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East Coast Salt Marsh Response To Sea Level Rise: Microbial Community Function And Structure

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EAST COAST SALT MARSH RESPONSE TO SEA LEVEL RISE: MICROBIAL COMMUNITY
FUNCTION AND STRUCTURE

BY

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THESIS

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ABSTRACT

EAST COAST SALT MARSH RESPONSE TO SEA LEVEL RISE: MICROBIAL COMMUNITY FUNCTION AND STRUCTURE

by

Matt R. Simon

University of New England, December, 2013

Coastal salt marshes are under stress from anthropogenic climate change-induced sea level rise (SLR). Sediment microbial decomposition is a major driver of marsh subsidence and any impact of SLR on this biotic process would have a direct effect on marsh surface elevation relative to sea level. Furthermore, sensitivity to SLR of microbial community composition may play a role in the functional response. I collected sediment from six coastal marshes on the United States Atlantic East coast, exposed it to simulated sea level rise and measured total respired carbon over a three week period. My results indicated that SLR caused a decrease in microbial decomposition but that this functional response varied among sites and between elevations within sites (Chapter 1 of this thesis). Although differences in decomposition rates among sites were related to organic matter content, differential functional responses to sea-level rise among sites and elevations could not be explained by organic matter, nor a suite of environmental variables that have the potential to effect microbial activity (i.e., porewater pH, salinity and redox potential). In order to determine if changes in community composition might explain the functional response that I observed, I conducted a terminal-restriction fragment length polymorphism analysis of 16S rDNA extracted from sediment from the Massachusetts and New Hampshire sites (Chapter 2 of this thesis). I found that microbial community composition varied between the two sites. Furthermore, increased inundation caused a decrease in microbial community compositional shift that corresponded to a decline in decomposition rate. My results suggest that microbial functional response to SLR may be linked to changes in community composition.

CHAPTER 1

EFFECT OF SEA LEVEL RISE ON SEDIMENT MICROBIAL DECOMPOSITION IN SALT MARSHES ALONG THE UNITED STATES EAST COAST

Introduction

The viability of coastal salt marshes is being threatened by rising sea levels caused by global climate warming (Kearney et al. 1994; Reed 1995; Nicholls et al. 1999). The 2007 Intergovernmental Panel on Climate Change report predicted as much as a 40 centimeter increase in global sea levels over the next century. Rapid sea level rise (SLR) may outpace natural sediment accretion rates in salt marshes and render them unable to survive (Reed 1995).

Salt marshes maintain elevation through a balance of forces that build up (accretion) and draw down (subsidence) the surface of the marsh. Accretion is driven largely by: (1) deposition of inorganic and organic particles from water inflow (tidal or riverine) and (2) production of organic matter within the marsh itself (Redfield 1972). A highly productive marsh accretes sediment by producing a large amount of organic matter and by capturing sediment particles in its root systems (Redfield 1972; Miller et al. 2001). Accretion is balanced by subsidence owing to sediment erosion and organic matter decomposition, the latter increasing with marsh elevation due to the greater availability of oxygen. This ongoing accretion-subsidence cycle allows salt marshes to maintain surface elevation in the face of gradual changes in sea level (Kirwan & Megonigal 2013). However, at the current rate of SLR, marsh surfaces may not be able to

accrete rapidly enough to keep pace with sea level, leading to marsh submergence and vegetation die-off (Kirwan & Temmerman 2009; Kearney et al 1994; Reed 1995). However, these losses may be offset if microbial decomposition rates are slowed by increased inundation.

Thus, while both plants and microbes have important roles in the accretion-subsidence cycle in salt marshes, microbial sensitivity to hydrological alterations may be a crucial driver of marsh elevation maintenance. The direct effects of SLR on salt marsh sediment microbial decomposition are poorly understood. Nyman and DeLaune (1991) reported that wetter salt marsh sediments have slower rates of CO₂ emission, indicating a decrease in microbial respiration rates. Miller et al (2001) found increased tidal inundation actually resulted in a *decreased* input of organic carbon, particularly in wrack-affected areas. While this would obviously slow accretion, it could also potentially slow microbial decomposition rates by limiting available decomposable substrate. If the effect of increased flooding is to slow microbial decomposition then marsh elevation loss in the face of SLR would be slowed.

Although SLR is likely to decrease microbial decomposition rates (e.g., Nyman and DeLaune 1991; Miller et al. 2001), it is possible that sediment microbial communities will vary in their sensitivity to increased tidal inundation. Experiments studying the effects of increased inundation on marsh plant performance suggest that response to SLR varies depending on specific marsh features, such as the local plant community assemblage and marsh geomorphology (Sinicrope et al. 1990; Warren et al. 2002; Konisky & Burdick 2004). Likewise, any response that microbes show to SLR must necessarily occur within a specific environmental context, which may vary considerably among marshes. For instance, the organic matter content in East Coast salt marshes varies substantially, generally increasing from south to north (Gallagher & Plumley 1979), which could cause microbes to differ in their responses to SLR

because of differences in bioavailable food. A variety of water quality parameters, such as pH, salinity, and redox potential as well as other marsh physical characteristics like elevation and aboveground biomass, could also affect microbial decomposition (e.g., Hemminga & Buth 1991; Li 2010).

The goal of this study was to examine the response of microbial decomposition rate to simulated sea level rise in sediments originating from a variety of latitudes and tidal elevations along the East coast of the United States, while also accounting for variation in a number of other potential environmental influences. I hypothesize that exposure to SLR will reduce microbial decomposition rates but that this reduction will be more pronounced in sediments originating from higher in the tidal frame because those microbial communities are less adapted for dealing with inundation. I also expect that sediments with high organic matter content will support higher rates of microbial decomposition under conditions of SLR by providing a labile food source even during times of inundation stress.

Methods

Study Sites

I collected low marsh sediment from six coastal salt marshes on the United States East Coast during July and August of 2011 (Figure 1.1). The six marshes included (in order from south to north): Virginia Coast Reserve-Long Term Ecological Reserve (LTER) (Virginia, 37.394N, 75.870W); Chesapeake Bay National Estuarine Research Reserve (NERR) (Maryland, 38.223N, 75.761W); Delaware NERR (Delaware, 39.088N, 75.338W); Waquoit Bay NERR (Massachusetts, 41.557N, 70.504W); Great Bay NERR (New Hampshire, 43.137N, 70.888W); and Wells NERR (Maine, 43.339N, 70.543W).

