a loss was significantly lower for MPB than macroalgae+MPB mesocosms.

5.3.3 Phytoplankton Abundance

Chl-a concentrations in the water column were measured as a proxy for phytoplankton abundance. Measured values ranged from 0.06-0.56 µg/L for macroalgae+MPB, 0.02-0.69 µg/L for macroalgae, and 0.01-0.72 µg/L for MPB (Figure 5.4). Phytoplankton abundance was not significantly related to temperature or algae presence (Kruskal-Wallis $X^2=2.15$, $df=2$, $p=0.34$). However, macroalgae+MPB and macroalgae mesocosms tended to have lower levels of water column chl-a than mesocosms without macroalgae.

5.3.4 Sediment Organic Matter

Overall SOM content was low with values ranging from 0.24%-0.59% in macroalgae+MPB samples and 0.42%-1.10% in MPB mesocosms (Figure 5.5). SOM content was not significantly related to algae presence (Kruskal-Wallis X^2=3.04, df=1, p=0.08) or temperature of incubation (Kruskal-Wallis X^2=4.35, df=2, p=0.11). However, SOM% was generally higher for MPB only treatments and appeared to increase with temperature for both macroalgae+MPB and MPB mesocosms.

5.3.5 Photosynthesis

The photosynthetic efficiency, or α, tended to decrease with temperature for MPB (Table 5.1) but increased with temperature for macroalgae (Table 5.2) treatments. Photosynthetic efficiency for macroalgae ranged from 0.1-0.5 mgC (mg chl-a)$^{-1}$ h^{-1} and 29-21 mgC (mg chl-a)$^{-1}$ hr^{-1} for MPB samples. Both algal treatments had comparable I_k values. I_k was inversely related with temperature for macroalgae, but greatest for +2 treatments in MPB incubations. Maximum photosynthetic rate increased with increasing temperature for both macroalgae (12-40 mgC gdw^{-1} hr^{-1} and MPB (6-10 mgC (mg chl-a)$^{-1}$ hr^{-1}).

5.4 Discussion

Our results suggest that macroalgae and MPB competitively interact, decreasing the survival of both, at moderate temperature increases, but macroalgae appears to facilitate growth at high levels of thermal stress (+4°). While macroalgae growth in macroalgae+MPB mesocosms showed no relationship with temperature, growth in macroalgae grown alone was significantly higher in +2°C treatment. This suggests that macroalgae growth may be arrested by competition with MPB at +2°C. Macroalgae and MPB, in our simplified systems, primarily compete for light

and nutrients. Macroalgal mats floated above MPB and could outcompete MPB for light. While personal observation and measures of total irradiance indicated the light levels were lower beneath macroalgal canopies, further studies on shading are necessary. Additionally, the algae were likely competing for nutrients. NO_x^- (nitrate + nitrite) data from intertidal marshes in Bogue Sound trends positively with benthic chl-a (indicator of MPB) and negatively with macroalgae percent cover (Figure 5.6; O'Meara et al. *in prep*). One explanation is organism size, which can play a significant role in nutrient uptake. According to Nielsen and Sand-Jensen 1990, maximum growth rate of primary producers is directly related to the surface area/volume ratio (SA:V). As SA:V increases, so does efficiency in nutrient uptake based on diffusion. According to Hein et al. 1995, microalgae typically have an SA:V on the scale of $\sim 10^4$ and macroalgae have an SA:V of $\sim 10^2$. Since smaller organisms have a higher SA:V than larger organisms, MPB are expected to more efficiently incorporate nutrients than macroalgae. Therefore, MPB may hinder macroalgae growth via nutrient limitation. The addition of sediments (for MPB) may also be a source of nutrients, which could promote MA growth. However, since we observed the opposite in our samples, this supports the idea of competition between the two algal types. According to Largo et al. 2004, *Ulva lactuca* growth rates are optimized between 20-22°C and decrease drastically with increasing temperature when grown in a controlled laboratory setting. Because average ambient temperature was 18°C, our higher temperature treatments should have optimized macroalgae growth. However, a decline in growth at the +4°C incubation was observed. Under our experimental conditions, optimum growth temperature in our mesocosms may be lower or, at higher temperatures, macroalgae was able to reach is carrying capacity. Increased algal biomass could cause a decrease in available oxygen, which would be exacerbated

at higher temperatures based on oxygen solubility. This oxygen limitation could explain the decline in macroalgal biomass at higher temperature treatments.

