ANALYSIS OF ATLANTIC AND NORTHERN GULF COAST WETLAND

BACTERIAL EXTRACELLULAR ENZYME ACTIVITY

By
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ABSTRACT

Sea-level rise is projected to cause saltwater marshes to migrate landward replacing brackish and freshwater marshes. Coastal wetlands are important sinks of carbon, phosphorous, and nitrogen, so it is important to understand the function of their microbial communities. This study aims to categorize the difference in function between different spatially distinct wetland marsh types in advance of the expected alteration of the wetland ecosystems. Extracellular microbial enzymatic activity was measured to understand organic matter decomposition and nutrient mineralization in different marsh types. We measured the activities of the extracellular enzymes β-glucosidase, NAGase, peroxidase, phenol oxidase, and phosphatase across sites along the Northern Gulf of Mexico and Atlantic coast. Both tidal salt and tidal fresh marsh sediment were sampled at each location. Higher salinity depressed the activity of NAGase. Salinity did not have a significant effect on phosphatase activity. High salinity slightly repressed carbon-degrading enzyme β-glucosidase activity but increased peroxidase and phenol oxidase activities. Sediments with high organic matter content had lower enzyme activities. Warmer water temperature sites tended to exhibit higher overall enzyme activity. This study finds that increasingly saline wetlands will cause a change in nutrient cycling functionality. Saltwater intrusion into fresh marsh will reduce the capacity for nitrate removal leading to potential coastal eutrophication, and saltwater intrusion will increase carbon metabolism leading to less accretion than in freshwater marshes further amplifying the effect of sea-level rise.

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Introduction

With the changing global climate, the oceans are predicted to rise by
approximately 0.5 m on average with a range of between 0.26-0.82 m by year 2100
according to various Intergovernmental Panel on Climate Change models (Church et al.,
2013). The thermal expansion of the ocean waters due to heat retention and the
displacement of melted glacial ice are the two main contributors to sea-level rise (SLR)
(Nicholls and Cazenave, 2010). Current climate change models predict sea-level rise will
increasingly impact coastal wetlands. The increase in sea-level will cause an inward
migration of ocean water, and an increase in SLR of 0.5 m could cause as much as a 46-
59% loss of global coastal wetlands by 2100 if environmental policy is maintained and
coastal dike construction continues (Spencer et al., 2016). Saltwater marshes will migrate
landward replacing areas previously occupied by brackish and freshwater marshes (Craft
et al., 2009)

Along with the increased salt water intrusion landward due to SLR, climate
models also predict an increase in frequency of extreme coastal storm surges along the
Gulf Coast and even more so along the Atlantic Coast over the next hundred years. The
frequency of the storms will not be equivalent in all coastal ranges, however, but will
vary based on geographic location and characteristics (Tebaldi et al., 2012). These storm
surges will temporarily cause saltwater intrusion into brackish and salt marsh before
receding, potentially altering wetland sediment bacterial communities (Amaral-Zettler et

al., 2008). The extent to which SLR and these storms affect wetlands will be in part determined by the rate of organic matter build up that has occurred in the wetlands. As SLR increases, one mechanism for countering this is to increase the vertical sediment buildup of the wetlands through accumulation of organic matter and sediment. This increase in land, coinciding with SLR, would allow for salt marsh intrusion to occur at a slower pace (Spencer et al., 2016)

Rate of organic matter accumulation is related to how fast this material decomposes, which is dependent on the composition and activity of the sediment microbial community. Of particular concern is the impact a shift in marsh salinity and temperature may have on altering these microbial communities. Wetland bacteria are important in nutrient cycling, primary productivity, and decomposition (Brinson et al., 1981) and different marsh salinity grades contain differences in taxonomic bacterial diversity (Santoro et al., 2006). Along with a potential change to wetland bacterial community composition, changes from SLR could result in functional changes in bacterial activity because of a higher salinity environment. When microbes are subject to limits of survival, such as in higher salinity situations, certain functions are emphasized at the cost of efficiency in other aspects (Dupoint et al., 2014). One of the functional trade-offs bacteria may make in adjusting to a higher salinity would be a reduction in extracellular enzymatic activity. An increase in salinity could lead to a depression in microbial enzyme activity and a change in community composition to salinity tolerant microbes, potentially reduced rates of nutrient cycling and organic matter decomposition following saltwater intrusion events (Jackson and Vallaire, 2009).