Sediment cores (6 cm diameter X 10 cm depth) were collected at each site from two tidal elevations (which will be referred to hereafter as “low elevation” and “high elevation”) within the low marsh zone. Sixteen low elevation cores were taken within 10 m of an area of tall form *S. alterniflora*, which grows along tidal creeks and in other frequently flooded areas of the marsh. In addition, sixteen high elevation cores were taken from within 10 m of the transition zone, where *Spartina alterniflora* gives way to *Spartina patens*. Within each elevation, I collected the cores in a haphazard fashion, avoiding bare patches of marsh and panes, while maintaining a minimum of 2 m of spacing between all cores. Cores were stored on ice while being transported to a lab facility where they were processed – see *Sediment Microbial Decomposition Response to Sea Level Rise* section below.

I also measured four environmental variables across an elevational gradient at each site that could help in explaining any observed variation in decomposition rates between sites not directly related to hydrology. At each site I established 4 transects perpendicular to the seaside edge of the marsh, each 20-50 m long (depending on the width of the marsh) with 5 m spacing between transects which encompassed the core sediment collection area. Environmental variables were sampled every 5 meters along each transect, resulting in 16-40 sample points per marsh (once again, depending on the width of the marsh). At a depth of 4 cm below the marsh surface, porewater pH was measured using a handheld pH meter (Omega Engineering Inc., Stamford, CT), porewater salinity was measured using a handheld refractometer (LW Scientific, Lawrenceville, GA), and soil redox potential was measured using a handheld oxidation-reduction potential (ORP) meter (Oakton Instruments, Vernon Hills, IL). Finally, standing aboveground biomass was measured by clipping all live standing plant matter within 10cm x 10cm quadrats.

Sediment Microbial Decomposition Response to Sea Level Rise

Live roots and rhizomes were removed from sediment cores by hand after which I enclosed the sediment from each core in a 5 cm x 9 cm section of dialysis tubing (Sigma-Aldrich; St. Louis, MO). The purpose of these “microbial cages” (after Gasol et al. 2005) was to hold the microbes inside while allowing the free passage of water, solutes, and gases. In addition, approximately 5 grams of sediment was removed from each core and dried to a constant mass at 105° C to determine the approximate dry mass in each cage. After drying, subsamples were combusted in a muffle furnace at 550° C for four hours to determine the organic matter content of each core by way of the resulting mass loss.

To manipulate hydrology, I connected electronic timers to solenoid drain valves in circuit within a flow-through seawater system and set the timers to fill and drain plastic tanks at specific time intervals. Eight replicate microbial cages from each site x elevation combination were exposed to “normal hydrology” by flooding the cages for approximately 3.5 hours twice per day (duration of flooding was determined based on the average daily inundation time for the “high elevation” samples collected from the Wells NERR site over a 3 month period spanning the dates of the experiment). I simulated sea level rise (SLR) of forty centimeters (after the 2007 IPCC report) by flooding the cages for five hours twice per day (based on the estimated additional time of inundation at the Wells NERR site). Prior to exposure to hydrological treatments, all cages were acclimated for forty-eight hours, during which time the seawater tanks were flooded twice per day for four hours.

Starting on day zero (end of acclimation period), I measured carbon dioxide efflux (μm of $\text{CO}_2/\text{m}^2/\text{sec}$) from each cage once per week for three weeks. Carbon dioxide efflux was measured using a Li-Cor 6400 Infrared Gas Analyzer (Lincoln, NE).

Statistical Analysis

Total respired carbon (also referred to as decomposition hereafter) for each microbial cage was determined by plotting the individual carbon efflux data points for each cage versus time and calculating the area under the curve by summing the areas of the trapezoids generated by each pair of adjacent data points. I used a fully factorial analysis of variance (ANOVA) to model variation in total respired carbon as a function of site, elevation, and hydrology. In the event of significant interactions involving site, I further explored the effects of elevation and hydrology on total respired carbon by running site-specific ANOVAs with elevation and hydrology as fully-crossed factors. Statistical comparisons between hydrological treatments within elevations at each site were conducted using Tukey multiple comparison tests. As only comparisons between normal and SLR hydrologies within elevations were considered relevant to my analysis of the hydrological effects of SLR on microbial decomposition, I did not compare the effects of hydrological treatments across elevations.

I used analysis of covariance (ANCOVA) to model total respired carbon as a function of hydrology (normal vs. SLR) and organic matter content (as a continuously distributed covariate), where a significant hydrology X organic matter interaction would indicate a difference between the regression slopes of the two hydrological treatments. I used simple linear regressions drawn from this ANCOVA model to examine total respired carbon as a function of organic matter content for each site-hydrology treatment combination. In order to test for significant effects of

elevation on a variety of environmental variables that could potentially impact sediment decomposition rates I conducted a series of simple linear regressions within each site of pH, salinity, redox, and biomass on elevation. All statistical analyses were carried out using R statistical software (R 2.13.1, Vienna, Austria) and significance was determined based on an alpha of 0.05.



Figure 1.1. Map of six field collection sites. Sites are numbered north to south: 1 – Wells NERR, 2 – Great Bay NERR, 3 – Waquoit Bay NERR, 4 – Delaware NERR, 5 – Chesapeake Bay NERR, 6 – Virginia Coast Research LTER.

Results

I found that total respired carbon from salt marsh sediment microbial communities significantly differed among Atlantic Coast sites, between elevations, and in response to hydrological treatments (main effects, Table 1.1). Across the six sites surveyed, total respired carbon over the 22 day experiment ranged from a low of 1.58 $\mu\text{molC/kg}$ in Virginia, to a high of 12.60 $\mu\text{molC/kg}$ in Massachusetts. The differences among sites appeared to be linked to sediment organic matter content (see below). Total respired carbon was consistently greater at

high elevations, averaging 6.53 $\mu\text{molC/kg}$ at high elevations compared to 5.41 $\mu\text{molC/kg}$ at low elevations. In addition, sea level rise (SLR) caused a 15% decrease in total respired carbon from an average of 6.47 $\mu\text{molC/kg}$ (at normal hydrology) to 5.48 $\mu\text{molC/kg}$ (with SLR).

More importantly, the impact of SLR on total respired carbon varied depending on site and elevation (interaction terms, Table 1.1). SLR led to significant declines in respired carbon in two of the North Atlantic sites (Massachusetts and Maine), but no significant effect was observed in any of the Mid-Atlantic sites (Table 1.2, Figure 1.2). Furthermore, at these two North Atlantic sites the response to SLR was influenced by tidal elevation (Table 1.2, Figure 1.2). Within the Massachusetts site, SLR reduced respired carbon in samples from both tidal elevations, whereas in Maine only microbial communities from low elevations were affected. Although a significant elevation X hydrology interaction was also observed in the New Hampshire site (Table 1.2), SLR had no effect on decomposition at either elevation. In addition, although not my primary focus, it should be noted that Maryland, Delaware and New Hampshire showed significant differences in total respired carbon between elevations irrespective of hydrological treatment (Table 1.2).