MPB biomass declined during the incubation (relative to initial levels) for all treatments. MPB survival was greatest at ambient conditions, but macroalgae growth peak at +2°C. This indicates that MPB are not as heat tolerant as macroalgae. Presence of macroalgae may decrease light intensity at the sediment surface through shading, which could benefit MPB by preventing photoinhibition. According to studies by Morelissen and Harley 2007, Thompson et al. 2004, and Underwood 2002, MPB can be significantly hindered by exposure to high irradiances. Photoinhibition can be exacerbated at higher temperatures (Havaux 1994; Falk et al. 2006). Therefore, MPB may benefit from shading as temperature increases. In this view, growth of macroalgae and MPB may reflect competition and facilitation working in unison. At lower temperatures, MPB and macroalgae moderate each other's growth though competition for nutrients and light. As temperatures rise, macroalgae facilitates MPB survival by reducing insolation stress through shading. In addition, the slight increase in macroalgae and MPB growth in macroalgae+MPB mesocosms may indicate mutualism at higher temperatures (+4°) temperatures. According to Bruno et al. 2003, facilitation can increase survival at high levels of environmental stress. The evidence of increased survival (MPB) and increased growth rate (macroalgae) when present together at high temperatures supports a hypothesis of mutual facilitation of both algal types. However, the variability is too high to demonstrate this with certainty.

Measuring photosynthetic parameters provided information on the rates of carbon uptake and response to light. This information provides a common basis for comparison between the two algae types, which is particularly important for MPB since growth could not be measured

directly. Our values of α, I_k, and P_{max} were similar to previously reported values for both MPB (Reynolds 2006) and macroalgae (Coutinho and Zingmark 1987). Photosynthetic efficiency (α) correlated positively with temperature only for macroalgal incubations. MPB α values were highest at ambient conditions and lowest at +2°C. Maximum photosynthetic rate (P_{max}) increased with temperature for both macroalgae and MPB. Both photosynthetic efficiency and P_{max} are expected to increase with temperature because metabolic rate increases with temperature (Gillooly et al. 2001). Photosynthesis relies on two types of reactions: photochemical reactions (temperature independent) and biochemical reactions (temperature dependent; Huner et al. 2008). Therefore, as temperature increases, and metabolic rate rises, the overall process of photosynthesis is enhanced in terms of α and P_{max} for all photosynthesizers. Since MPB are often shaded, they are more inclined to opportunistically respond light. On the sediment surface, light is not always available. Therefore, when MPB do receive light, they need to respond rapidly to utilize the resource. These results agreed with Barranguet et al. 1998 and MacIntyre and Cullen 1996 who showed that temperature significantly impacted α values. P_{max}, overall, were typically higher for macroalgae than MPB. According to Nielsen et al. 1996, the thickness of photosynthetic tissue plays an important role in maximum growth rate. Current findings support this assertion since MPB have thinner tissues than macroalgae, MPB have higher maximum growth rates. Therefore, our findings agree with previous literature.

Saturation irradiance, I_k peaked at +2°C for MPB, but increased with temperature for macroalgae. Because I_k is calculated based on P_{max} and α, the relationship between these variables affects I_k. If change in P_{max} is small, I_k and α are inversely related. If α is relatively constant, I_k is directly proportional to P_{max}. If P_{max} and α proportionally increase, then I_k will remain the same. For both macroalgae and MPB, it appears that I_k is more closely associated

with α. These results indicate that MPB photosynthetic response is greatest under ambient conditions and decreases at both temperature additions. On the other hand, macroalgae photosynthetic response increases with temperature. Therefore, macroalgae appear to benefit from increasing temperature, but MPB photosynthetic performance appears to decrease at increasing temperature treatments. As sea level rises and average temperature increases, these results indicate that benthic macroalgae may be less tolerant to loss of light (due to increased depth and light attenuation), but more tolerant to temperature increases than MPB.