The coastal wetland microbiome and its ability to maintain enzymatic functions through increased environmental stress has been understudied yet is vital to understanding the impact of climate change on coastal ecosystems. Changes in the rate of nutrient cycling and carbon mineralization will also affect the wetland's role as a sink for nitrogen, phosphorus, and carbon. If the enzymatic function of bacteria in the marsh ecosystem is altered by changing salinity, eutrophication may be impacted due to the reduced effectiveness of nutrient removal (Loomis and Craft, 2010). The extracellular enzymes phenol oxidase and peroxidase (Sinsabaugh, 2010) as well as β-glucosidase and β-N-acetylglucosaminidase (NAGase) are produced by microorganisms, and the activity of these enzymes correlates with increased rates of particulate organic matter decomposition in wetlands (Jackson and Vallaire, 2007). Yet, an increase in salinity can reduce bacterial enzyme activity, which may suggest a reduction in rates of nutrient cycling (Jackson and Vallaire, 2009). Enzymatic breakdown of organic matter also reduces the carbon storage in wetlands, and salt marshes are efficient sinks for sequestering carbon in the soil and above-ground and below-ground biomass (Ouyang and Lee, 2014).

Understanding rates of enzymatic activity in coastal wetlands is valuable in predicting how estimated SLR may impact future wetland functions, as fresh and brackish marsh will gradually be replaced by landward encroaching salt marsh and salt marsh is replaced by ocean (Keddy et al., 2007). Despite this, there has been little research into the extracellular enzymatic activity of bacteria across different marsh types along severally distinct geographic locations on the United States' Atlantic and Gulf Coasts. With SLR and increasing saltwater intrusion events from storms, it is imperative

to understand the function of wetland microbial communities, especially in the context of organic matter decomposition and nutrient mineralization. This research aims to categorize the difference in function between different spatially distinct wetland marsh types in advance of the expected alteration of the wetland ecosystems. To achieve this aim, soil samples were taken along salinity gradients observed across a wide spatial range on the northern Gulf Coast and Atlantic Coast to compare the microbial extracellular enzyme activities between sediment of fresh (<0.5 ppt salt), intermediate (0.5-5 ppt), brackish (5-18 ppt), and saltwater marshes (18-40 ppt). The activities of the enzymes β-glucosidase, peroxidase, and phenol oxidase were measured to assess rates of organic matter decomposition. NAGase and phosphatase activities were used to estimate the potential for nitrogen and phosphorus mineralization, respectively.

Methods

Sample Collection:

Soil sediment samples were taken from thirteen locations along the northern Gulf Coast and Atlantic Coasts during June 2018. Samples were acquired from wetlands at the confluence of salt marsh and brackish or fresh marsh. The sampling sites along the Gulf Coast were Cocodrie, LA; New Orleans, LA; Grand Bay, MS; Weeks Bay, AL; Apalachicola, FL; St. Marks, FL; Cedar Key, FL; and St. Augustine, FL, and the Atlantic coast locations were Sapelo Island, GA; Charleston, SC; Wilmington, NC; Morehead City, NC; and Gloucester Point, VA. Each sampling location was subdivided into two sediment sampling sites of salt marsh and fresh marsh or brackish marsh. Fresh marsh, brackish marsh, and salt marsh were identified by measuring salinity level using a YSI meter (YSI Inc, Yellow Springs, OH) and by the observed dominant vegetation type. Fresh marsh was categorized as having a salinity of less than 0.5 ppt with the dominant vegetation being *Zizaniopsis miliacea*. *Juncus roemerianus* and a salinity between 5-18 ppt discriminated brackish marsh. Salt marsh was identified by *Spartina alterniflora* and a salinity being greater than 18 ppt (Craft et al., 2009, Elmer et al., 2013).

Five soil cores were extracted using a 38 cm x 2 cm soil corer at each of the eighteen sample sites differentiated by geographic location and marsh type. Between each sample taken, the corer was sterilized with 70% ethanol. Each soil core sample extended from surface to root depth 30 cm deep. Each core had soil from the top and bottom of the

sampling collected and isolated for further differentiation of bacterial microenvironments. Soil subsamples were stored in sterile, labeled 50mL microtubes and placed on ice until lab analysis. To account for differing times of sample collection, once back in the laboratory, all samples were analyzed in the order they were collected to normalize the time each soil sample spent on ice. At each collection site the salinity, pH, and temperature of the marsh was recorded using a Model 30 and a Model 60 YSI meter.

Sample Analysis:

Once back in the laboratory at the University of Mississippi, colorimetric enzyme assays for β-glucosidase, peroxidase, phenol oxidase, β-N-acetylglucosaminidase (NAGase), and phosphatase were performed to measure microbial activity rates for each soil sample. β-glucosidase, peroxidase, and phenol oxidase activities were measured to evaluate potential for organic matter decomposition. NAGase and phosphatase activities were obtained to estimate potential for organic nitrogen and phosphorus mineralization. For the enzyme assays, preparation of the soil samples and artificial enzymatic substrates and buffers followed protocol outlined by Jackson et al. (2013).