Sediment organic matter content appeared to explain much of the variation in total respired carbon among sites, but not the observed responses to SLR. There was a linear relationship between average total respired carbon and percent organic matter content within sites (Figure 1.3; $p < 0.0001$, $F = 49.09$, $df = 1, 10$) but no difference between samples subjected to normal hydrology or SLR (ANCOVA, $p = 0.34$, $F = 1.05$, $df = 1, 8$) Other measured environmental variables that could have played a role in determining microbial response to SLR included porewater pH, salinity, and redox potential, as well as aboveground biomass. Although I observed several site-specific effects of elevation on these environmental variables (Table 1.3),

the patterns were not consistent among sites. In particular, at the one site that showed significant differential responses in total respired carbon in response to SLR at low and high elevations (Maine, Figure 1.2) no environmental parameter was significantly correlated with elevation (Maine, Table 1.3).

Table 1.1. Results of ANOVA for full model: Total Respired Carbon as a function of Site X Elevation X Hydrology. Bold indicates significance ($p < 0.05$).

Treatment	Degrees of Freedom	Sum of Squares	Mean Square	F	p
Site	5	46.480	9.296	99.591	<0.0001
Elevation	1	1.169	1.169	12.524	0.0005
Hydrology	1	0.920	0.920	9.857	0.002
Site X Elevation	5	1.594	0.319	3.416	0.006
Site X Hydrology	5	2.767	0.553	5.929	<0.0001
Elevation X Hydrology	1	0.032	0.032	0.339	0.561
Site X Elevation X Hydrology	5	1.481	0.296	3.174	0.009
Residuals	168	15.681	0.093		

Table 1.2. Results of site-specific ANOVAs on the effects of hydrology and elevation on total respired carbon. Bold indicates significance where * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

Site	Elevation Main Effect	Hydrology Main Effect	Elevation X Hydrology Interactive Effect
Virginia	$F_{1,28} = 2.60$	$F_{1,28} = 0.35$	$F_{1,28} = 0.68$
Maryland	$F_{1,28} = 20.59^{***}$	$F_{1,28} = 0.04$	$F_{1,28} = 0.01$
Delaware	$F_{1,28} = 6.07^*$	$F_{1,28} = 1.18$	$F_{1,28} = 0.06$
Massachusetts	$F_{1,28} = 2.76$	$F_{1,28} = 8.76^{**}$	$F_{1,28} = 1.83$
New Hampshire	$F_{1,28} = 23.33^{***}$	$F_{1,28} = 0.15$	$F_{1,28} = 4.59^*$
Maine	$F_{1,28} = 1.30$	$F_{1,28} = 4.69^*$	$F_{1,28} = 6.83^*$

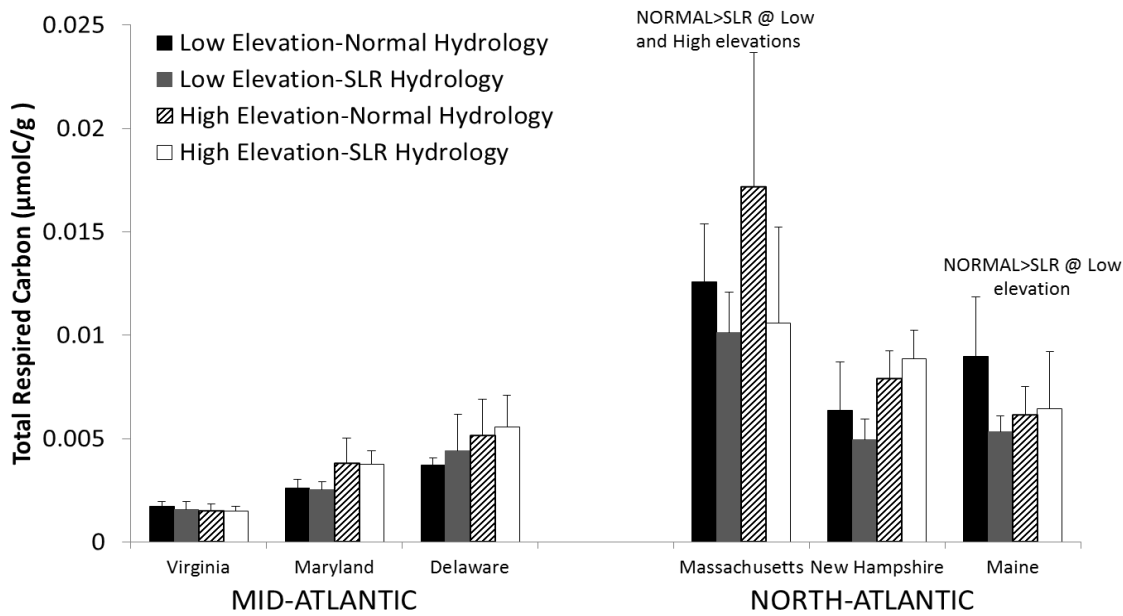


Figure 1.2. Average total respired carbon for each elevation X hydrology combination at each site. Error bars indicate standard deviations. Significant differences between hydrological treatments within elevations as found by Tukey tests are noted on the figure (see Table 1.2 for ANOVA results).

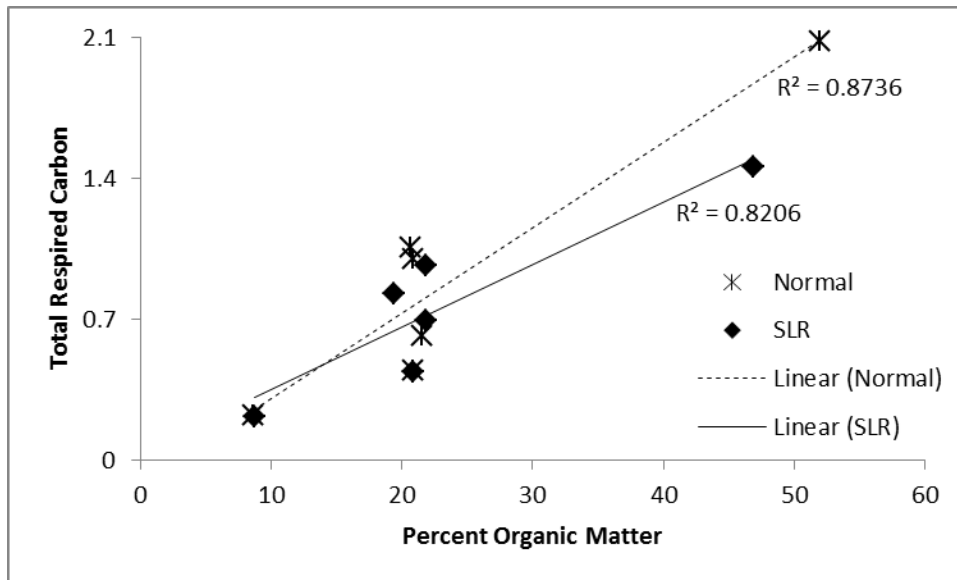


Figure 1.3. Average total respired carbon for each site X hydrology combination plotted against average percent organic matter.