Water column chl-a measurements for our mesocosms were lower than expected for Bogue Sound, NC. According to data collected from the IMS pier biweekly (unpublished data) from 2008-2014, average water column chl-a is 3.8±0.13 μg/L. This could indicate that nutrient concentrations were significantly reduced by macroalgae and MPB growth. Since phytoplankton can quickly bloom when nutrients are pulsed though estuarine waters (Rudek et al. 1991; Hecky and Kilham 1988), we would expect an increase in chl-a concentrations if nutrients were abundant. However, chl-a concentrations were not significantly different from seawater controls, which indicate that nutrients within mescosms were sufficient for algal growth. Within each treatment, variability in SOM was low (~7.7% average error), which indicates that the sediments were relatively homogenous in substrate content. SOM content can be an indicator of MPB abundance. According to Fabiano and Danovaro 2004, MPB biomass can account for 18.1% of total SOM%. While we cannot assume that MPB biomass is solely responsible for the rise in SOM, there is evidence to suggest it could contribute to SOM%.

While this small-scale study does have big picture implications, no study is without limitations. Our mesocosm incubations were limited to a single macroalga, in a single season. Since algal diversity is based on season and growth rates are not consistent between species,

seasonal variations in algae would significantly alter the results of this study. In addition, macrograzers were removed, but zooplankton, viruses, and heterotrophic bacteria were not. In our mesocosms, these micrograzers can have a significant impact, especially considering organisms with an exponential growth curve. Temperature increases strengthen the interaction between herbivores and plants (O'Connor 2009; O'Connor et al. 2009). As temperature increases, we would expect consumption of algae to also increase. Zooplankton impacts were accounted for in our seawater controls, but viruses and heterotrophic bacteria were not quantified based on methodological constraints. Bacterial production increases with temperature and algal biomass (White et al. 1991; Fuhrman et al. 1985) and viruses can significantly impact algal diversity (Baudoux and Brussard 2005). Future experiments should include quantifying micrograzer consumption rates, characterizing micrograzer diversity, sampling growth several times during the incubation, and measuring growth across seasons.

5.5 Conclusions

Temperature significantly affected interactions between the macroalga, *Ulva lactuca*, and MPB. Macroalgae was shown to be more tolerant of increased temperature than MPB, which may be attributed to photoinhibition. Since water attenuates light, if temperature and sea level rise together, the mitigation of insolation stress through light attenuation may help maintain MPB concentrations. Under current light regimes, macroalgae appears to decrease insolation stress and facilitate MPB growth. In addition, since macroalgae abundance peaked at +2°C, it is possible that macroalgae growth will increase as temperature increases. Further studies are necessary to determine the impacts of higher trophic levels on algal abundances, but currently our study suggests that a balance between competition and facilitation may control MPB and

macroalgae abundance under the following scenarios (without predators). At ambient temperatures, macroalgae can outcompete MPB for light and/or nutrients. At moderate temperature increases, MPB regulates macroalgae growth through competition for nutrients. Finally, at higher temperatures macroalgae facilitates growth of MPB through mitigation of photoinhibition by shading. In the Sanders stability-time hypothesis (Sanders 1968), organisms typically adapt to the stressor. In these physically controlled environments, biological interactions are likely to be of lower intensity (Menge and Sutherland 1976). However, we found that biological interactions between macroalgae and MPB were the primary controlling factors for growth. Both competition and facilitation were important for the survival of both algal types. According to Menge and Sutherland 1976, competition reduces diversity through competitive exclusion in structurally simple environments. In our simple, single trophic level experiment, competition did not reduce diversity, but instead facilitated the growth of algae under high thermal stress. We did find that facilitation can significantly increase tolerance to environmental stress as described in Bruno et al. 2003. These basic ecological principles are based on predator/prey interactions and are related to zonation, which were absent from our mesocosm design. However, even in this highly simplified and small-scale system, we observed both competition and facilitation.

TABLE 5.1: MPB photosynthetron data. The units for α are mgC (mgChl-*a*)$^{-1}$ (mol photons m^{-2}). I_k is expressed in μmol photons m^{-2} s^{-1} and P_{max} is shown in mgC (mgChl-*a*)$^{-1}$ h^{-1}

Treatment	α	I_k	P_{max}
+0	29	53	6
+2	19	172	7
+4	21	135	10

TABLE 5.2: MA photosynthetron data. The units for α are mgC m^{-3} hr^{-1} (μmol m^{-2} s^{-1})$^{-1}$. I_k is expressed in μmol m^{-2} s^{-1} and P_{max} is shown in mgC gdw^{-1} hr^{-1}

Treatment	α	I_k	P_{max}
+0	0.1	159	12
+2	0.3	143	41
+4	0.5	88	40

FIGURE 5.1: Difference in rate of nitrogen assimilation (NA, primary axis) between macroalgae (MA) and microphytobenthos (MPB) plotted against season. Average temperature shown on secondary axis. Note that the difference in NA is determined by subtracting MPB NA from MA NA. Negative NA values show that MPB NA are higher than MA NA. Adapted from McGlathery, 2004 and climate data from NOAA.