5mL of sediment samples, avoiding roots, were prepared as a 15mL homogenous slurry with R.O. water. 150μL of each slurry were combined with 150μL of their corresponding substrate in a deepwell block for reaction incubation. Phenol oxidase and peroxidase assays used the artificial substrate L-3,4,-dihydroxyphenylalanine (L-DOPA) which oxidizes to a brown complex in the presence of phenol oxidase or peroxidase. Peroxidase assays had an addition of 15μL of 0.3% H_2O_2 added to samples. Acid

phosphatase, β-glucosidase, and NAGase reactions used the substrates pNP-phosphate, pNP- β-glucopyranoside, and pNP- β-N-acetylglucosamide, respectively, all of which yield p-nitrophenol (pNP) which is yellow under basic conditions. Four replicates of each substrate and sample reaction, two sample controls (sample included, no substrate), and two substrate controls (substrate included, no sample) were conducted per sample for each assay. Phosphatase reactions ran for 1 hour, β-glucosidase for 1.5 hours, NAGase for 2 hours and phenol oxidase and peroxidase ran for 2.5 hours. After the reactions ran to completion, the samples were centrifuged and 150µL of their supernatant was transferred to a microplate for analysis. Colorimetric absorbance was read using a BioTek Synergy 2 microplate reader. Phenol oxidase and peroxidase assays were read at absorbance of 460nm; phosphatase, β-glucosidase, and NAGase assays were read at 410nm.

Organic matter content of soil was also measured to standardize enzyme activity rates. Organic matter was determined by weighing the mass of original soil samples before and after dehydrating the soil in an oven at 70°C for 48 hours and then again after being ashed in a muffle furnace for 2 hours at 500°C. The difference between the pre-ignition weight and the ashed weight divided by the pre-ignition weight was the percent organic matter per sample. Enzyme activity was calculated by dividing a sample's colorimetric absorbance value by its corresponding organic matter per reaction, length of reaction time, and a control value.

Statistical Analysis:

After gathering the data, ANOVA was used to analyze the significance of different soil organic matter content levels between marsh types.

Results

The activities of β-glucosidase, phosphatase, phenol oxidase, peroxidase, and NAGase varied greatly across sediment samples from the different locations, although there were some general patterns (Figure 1). Peroxidase activity was generally the highest while β-glucosidase and NAGase were often the enzymes with the least activity. St. Augustine, FL had by far the highest peroxidase and phenol oxidase activity, but had low activities of β-glucosidase, NAGase, and phosphatase compared to other sample locations. Phosphatase activity was highest in Grand Bay, MS. Cocodrie and New Orleans, LA, and Sapelo Island, GA, had low activities for all measured enzymes (Figure 1). NAGase and β-glucosidase activity levels had a smaller range throughout all sampling sites compared to wide variation seen in activity levels of phosphatase, peroxidase, and phenol oxidase.