Table 1.3. Results of a series of simple linear regressions of environmental variables on elevation within sites. Bold indicates significance where * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

Site	pH	Salinity	Redox Potential	Aboveground Biomass
Virginia	INCREASES w/ELEVATION $F_{1,14} = 8.14^*$	INCREASES w/ELEVATION $F_{1,14} = 10.13^{**}$	$F_{1,14} = 0.33$	$F_{1,14} = 0.08$
Maryland	$F_{1,2} = 0.19$	$F_{1,2} = 1.38$	$F_{1,2} = 0.05$	$F_{1,7} = 1.65$
Delaware	$F_{1,28} = 1.57$	$F_{1,6} = 0.54$	$F_{1,24} = 1.79$	$F_{1,32} = 1.10$
Massachusetts	$F_{1,37} = 0.65$	$F_{1,32} = 1.32$	INCREASES w/ELEVATION $F_{1,37} = 7.46^*$	$F_{1,37} = 0.01$
New Hampshire	DECREASES w/ELEVATION $F_{1,25} = 9.46^{**}$	$F_{1,25} = 0.95$	$F_{1,24} = 0.30$	DECREASES w/ELEVATION $F_{1,35} = 7.72^{**}$
Maine	$F_{1,23} = 0.02$	$F_{1,23} = 3.25$	$F_{1,23} = 0.20$	$F_{1,33} = 3.14$

Discussion

The goal of my study was to determine how increased inundation impacts sediment microbial decomposition dynamics in salt marshes. While past work has shown that increased inundation may decrease overall plant productivity in salt marshes (Warren et al. 2002; Konisky & Burdick 2004), relatively little is known about how SLR will impact sediment microbial decomposition rates. Other studies have suggested that water-logging is likely to reduce microbial activity in freshwater wetlands (Day 1983; Lenssen et al. 1999) and terrestrial soils (Gaunt et al. 1995; Kimura & Tun 1999), a pattern that could enhance accretion rates if seen in salt marshes.

My results indicated that microbial communities generally exhibit slower rates of decomposition in the face of SLR; I detected an overall 15% decrease in total respired carbon in response to increased inundation. Decreased marsh metabolism in the face of SLR has been observed before in high marsh sediments (see Miller et al. 2001; Nyman & DeLaune 1991). Miller et al (2001) reported a 28% decrease in soil respiration in flooded high marsh sediments as compared to their control group. The sediment collected in my study however, all came from low marsh areas. Although flooded for a shorter duration, sediment from my high elevation locations was still flooded twice per day. Sediment collected from the high marsh zone is only naturally flooded during particularly high tides (i.e., spring tides, storm events). Interestingly, the response to SLR in my study varied according to latitude as evidenced by two of my North Atlantic locations (Massachusetts and Maine) showing significant negative responses to SLR, while no such response was observed in any of the Mid-Atlantic sites (Table 1.2, Figure 1.2).

I also observed a high degree of latitudinal variation in total respired carbon, with a maximum 87% higher total respired carbon in Massachusetts sediments as compared to those seen from Virginia samples (Figure 1.2). Nyman & DeLaune (1991) reported as much as a 70% difference in carbon dioxide emissions from sediments collected at different locations in their study. The latitudinal variation in my study appeared to be tightly linked with sediment organic matter content (Figure 1.3). That microbial decomposition would vary with organic matter content is hardly surprising; a greater amount of decomposable substrate in the sediment would logically lead to greater microbial decomposition. However, despite correlating strongly with decomposition rates among sites, the site to site variation in microbial response to SLR was not well explained by organic matter content (Figure 1.3). Therefore, while certainly a driver of microbial decomposition rates in marshes, higher or lower organic matter content alone does not appear to drive sediment microbial response to hydrological change.

Although I found significant differences in decomposition between different tidal elevations, I found no evidence that elevation influenced the response to SLR. In particular, I observed differences in decomposition rates between low and high tidal elevations within the low marsh zone in all sites except Virginia, with high elevation communities tending to exhibit greater total respired carbon (Table 1.2, Figure 1.2). At the site in Maine, the only site in which microbial response to SLR was directly impacted by tidal elevation (Table 1.2, Figure 1.2), low elevation Maine samples showed significantly reduced total respired carbon under SLR conditions while high elevation communities showed no significant response to SLR (Figure 1.2). I expected to see a stronger negative response elicited from high elevation microbial communities on the assumption that these microbes would be less conditioned to dealing with inundation stress but SLR only reduced total respired carbon at high elevations in one site

(Massachusetts). Perhaps differences in elevation within the low marsh zone are not as important to microbial response to SLR as that between low marsh and high marsh zones.

I also attempted to explain the overall reduction in total respired carbon in the face of SLR (and variable patterns between sites and elevations) with several environmental variables: porewater salinity, pH and redox potential as well as aboveground biomass. More saline marsh sediments have been shown to cause decreases in microbial decomposition (Hemminga & Buth 1991). While little-studied in salt marshes, terrestrial soil decomposition is known to be depressed as soils become more acidic (Rousk et al. 2009). Aerobic decomposers tend to be more active in soils with a positive reduction potential while anaerobic decomposition proceeds more readily when redox conditions are negative (Li 2010). This fact would suggest that variations in reduction potential would alter the active microbes in sediments and thus likely impact decomposition rates. More productive marshes (greater aboveground biomass) tend to have greater soil organic matter contents, which as seen in my results above can be an important driver of microbial function. However, despite the fact that I did observe some site-specific variation with elevation in porewater salinity, pH, redox potential and aboveground biomass (Table 1.3), there was no consistency among these patterns that might have helped explain the patterns of SLR impact that I observed (Table 1.3). For instance, the only site that showed significant elevation differences in response to SLR (Maine) did not show significant elevational variation in any of the measured variables (Table 1.3). Further exploration of the effect of these environmental parameters, and a sampling approach that would allow comparisons between sites would help illuminate these effects further.

In summary, I found that decomposition rates generally decreased in response to increased inundation stress. Furthermore, the effect of SLR was strongest in the North Atlantic

sites and sometimes varied with tidal elevation within a given site. I attempted to explain the overall negative impact of SLR and observed latitudinal and elevational variation in that response with a suite of environmental variables. However, although organic matter content varied between sites, it did not satisfactorily explain the latitudinal variation in response to SLR. In addition, although I noted some site-specific elevational gradients of porewater salinity, pH, redox potential and aboveground biomass, none of these helped to explain the observed SLR responses. However, I did observe differences in decomposition among sites and elevations in this study. Understanding what drives these inherent differences may go a long way towards explaining why the impact of SLR varied with both latitude and elevation. While further study into the variables I measured could help elucidate these differences, there is some evidence that microbial community function is driven by microbial community structure (e.g., Zogg et al. 1997; Reed & Martiny 2007; Strickland et al. 2009). An examination of this structure-function link may shed more light on what drives the variable responses to SLR observed in this study, and has the potential to yield valuable insight into the importance of microbial communities to maintaining salt marsh elevation in the face of SLR.