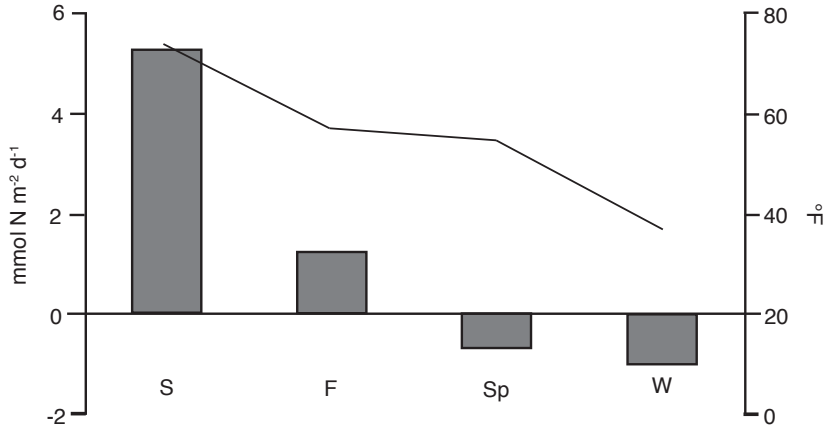


FIGURE 5.2: Experimental Design. Circles represent mesocosms. Nutrient and algae treatments were randomly assigned to mesocosms within each temperature treatment (+0C, +4C, +8C). MPB = microphytobenthos and MA = macroalgae

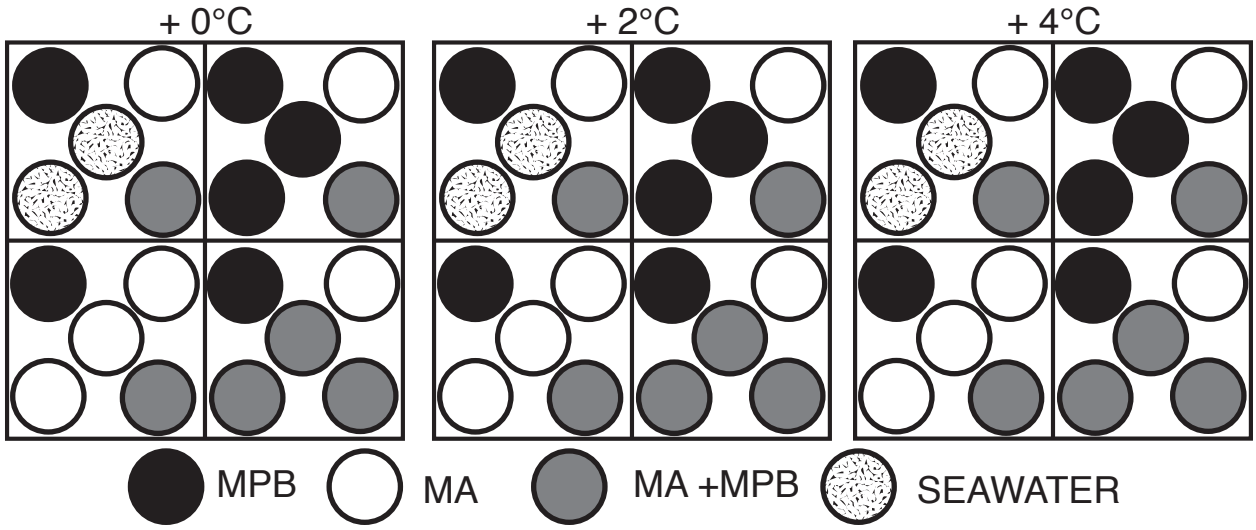


FIGURE 5.3: Algal growth. a.) represents MA wet weights for MA and MA+MPB mesocosms and b.) represents change in benthic chlorophyll-a concentrations for MPB and MA+MPB mesocosms as a proxy for MPB growth.