The relationship of the activity of each enzyme to water temperature at the samples sites was examined (Figure 2). A general measure of combined enzyme activity was determined by averaging the individual activities of all five enzymes at each site. Activities of peroxidase (Figure 2A, R= 0.288) and phenol oxidase (Figure 2B, R= 0.297) were higher in sites of warmer water compared to cooler water. NAGase activity was constant across all ranges of water temperature (Figure 2C, R= 0.083). Phosphatase (Figure 2D, R= -0.054) and β-glucosidase (Figure 2E, R= -0.230) showed slightly higher rates of activity in cooler water.
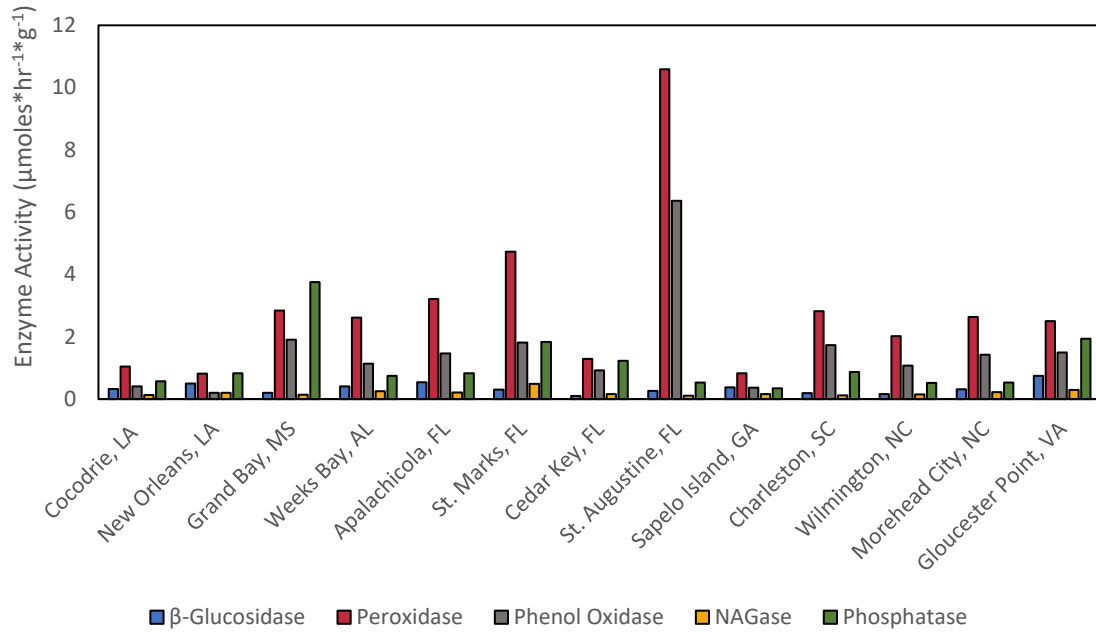
Figure 1. Activities of enzymes involved in organic carbon, nitrogen, phosphorus mineralization in coastal wetland sediments along the US Gulf and Atlantic coasts. Mean specific enzyme activity was measured for each geographic location irrespective of marsh types and salinity levels at each location. Enzyme activity was determined colorimetrically and expressed per g of organic matter in sediment.
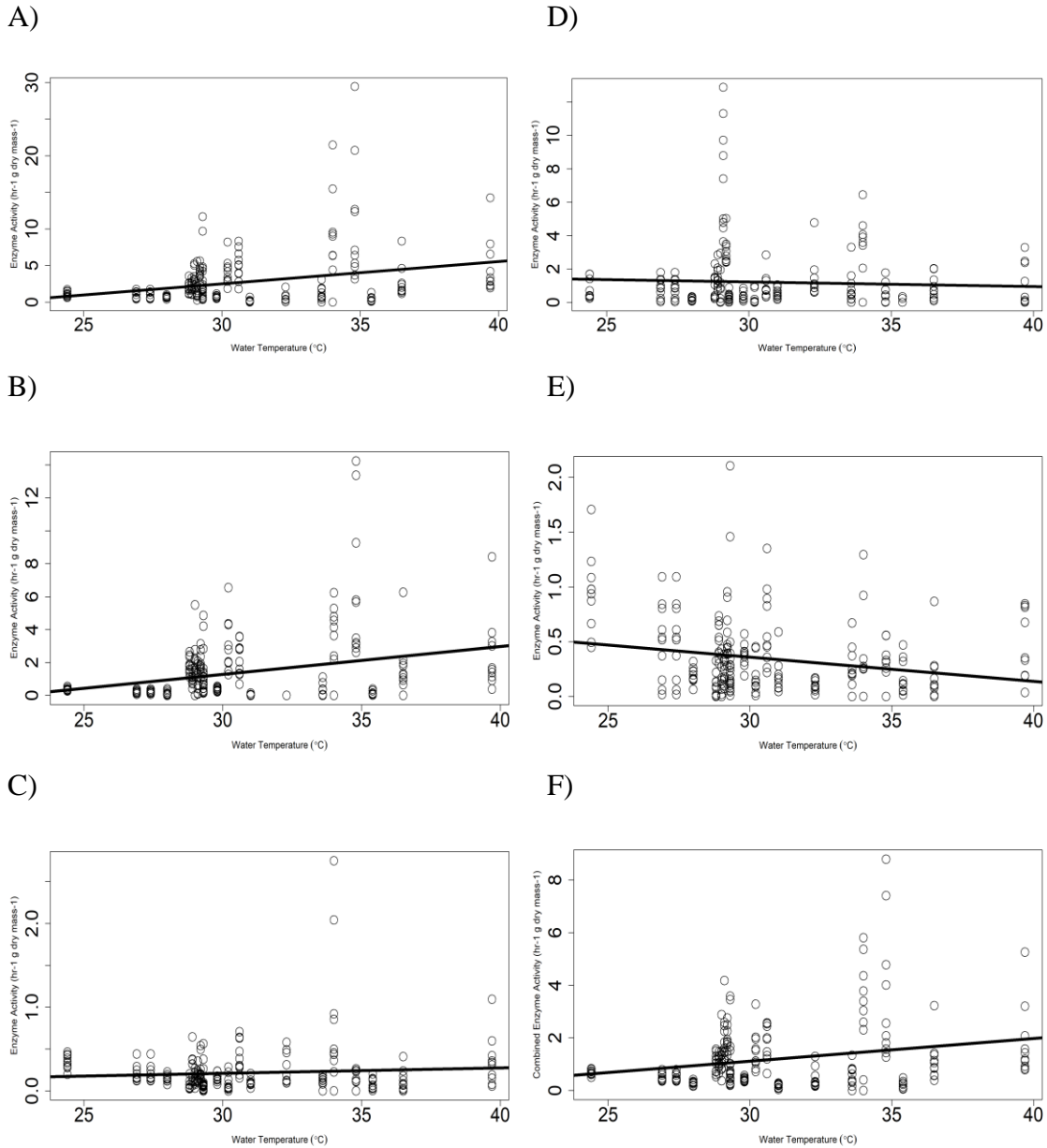
A)

B)

C)

D)

E)

F)

Figure 2. The relationship between sediment microbial enzyme activity and in situ water temperature for sediment collected from 13 sites along the US Gulf and Atlantic coasts. The combined average of the individual activity of peroxidase (A), phenol oxidase (B), NAGase (C), phosphatase (D), and β-glucosidase (E) at each site determined combined enzyme activity (F). Enzyme activity was calculated by dividing a sample's colorimetric absorbance value by its organic matter content, and length of reaction time.