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CHAPTER 2

SEA LEVEL RISE IMPACT ON SEDIMENT MICROBIAL COMMUNITY FUNCTION AND STRUCTURE IN TWO NEW ENGLAND SALT MARSHES

Introduction

That increased biodiversity will help ecosystems maintain viability in the face of environmental change is a widely accepted notion in ecology – the more species and genetic diversity that is present in an ecosystem, the more likely it is that some members will continue to thrive when conditions change. While this principle has been supported by several studies on plants (e.g. Tillman et al. 1997; Spehn et al. 2005) and animals (see Ehrlich & Ehrlich 1992), it has been relatively understudied in microbes (see Alison & Martiny 2008). Given the central importance of microbial community function in salt marsh ecosystems, understanding how microbial community composition impacts functionality is critical – especially when considering the current environmental changes being brought about by sea level rise (SLR).

Coastal salt marshes provide unique habitats for many migratory birds (Tiner 1984) and transient fish species (Shenker & Dean 1979), as well as flood protection for human infrastructure (see Reed 1990). Anthropogenic rises in sea level pose a threat to marsh viability by potentially interfering with biotic controls over maintenance of marsh surface elevation. Plant productivity drives salt marsh accretion directly via the input of dead organic matter onto the surface of the marsh and indirectly by intercepting particulate matter within the water column. Microbial activity also influences marsh surface elevation. For example, increased inundation

typically causes a decline in microbial respiration (Nyman & DeLaune 1991; Miller et al. 2001), which decreases the breakdown rate of sediment organic matter (Hargrave 1972) and thus increases accretion rates. However, several modeling studies have suggested that the accelerated SLR rates associated with anthropogenic global climate change will be too great for marsh accretion rates to keep pace with and may ultimately result in loss of marsh habitat (van Wijnen et al. 2001; Morris et al. 2002; Kirwan & Temmerman 2009).

Estuarine microbial communities are known to vary in structure according to environmental factors (Martiny et al. 2006); however, the potential link between structure and function is less understood. Changes in season (Keith-Roach et al. 2002; Crump et al. 2004), heavy metal concentration (Cordova-Kreylos et al. 2006), elevation (Franklin et al. 2002) and hydrology (Ravit et al. 2007) have all been shown to impact microbial community structure in estuarine ecosystems. There is also some evidence that sediment microbial community composition may be influenced by soil water content in terrestrial soils (Drenovsky et al. 2004; Sylvia et al. 1999; Schimel et al. 1999). The association between microbial community structure and function, however, has been more studied in terrestrial systems (see Torsvik & Ovreas 2002). For example, compositional shifts and concomitant changes in function have been observed in terrestrial microbial communities in response to temperature (Zogg et al. 1997), land use (Waldrop et al. 2000), dissolved organic matter content (Cleveland et al. 2007) and phytoremediation (Siciliano et al. 2003). Terrestrial soil microbial community composition has also been shown to play a role in litter decomposition rate (Strickland et al. 2009). The evidence for a structure-function association in microbial communities in terrestrial systems suggests that the existence of such a link in salt marshes is likely.

The primary goal of this study was to determine whether microbial community functional response to SLR (as determined by decomposition rate) was driven by shifts in community composition. I hypothesized that SLR would have a direct impact on microbial community structure and that changes in structure would determine changes in decomposition rates. A better understanding of the structure-function link in salt marsh microbial communities would enhance the ability to predict how marshes will respond to SLR.

Methods

Study Sites

I collected sediment from the low marsh zone (dominated by *Spartina alterniflora*) of two New England salt marshes in July, 2011: Waquoit Bay National Estuarine Research Reserve (NERR) in Massachusetts (41.557N, 70.504W) and Great Bay NERR in New Hampshire (43.137N, 70.888W). Average summertime (July-August, 2011) air temperature was 23.0°C at Waquoit Bay NERR and 21.7°C at Great Bay NERR (NERR System Centralized Data Management Office). A loss on ignition protocol of thirty-two low marsh sediment samples from each site (after Heiri et al. 2001) showed average organic matter content to be 49% at Waquoit Bay NERR and 21% at Great Bay NERR. I collected sixteen sediment cores (6 cm diameter X 10 cm depth) at each of two tidal elevations within the low marsh zone (referred to hereafter as “low elevation” and “high elevation”) for a total of thirty-two samples per site. Low elevation cores were collected from within 10 m of a creek edge or tidal mudflat, whereas high elevation cores were collected from within 10 m of the *Spartina alterniflora*-*Spartina patens* transition zone. Within each elevation, I collected the cores in a haphazard fashion from an area of ~300 m² while avoiding bare patches of marsh, pools and panes and maintaining a minimum

of 2 m of spacing between all cores. Cores were stored on ice while being transported to a lab facility where they were processed as described below.

Microbial Functional and Structural Response to Sea Level Rise

I enclosed sediment from each core in a “microbial cage” (after Gasol et al. 2005) of dialysis tubing (Sigma-Aldrich; St. Louis, MO) in order to hold the microbes inside while allowing the free passage of water, solutes, and gases. In a flow-through seawater lab (University of New England, Biddeford, ME) eight replicates from each site x elevation combination were exposed to “normal hydrology” by flooding the cages for approximately 3.5 hours twice per day (flood time was determined according to the average daily inundation time at Wells NERR in Maine over a 3 month period spanning the dates of the experiment). The second set of eight replicates was exposed to simulated SLR of forty centimeters (after the 2007 IPCC report) by flooding the cages for five hours twice per day (based on estimated additional time of inundation at Wells NERR). To measure microbial functional response to SLR, I used a Li-Cor 6400 Infrared Gas Analyzer (Lincoln, NE), to determine instantaneous carbon dioxide efflux ($\mu\text{m of C/sec}$) for each cage weekly for a period of three weeks. In order to characterize microbial community structural response to SLR, I conducted a terminal-restriction fragment length polymorphism (t-RFLP) analysis on sediment from each cage immediately prior to and following the experiment (see *Molecular Analysis* below).

Molecular Analysis

I removed 0.5 grams of sediment from each core prior to beginning my experiment, and from each corresponding cage following completion of my experiment in order to characterize shifts in community composition using a t-RFLP molecular analysis. All samples were stored at

-80°C within one day of collection. I extracted total bulk DNA from each sample using a Powersoil DNA isolation kit for soils (MO-BIO, Carlsbad, CA) according to the manufacturer's instructions.