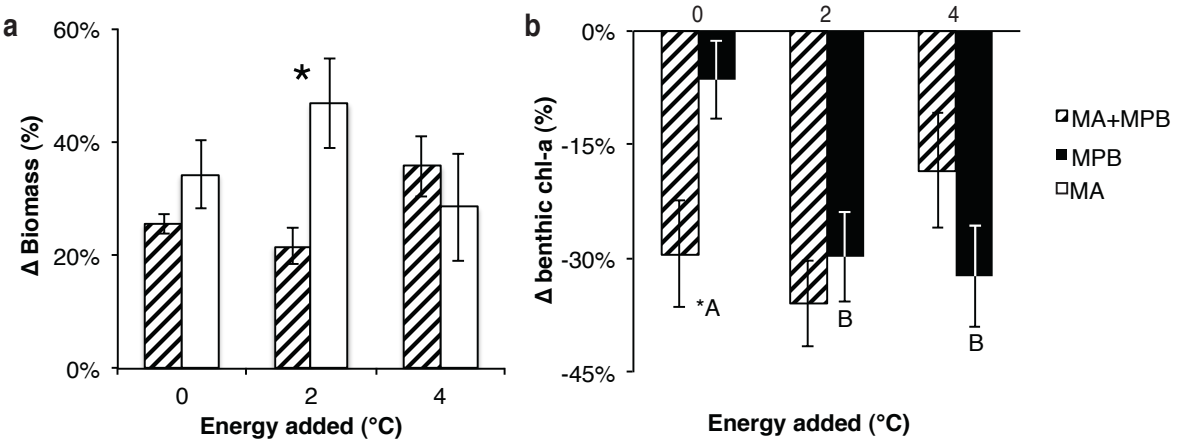


FIGURE 5.4: Water column chlorophyll-a concentrations for each algal treatment. Temperature data was pooled for each mesocosm type due to lack of temperature trend.

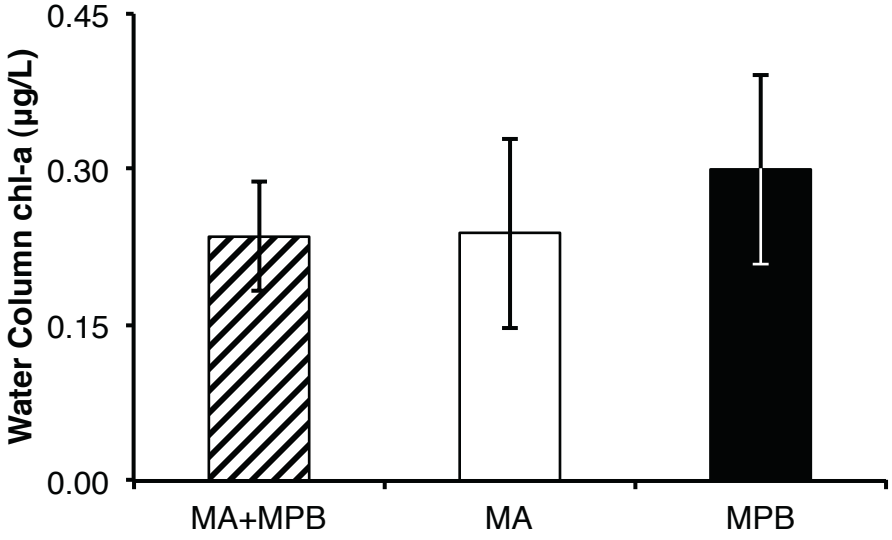


FIGURE 5.5: Sediment organic matter content for MA+ MPB and MPB samples. Note the general increase in MPB SOM which is not present for MA+MPB samples.