The combined site activity very significantly ($p < 0.001$, R= 0.242) correlated with water temperature at time of sample collection (Figure 2F). Samples from marshes with warmer water tended to have higher combined enzyme activity, whereas cooler sites had the lowest enzyme activities.

The relationship between salinity and enzyme activity varied for each enzyme tested (Figure 3). NAGase activity (Figure 3A) was significantly lower at higher salinity ($p<0.01$, R= -0.233). β-glucosidase (Figure 3B) showed the same trend of decreasing activity with increasing salinity but was only moderately significant ($p=0.08$, R= -0.215). Phosphatase activity (Figure 3C) was largely unaffected by marsh salinity levels ($p=0.82$, R= -0.014). The activity of peroxidase (Figure 3D, $p=0.47$, R= 0.184) and phenol oxidase (Figure 3E) increased with salinity, although the effect was only borderline significant for phenol oxidase ($p=0.07$, R=0.249). The combined enzyme activity showed a trend of being larger in wetlands high in salinity (Figure 3F, R=0.163). There was a marginally significant ($p=0.0525$) difference between overall enzyme activity in the lower salinity, fresh and intermediate marsh (0-5 ppt), and higher salinity marsh types, brackish and salt marsh (5-40 ppt).

Fresh marsh sediments (those with salinities <0.5 ppt) had higher organic matter content than sediments from intermediate, brackish, or saltwater marshes (Figure 4). Intermediate and brackish marsh had similar organic matter content to each other. Salt marsh (18-40 ppt salinity) soil had the lowest median percent organic matter. Intermediate marsh soil had the largest range in organic matter content. The difference in organic matter content between each marsh type was significant ($p<0.001$).
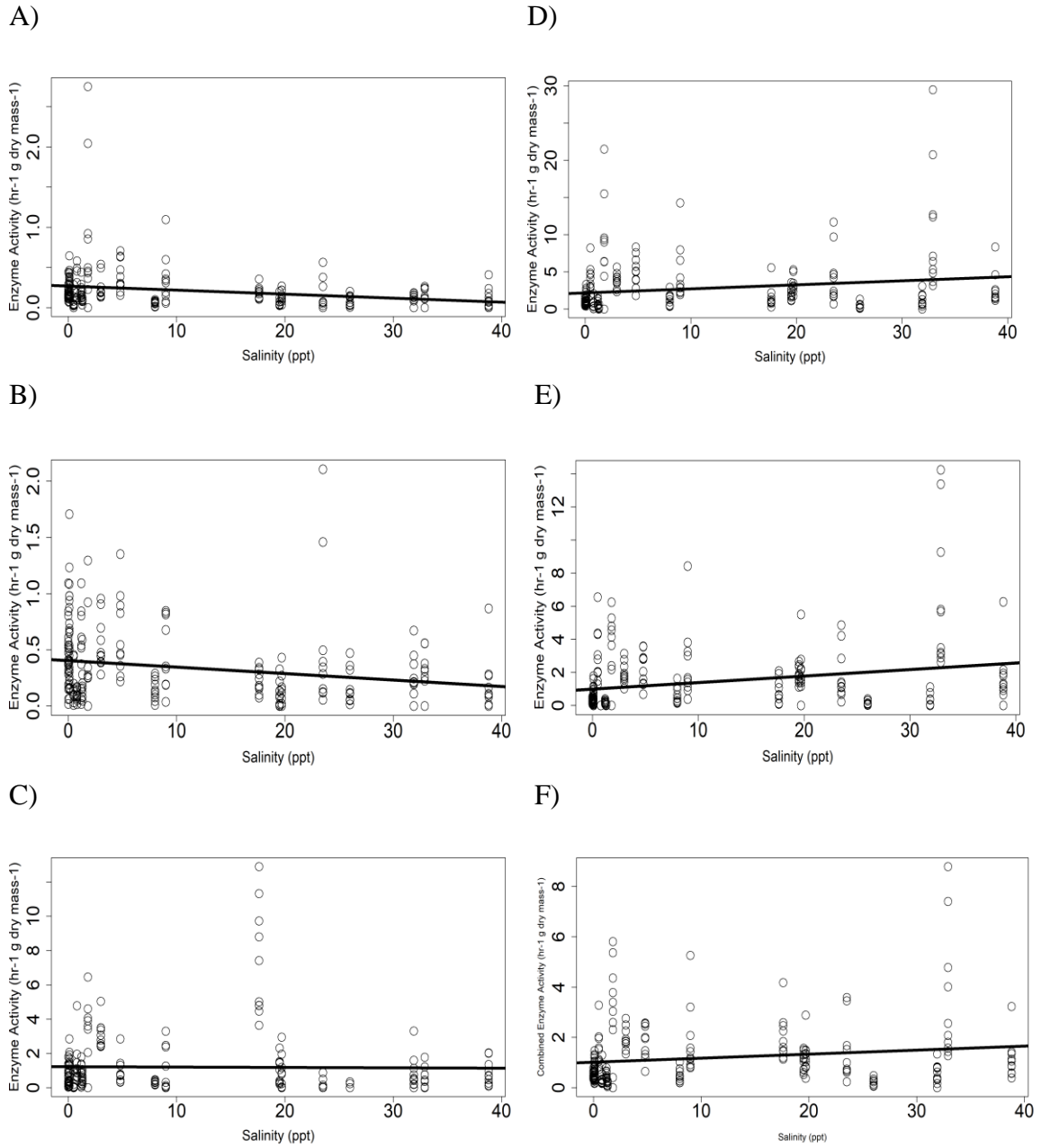
A)