As part of the t-RFLP protocol (after Kim & Marsh 2008), I amplified 16s rDNA from each bulk DNA sample using polymerase chain reaction (PCR) with one bacteria-specific primer (5' AGAGTTTGATCMTGGCTCAG) and one universal primer (5' ACCTTGTTACGACTT) (Kim & Marsh 2008). Each primer was labeled with a different fluorescent tag for visualization of terminal restriction fragments. I ran four PCR reactions on each sample to generate a total yield of approximately 200ng of amplicon prior to my first DNA purification step. Each PCR reaction included 1ng of bulk DNA, 1x PCR buffer, 10µg/µL bovine serum albumin (BSA), 4mM MgCl₂, 0.2mM deoxynucleotide triphosphates (dNTPs), 10 picomoles of each primer described above, and 2 Units of *Taq* DNA polymerase in a total volume of 50µL. Thermal cycler conditions followed the t-RFLP protocol from Kim and Marsh et al (2008). Following PCR, I used an ethanol precipitation to purify the DNA, in which I incubated the amplification product in two volumes of 100% ethanol at -20°C for two hours prior to spinning down the pellet and washing in 70% ethanol. All samples were run on 1% agarose for quantification, and concentrated to roughly 16-17ng/µL solution volume.

Concentrated samples were digested with 10 units of *RsaI* restriction endonuclease in 15µL reaction volumes at 37°C for 16 hours. Reactions were terminated by heating to 75°C for 20 minutes. Following digestion, DNA was again purified using the same ethanol wash protocol as described above. Restriction fragments were separated using an ABI 310 Genetic Analyzer (Applied Biosystems, Inc., Foster City, CA). Fluorescently tagged terminal fragments were sized using Genemapper v.4.0. Presence or absence of t-RFLP markers was scored using

Genographer v.2.1.4, and then this data was used to characterize the microbial community structure of each sample.

Statistical Analysis

I calculated total respired carbon for each microbial cage by plotting the individual carbon efflux data points for each cage against time and determining the area under the curve. I used a fully factorial analysis of variance (ANOVA) to examine variation in total respired carbon as explained by site, elevation, and hydrology. Pairwise comparisons between hydrological treatments within elevations from each site were conducted via t-tests after adjusting total respired carbon for initial carbon efflux in order to factor out differences in initial decomposition rates (prior to treatment). Since only comparisons between hydrological treatments within elevations were relevant to the effects of SLR on microbial function, those were the only *posthoc* comparisons conducted.

I applied a principal coordinates analysis (PCO) to my t-RFLP data to visualize differences in microbial community composition between sites and elevations pre-experiment, and also shifts in community composition due to hydrological treatments, i.e., from pre- to post-experiment. In order to examine any initial differences in community composition between sites, I conducted a series of one-way ANOVA tests on the eigenvectors from my PCO analysis to determine which principal coordinates loaded significantly ($p < 0.05$) on the main effect of site, and plotted the eigenvectors for the two that were most informative (i.e., that returned the smallest p-values). To examine the degree of shift that occurred in sediment microbial communities as a result of the hydrological treatments, I used one-way ANOVAs to identify principal coordinates that loaded significantly ($p < 0.05$) on the site x elevation x hydrology

interaction. Any principal coordinates with significant loadings were then used to calculate Euclidian distances between the pre-experimental and post-experimental communities for each sample so that I could plot and visually evaluate how patterns of shift mirrored functional changes from my decomposition rate experiment.

Results

My t-RFLP analysis included a restriction digest with one restriction endonuclease (*RsaI*) and provided me substantial informative power to differentiate community composition between samples. The t-RFLP analysis yielded 88 scorable terminal fragments, ranging from 50-489 base pairs in length. All fragments varied across samples: each scored band was found in at least 17% of all samples analyzed with the most common fragment being present in 44% of all samples.

I found significant differences in community composition between sites and to a lesser extent between elevations. My principle coordinates analysis of these t-RFLP data showed that microbial community composition varied between my two collection sites (Figure 2.1). I found two principle coordinates with significant loadings on the main effect of site (PCO axis 1: $F(1,42)=20.47$, $p<0.001$, explained 11.2% of the variation; Axis 2: $F(1,42)=9.95$, $p=0.002$, explained 8.7% of the variation) for my pre-experiment samples. I did not find any principal coordinates that loaded significantly on the site x elevation interaction; however, there were minor differences between elevations within sites that were visually apparent from the first two PCO axes (see Figure 2.1).

In addition to site-specific differences in microbial community structure, I found that microbial communities from two of my four site x elevation combinations showed significant functional responses to SLR. In my Massachusetts samples, I observed a significant decrease in

microbial total respired carbon in high elevation communities under conditions of SLR, as compared to the normal hydrology ($t=-3.902$, $df=27$, $p<0.001$; Figure 2.2A). I observed a similar decrease in total respired carbon under SLR in my low elevation New Hampshire samples ($t=-2.079$, $df=27$, $p=0.047$; Figure 2.2A). Although SLR appeared to decrease decomposition rates in the low elevation Massachusetts communities as well (Figure 2.2A), this difference (as well as that in the high elevation New Hampshire samples) was non-significant.

Furthermore, the functional responses that I observed appeared to be associated with the amount of shift in community composition that occurred during my experiment. I detected four principal coordinates that loaded significantly on the site x elevation x hydrology interaction (PCO Axis 1: $F(15,68)=3.95$, $p<0.001$, Axis 3: $F(15,68)=2.80$, $p=0.002$, Axis 5: $F(15,68)=5.02$, $p<0.001$, Axis 6: $F(15,68)=3.67$, $p<0.001$). Using these axes to calculate a total Euclidian distance (4-dimensional) for each sample between pre-experiment and post-experiment, I found that the average degree of shift in community composition over the course of the experiment was smaller in samples that had been exposed to SLR (Figure 2.2B). The microbial communities in the normal hydrology treatment changed to a greater degree and generally maintained higher decomposition rates than those exposed to SLR (Figure 2.2A, 2.2B). The reduction in microbial community compositional shift with SLR corresponded to the significant decreases in decomposition rate I observed as a response to SLR in my functional experiment in three of my four site x elevation combinations (all but New Hampshire high elevation; Figures 2.2A, 2.2B).