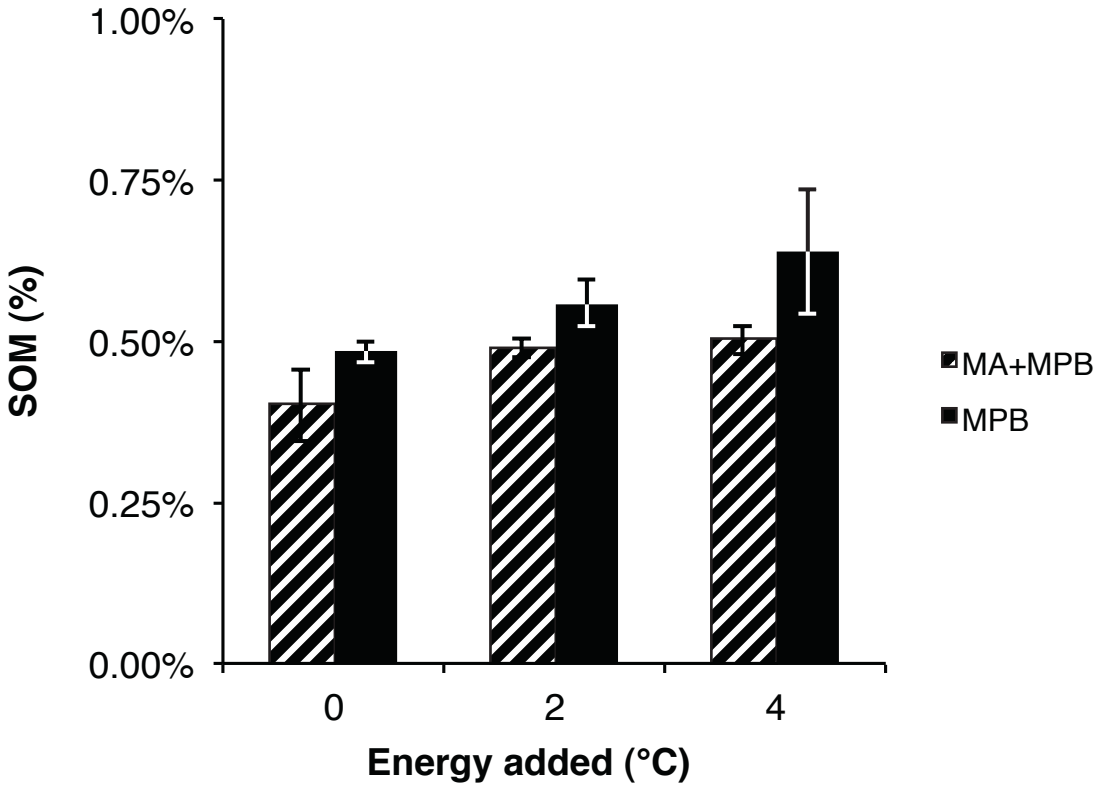
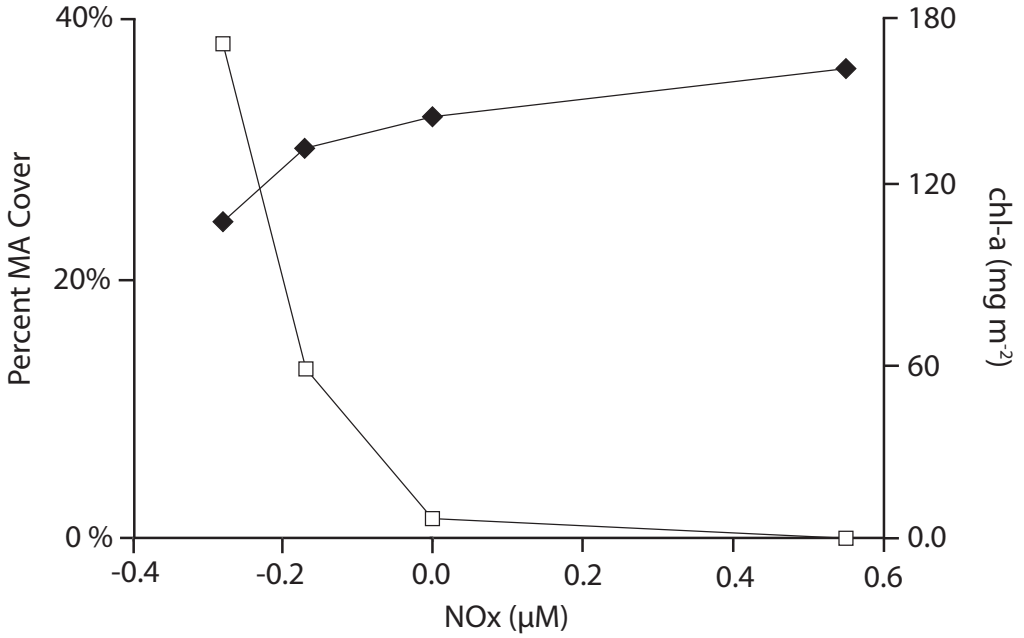


FIGURE 5.6: Trends in algal abundance/concentration with NO_x concentration in Bogue Sound, NC. Benthic chlorophyll-a concentrations (◆; proxy for MPB) and MA percent cover (□) have opposing trends with NO_x^- concentrations.