B)

C)

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Figure 3. The relationship of NAGase (A), β-glucosidase (B), phosphatase (C), peroxidase (D), phenol oxidase (E), and total combined enzyme activity (F) to sampling site salinity collected from 13 sites along the US Gulf and Atlantic coasts. Each circle represents enzyme activity of one sediment sample.
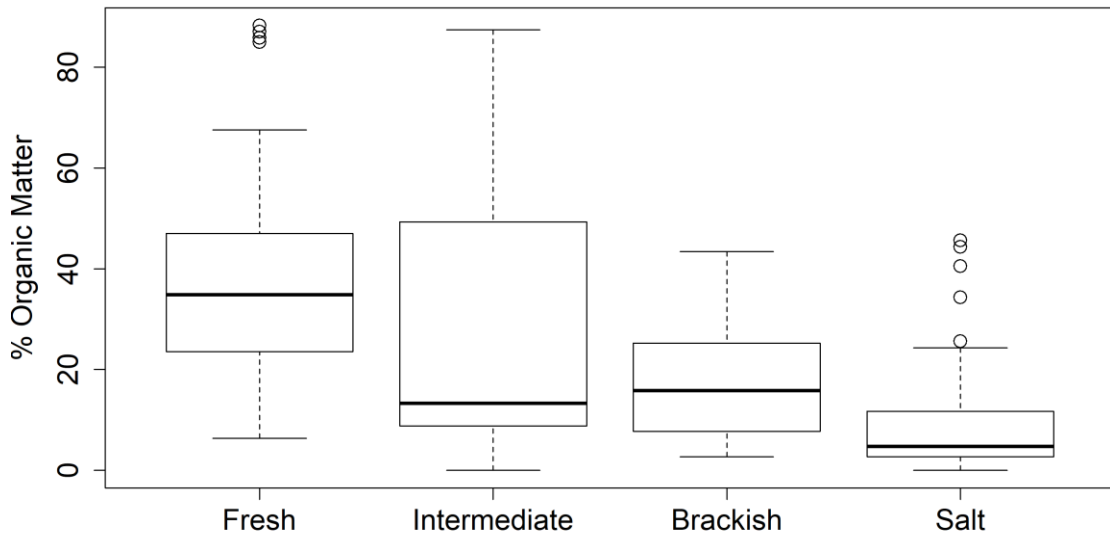
Figure 4. Organic matter content of wetland sediments taken along the US Gulf and Atlantic coasts. Fresh, intermediate, brackish, and salt marsh are defined as having a salinity range of 0-0.5 ppt, 0.5-5 ppt, 5-18 ppt, and 18-40 ppt, respectively. The box represents the second and third quartile being split by the median shown as a dark horizontal line. Circles represent extreme outliers while the tails of the graph represent the upper and lower quartile of results. Organic matter content was determined by combustion of dried samples.