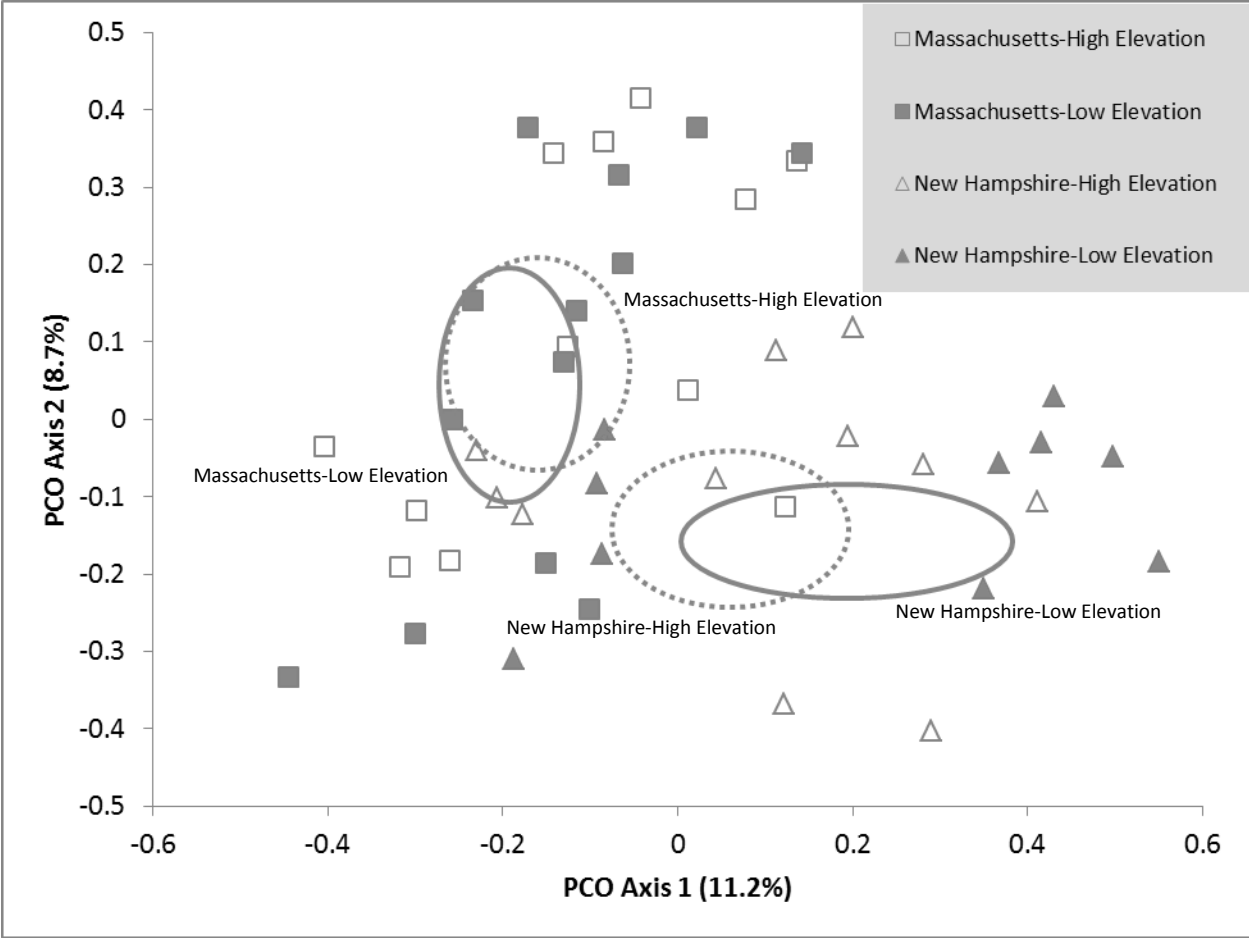


Figure 2.1. Differences in microbial community composition among sites and elevations prior to SLR experiment based upon PCO analysis of t-RFLP data. Ellipses indicate 95% confidence intervals around the centroid of each site x elevation combination. Axis values indicate percent variation explained by the specific PCO axis.

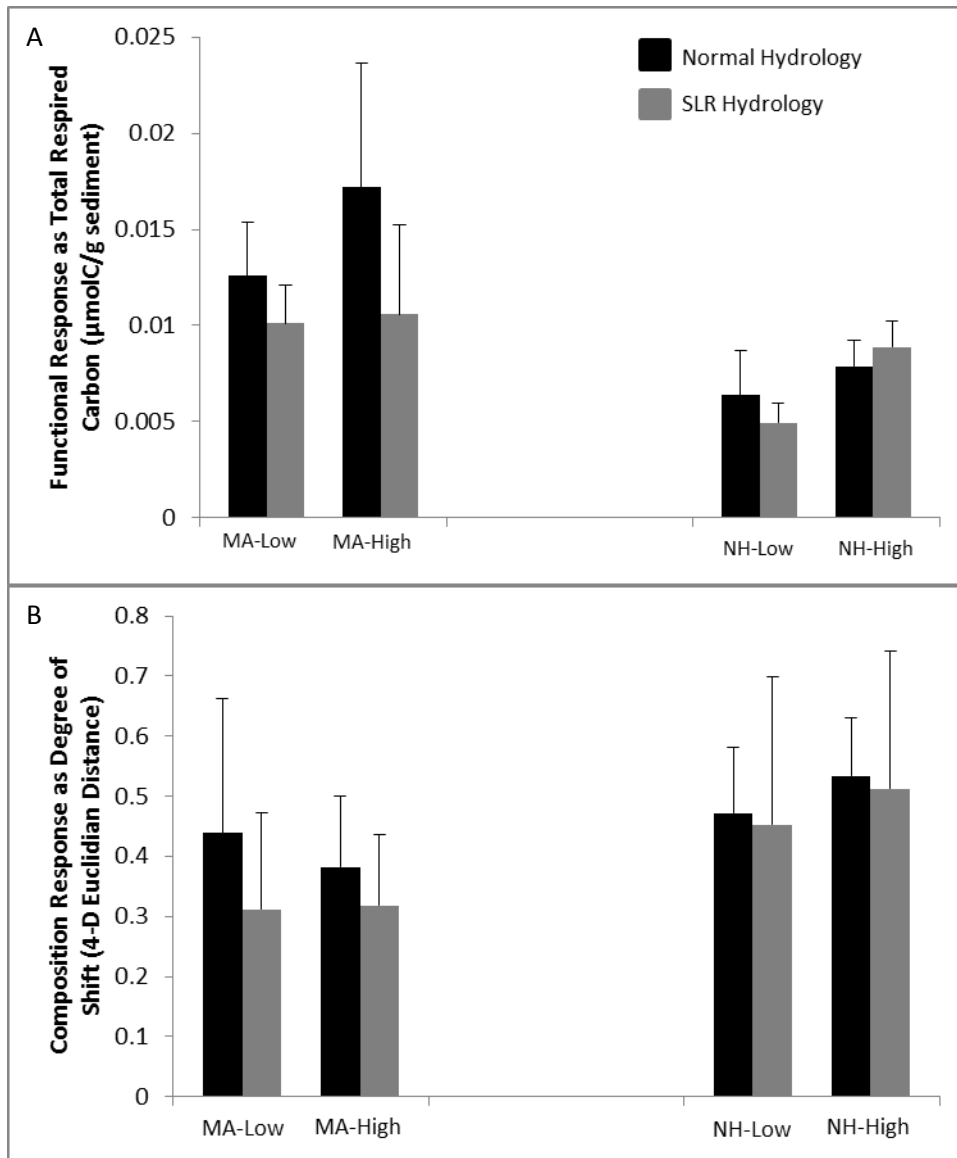


Figure 2.2. Effects of SLR on (A) microbial functional response over the 3-week experimental period and (B) microbial compositional response as determined by differences between pre- and post-experimental PCO eigenvalues of t-RFLP data. Error bars reflect standard deviations.