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CHAPTER 6
Conclusions

6.1 Nutrient processing in estuarine marshes

Many human activities result in ecological disturbance. Anthropogenic environmental change such as sea level rise, global warming, development, noise pollution, light pollution, and nutrient loading have all significantly affected coastal systems and caused potentially irreversible alterations to estuarine marshes (Strahlberg et al. 2011; Reed 1995; Roessig et al. 2005; Long et al. 2011; Banner and Hyatt 1973; Dwyer et al. 2013; Vitousek 1994). The loss of a single estuarine ecosystem service could significantly impact our way of life. For instance, if sea-level rise caused the loss of estuarine marshes through drowning, estuarine marsh habitat would be transformed to shallow subtidal habitat and the decrease in N removal through DEN would be on the order of Gg in the US and Tg globally (Table 6.1).

Although Kitty Hawk Bay exhibited an increase in N removal by DEN as a result of conversion of the HM and LM to the ST, this rate measured with MIMS, did not assess N_2O production. F-DEN rates typically increase with decreasing salinity, which may account for the significantly lower rates of DEN in Kitty Hawk Bay when compared to Ships Bay, which has similar salinity. Overall, the loss of estuarine marshes would severely decrease nitrogen removal via denitrification and resilience of estuarine ecosystems to anthropogenic eutrophication. Based on rates measured along the NC coast, the conversion of HM and LM to ST would result in an estimated loss of DEN processing ranging from 43-88% (excluding Kitty Hawk Bay, 39%

increase). While these results may seem extreme, rates measured for this dissertation are comparable to rates determined in other marshes worldwide (Table 2.3) and at least an order of magnitude smaller than DEN estimates measured by Tobias et al. 2001 (43.2-422 mmol N m^{-2} d^{-1}) and Dollhopf et al 2005 (4.7-79.9 mmol N m^{-2} d^{-1}). The overall reduction in DEN as a direct result of marsh loss would decrease the resilience of coastal wetlands to eutrophication and increase the export of nutrients from these habitats. According to estimates of the value of N removal, US and global losses range from $209-$604 million/yr and $18.1-52.3 billion/yr respectively (Piehler and Smyth 2011; Newell et al. 2002; Beseres Pollack et al. 2013). Additionally, when DEN is lost, so are other ecosystem services. Costanza et al 1997, estimates the value of ecosystems services provided by tidal marshes as $9,990 ha^{-1} yr^{-1}. Therefore, the value of US marshes alone is $16.1 billion/yr and worldwide, $1.4 trillion/yr. While there are issues with placing value on ecosystem services and debate over the reported values in Costanza et al. 1997 in particular (Gatto and De Leo 2000), these monetary values can be a useful metric particularly for policy makers and the general public. Without fully understanding the full range of ecosystem services provided by habitats there is no incentive to protect them and instead a tendency to exploit them.

As DEN rates decrease, the ratio of N_2 to N_2O produced may change. Sea level rise in marshes is associated with an increase in salinity (salt water intrusion; Taylor et al. 1989). Therefore, nearly fresh and brackish marshes may begin to resemble fully saline habitats (RCR). Although fungi were less important in the upland RCR habitats, they were the dominant microbes for DEN in the anoxic ST. This indicates that F-DEN may be a more significant contributor as sea level rises. In addition, nutrient loading to the estuary would potentially increase N_2O production by decreasing the efficiency of B-DEN. Therefore, as the estuarine

marshes are lost, DEN rates are predicted to decrease overall, but the proportion of N_2O produced would likely increase.

6.2 Primary production in estuarine marshes

While the scenario of drowning marshes seem extreme, significant marsh loss caused by sea level rise has been well documented (Kirwan et al. 2007; Wilson et al. 2007; Kearney et al. 2002; Day et al. 1995; Stevenson et al. 1988; Hackney and Cleary 1987) and we have already observed shifts in growing season, geographic location, and elevation of vegetation associated with changing climatic conditions. Along with the loss of nutrient processing, shifts in dominant vegetation will also occur. According to Kunkel 2013, the growing season (frost-free season) in the lower 48 states has increased by approximately 15 days from 1895 to 2012. Papers by Kelly and Goulden 2008, Crimmins et al. 2011, and Pucko et al. 2011 have reported shifts in plant elevation based on changing climate and water conditions. In marine systems, Harley et al. 2006 determined that climate change will alter ocean chemistry and circulation, including nutrient fate and transport. Under the "fully drowned" scenario, marsh grass dominated systems would be converted to algae dominated habitat. Currently, MPB and MA biomass is maintained in estuaries by competition for light, nutrients, substrate and predation. Each of these factors differentially affects algal abundance. O'Connor et al. 2009 showed that increased temperatures strengthen producer-herbivore interactions. Bruno et al. 2003 indicated that environmental stress can be reduced by interspecies facilitation. Experimental results presented in this dissertation have shown that changes in global temperature can impact distributions of MA and MPB (Figure 5.3) through competition and facilitation. High insolation and temperature stress environments favored MA growth over MPB. However, since MA presence facilitates MPB growth at higher