Individual enzyme activity showed very slight reductions between sites low in organic matter compared to sites high in organic matter with for β-glucosidase (Figure 5A, R= -0.062); NAGase (Figure 5B, R= -0.161); and phosphatase (Figure 5C, R= -0.157). Peroxidase (Figure 5D, R= -0.484) and phenol oxidase (Figure 5E, R= -0.474) activity showed much lower levels of activity in marshes with high percentages of organic matter compared to marshes with low organic matter content. Combined percent organic matter at sampling site decrease dramatically and significantly as combined enzyme activity increased (Figure 5F, R= -0.496, p<0.001). Sites with the highest combined enzyme activities averaged soil organic matter content of about 5% compared to soil with about 70% organic matter content at sites with the lowest combined enzyme activities.
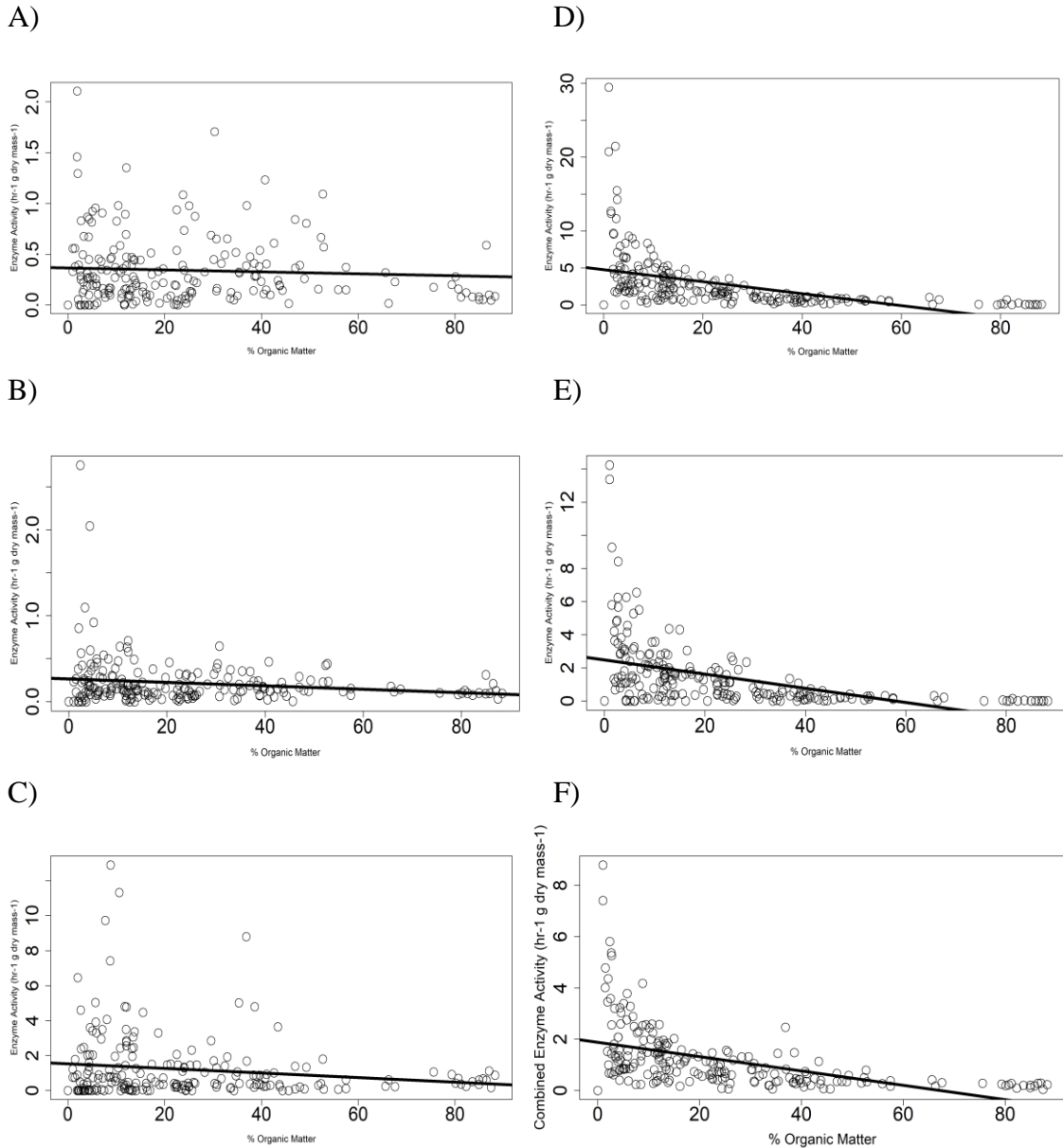
A)

D)

B)

E)

C)

F)

Figure 5.

The relationship between combined sediment microbial enzyme activity and organic

matter content for sediment collected from 13 sites along the US Gulf and Atlantic

coasts. The combined average of the individual enzyme activity of β-glucosidase (A),

NAGase (B), phosphatase (C), peroxidase (D), and phenol oxidase (E) at each site

determined combined enzyme activity (F). Organic matter content was determined by

combustion of dried samples.