Discussion

The goal of my study was to determine whether salt marsh sediment microbial community functional response to SLR was driven by shifts in community composition.

Evidence exists that suggests that variation in microbial community structure between sites is determined by environmental factors (see Martiny et al. 2006). Despite research to determine the

potential environmental conditions that impact microbial community composition (e.g. Cordova-Kreylos 2006; Ravit et al, 2007), little is known about the association between microbial community structure and function in salt marshes. With rising sea levels becoming increasingly threatening to these tidal habitats, it is important to understand what is driving functional response to this disturbance.

My results indicate that microbial communities in salt marshes vary significantly among sites and to a lesser extent between elevations within sites (Figure 2.1). There is some evidence that salt marsh sediment microbial community composition varies between sites with different moisture contents, and air and water temperatures (Martiny et al. 2011), as well as within sites based on differences in vertical elevation that may alter drainage patterns (Franklin et al. 2002). While I did observe minor differences in community composition between elevations in this study (Figure 2.1), they were not statistically significant. Perhaps the biggest difference between my two study sites was sediment organic matter content (49% in Massachusetts, 21% in New Hampshire). Other studies have found that the quantity of organic substrate available in terrestrial soils may drive variation in microbial community structure (Baath et al. 1995; Drenovsky et al. 2004), a trend that if extended to salt marshes may help explain the significant differences in microbial community composition between sites observed in this study (Figure 2.1).

I also found differences in microbial community functional response between communities exposed to SLR and those in the normal hydrological treatment (Figure 2.2A). In three of my four site x elevation combinations, SLR caused a decrease in microbial decomposition rate during the experiment, with two of these declines being statistically significant (Figure 2.2A). A flooding-induced decline in marsh metabolism has been observed

before in marsh sediments (Miller et al. 2001; Nyman & DeLaune 1991). Other studies have also shown that increased inundation can negatively impact microbial activity in terrestrial soils (e.g. Gaunt et al. 1995; Kimura & Tun 1999). I previously attempted to explain differential responses in decomposition rate to SLR among three mid-Atlantic and three north-Atlantic marshes (including the Massachusetts and New Hampshire sites in the current study) based on environmental variables such as porewater pH, salinity and redox potential but none of these adequately explained the functional response (see Chapter 1 of this thesis). While further study into these and other environmental factors may help explain the observed functional response to SLR, it is plausible that it is determined primarily by changes in microbial community composition.

The decline in decomposition rate observed in response to SLR (Figure 2.2A) appeared to be matched by a similar decline in magnitude of structural shift under SLR conditions (Figure 2.2B). In all four site x elevation combinations, a smaller degree of shift in composition occurred under SLR conditions as compared to in the normal hydrology treatment (Figure 2.2B). Because these average shifts were based on a series of ANOVAs to determine PCO's significantly loading on the site x elevation x hydrology interaction, further analyzing whether these shifts were significant between treatment combinations was avoided. However, the observed decreases in shift under SLR conditions suggest that increased inundation may inhibit microbial community plasticity, a factor that could limit the functionality of a given community when increased inundation occurs. In general, microbial communities tend to be sensitive to environmental perturbations (see Alison & Martiny 2008). In terrestrial soils for example, changes in temperature have been shown to cause shifts in microbial community composition that paralleled changes in respiration rates (Zogg et al. 1997; Monson et al. 2006). Increases in

dissolved organic matter in terrestrial soils have also been shown to cause major shifts in microbial communities that are concomitant with changes in microbial respiration (Cleveland et al. 2007). On the other hand, there is some evidence for microbial community resistance to certain environmental perturbations such as nutrient additions (Lovell et al. 2001; Bowen et al. 2009; Bowen et al. 2011) and elevated CO₂ (Gruter et al. 2006). In all of these studies, however, only the occurrence (or not) of a shift was determined. What is less well understood, and central to my study, is how the amount of shift in composition relates to functional alterations in response to an environmental stress. My results suggest that when microbial communities are exposed to SLR, the magnitude with which they can shift in response to the increased inundation is less than that with which they can shift in response to the lab setting without increased inundation (Figure 2.2B). Furthermore, this limited capacity for compositional change corresponded with a decline in decomposition rate under SLR conditions in three of my four site x elevation combinations (Figure 2.2A), perhaps indicating that the microbial communities there were unable to rapidly adjust to changes in sea level. The fourth site x elevation combination (New Hampshire, high elevation), while still showing a decline in degree of shift in response to SLR (Figure 2.2B), also showed a slight (not statistically significant) increase in total respired carbon (Figure 2.2A). This functional trend may have been an artifact of the generally increased decomposition rates at high elevation at this site (Figure 2.2A) which may have been attributable to the greater difference in initial microbial community composition between elevations in New Hampshire (as compared to Massachusetts; Figure 2.1).

From my study, I can conclude that salt marsh sediment microbial communities are structurally different between marshes (and to a lesser degree, between elevations within a marsh) and that SLR limits the degree to which those communities can change in the short term.

This restriction in shift corresponds to a functional decline in decomposition rate, perhaps as a sign that microbial communities are unable to rapidly adjust to SLR and maintain normal decomposition rates. The potential for microbial resistance to environmental disturbance is reviewed by Reed & Martiny (2007) and further study into the structure-function link in marsh sediments, particularly through direct taxonomical classification (to provide a true measure of diversity) of microbial communities, would allow for a more direct examination of the role of microbial biodiversity in functional response to an environmental stressor. Decreased decomposition rates in marshes being subjected to increased inundation would ultimately help increase accretion rates and combat the effects of SLR. However, results from my previous study indicate that microbial communities from different marshes may differ in their functional responses to SLR (Chapter 1 of this thesis). Therefore, a better understanding of what drives marsh and elevation-specific functional responses is critical to being able to predict how marshes will respond to this stressor. The results of this study indicate that microbial community composition varies between marshes and that SLR-induced limitations on shifts in structure correspond to declines in decomposition rates.

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