temperatures, we may not observe these changes unless temperature changes follow IPCC high emissions estimates (3-4°C). Therefore, algal abundance and growth is highly dependent on the interaction of several variables, which may range significantly between sites. In Bogue Sound, we determined that in the absence of predators, at moderately increased temperatures, MA and MPB competed for light and nutrients. At higher temperatures, MA was resistant to thermal stress while MPB had a lower tolerance threshold. However, at these elevated temperatures MPB survival was increased in the presence of MA due to the mitigation of insolation stress (facilitation). In the future, as temperatures rise, sea level rise and MA cover may mitigate some insolation stress to help to maintain MPB populations. The loss of MPB would be significant because it is a high quality food source. In the case of incomplete marsh drowning, we predict landward migration of algae. The edge effect created by algal wrack collected at the marsh toe would move higher in the marsh, particular where hardened shorelines are present prohibiting marsh migration. Wave energy and higher tidal reach would push algae further inland smothering vegetation and reducing marsh grass abundance. In addition, nutrient loading and increasing temperatures would potentially increase algal biomass and exacerbate stress on the marsh caused by algae. Therefore, if impacts are severe, we could see a shift from marsh vegetation to algae dominated habitats even if the marsh is not fully inundated.

6.3 Recouping Our Losses

Alterations to the environment are inevitable. Even if we stop all greenhouse gas emissions tomorrow, global temperatures will continue to rise (IPCC 2007). The effects of marsh loss would not be isolated to the coast. Nutrient processing, shoreline protection, and habitat for commercially important fish are all ecosystem services provided by the marsh that

would have far reaching inland effects. While we cannot fully predict the reaction of estuarine habitats to anthropogenic change, understanding potential outcomes can help us prepare for the future with mitigation strategies/restoration plans. One possible way to reduce our impact on the coastal environment includes shifting from bulkheads to living shorelines for stabilization. Living shorelines such as sills, constructed oyster reefs, and marsh planting stabilize shorelines by attenuating wave energy, thereby reducing erosion of upland sediment. While currently more expensive to install and difficult to permit, living shorelines, when installed correctly, receive the best of the both worlds. Marsh sills can maintain habitat function and size, but shorelines and developed property are also protected (Currin et al. 2010). Living shorelines have also been shown to be more effective at protecting shoreline than vertical hardening structures in extreme conditions, such as Hurricane Irene (Gittman et al. *in review*).

There is no "silver bullet" cure-all solution for habitat restoration, but with well-engineered solutions, we can balance our use of coastal habitats with the needs of the ecosystem. Understanding and defining a healthy ecosystem is important not only for comparison, but also as model and guide of a functional and natural solution. Some solutions developed with ecological engineering are riparian buffers, living shorelines, green roofs, and rain gardens. These structures all use natural structures to mitigate anthropogenic change. Research presented here can be used as baseline for comparison as well as a cautionary tale. If we continue to place our needs above that of the ecosystem, we will continue to see habitat degradation and loss. In the future, we need to move towards solutions that utilize healthy ecosystems as a model and natural structures to reduce human impacts on the environment.

TABLE 6.1 Change in N removal via DEN as a result of converting marsh habitat to subtidal zones. Negative values indicate loss and positive values indicate an increase in N removal.

Site	USA (Gg N/yr)	Global (Tg N/yr)
Kitty Hawk Bay (N)	16.4	1.42
Ships Bay (CBR)	-28.3	-2.5
Bogue Sound (C)	-47.8	-4.1
Carrot Island (RCR)	-20.1	-1.7
Wilmington (S)	-45.8	-4.0
AVERAGE	**-25.1 (35.5)**	**-2.2 (3.1)**

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