Discussion

Current climate change models predict sea-level rise will increasingly impact coastal wetlands (Craft et al., 2009). Understanding the coastal wetland sediment microbiome and its ability to maintain enzymatic functions through increased environmental stress is important for predicting the consequences of SLR on the wetland ecosystem. This study analyzed sediment from along the Atlantic and Northern Gulf coasts at differing salinity gradients of fresh, intermediate, brackish, and salt marshes. Microbial extracellular enzyme activity was measured to estimate potential nutrient cycling and organic matter degradation. Activities of β-glucosidase, peroxidase, phenol oxidase were measured to assess organic carbon decomposition. Activities of NAGase and phosphatase were determined as indicators of organic nitrogen and phosphorus mineralization, respectively.

Salinity may be an influencing factor for microbial extracellular enzyme activity in coastal wetland sediments. The effect of highly saline sites on specific enzymatic activity varied. The significant decrease of NAGase activity in high salinity locations suggests that saltwater intrusion into fresh marsh environments will reduce the capacity for organic nitrogen mineralization (Olsen et al., 2014). The lack of effect of salinity on phosphatase suggests that phosphate levels in coastal marsh may not be affected by salt water intrusion due to SLR. This is important as eutrophication of coastal water is due in large part to increased levels of phosphate and nitrate (Fennel and Laurent, 2018). If

phosphatase activity was not independent of salinity and was low at high salinity levels, eutrophication would likely increase because the salt marsh microbiome would be less efficient in processing phosphate for sequestration, leading to excess phosphate draining into coastal ocean waters. This situation does not correspond to this study's findings.

Lower NAGase activity levels in sites with high salinity signal that organic nitrogen processing bacteria may be less efficient in saline environments. Less NAGase activity suggests less organic nitrogen mineralization resulting in fewer gaseous nitrogen compounds being released from the wetland and leaving the coastal system (Hefting et al., 2013). Excess nitrogen, along with phosphate, results in eutrophication. As fresh marsh is converted into a less efficient nitrogen processing salt marsh, an increase in nitrogen runoff into coastal waters can be expected (Olsen et al., 2014). Nitrogen also affects carbon sequestration as high levels of soil nitrogen can restrict carbon cycling and promote plant growth leading to carbon accretion (Martina et al., 2016). This study found that not only did high salinity correlate with less organic nitrogen processing, but so did soil with a high percentage of organic matter. This leads to the question of whether NAGase activity is low because of a reduced need to actively process organic nitrogen as inorganic nitrogen is plentiful in the soil environment, or whether higher salinities depress NAGase activity. Water temperature did not relate to NAGase activity in this study.

β-glucosidase, peroxidase, and phenol oxidase activity are measures of organic carbon metabolism. While enzyme activity did not increase with salinity for every processing enzyme, combined carbon metabolizing enzyme activity was greater at sites of higher salinity. This implies the potential for freshwater marshes to build sediment

faster than saltwater marshes as carbon is not being cycled as quickly (Zhang et al., 2011). Increasing soil carbon metabolism acts to decrease the sink effect of the wetlands and will turn them into a source of carbon release. This is evident as fresh marsh soil samples show about a 700% higher average percent organic matter content than salt marsh. Fresh marshes' high retention of organic carbon may lead to increased marsh elevation. This outcome associated with fresh marshes may be at risk. If marsh salinity increases, the higher carbon metabolism associated with high salinity environments may lead to less build-up of land banks further amplifying the effect of salt water intrusion landward due to SLR (Luo et al., 2017).

The potential for more organic carbon mineralization to occur due to the observed increase in carbon based microbial enzyme activity is reflected by the relationship of enzyme activity and percent organic matter. Organic matter is carbon based, so the percent organic matter found at marsh sites gives insight into the prolonged carbon enzyme activity at that site. Organic matter content is related to carbon input from vegetation and carbon output from microbial activity (Wang et al., 2003). The organic carbon levels are likely due in part to the specific activities of carbon processing enzymes β-glucosidase, peroxidase, and phenol oxidase. Sediment organic matter decreased dramatically and significantly with combined enzyme activity. Low organic matter content may indicate high enzyme activity because the organics are consumed by the microbes depressing their levels. Low organic matter content in sediment could also indicate low enzyme activity levels as the nutrient poor soil supports less microbial biomass, which leads to low levels of activity (Hu et al., 2011). This supports the understanding that salinity will increase carbon metabolism which ultimately affects the

make-up of the marsh soil through decreasing carbon accumulation. Site location and in situ water temperature were also factors influencing carbon-based enzyme activity along with the covariate of salinity. This suggests that local environmental conditions such as vegetation are important to consider when predicting microbial activity in coastal wetlands (Xu et al., 2017).

Along with saltwater intrusion into wetlands from increased storm surges and SLR, climate change is expected to increase global water temperatures (Cheng et al., 2019). An increase in wetland water temperature has the potential to impact marsh microbial enzyme activity, and I found higher enzyme activity at sample sites with higher water temperatures for all enzymes except NAGase, which was largely not affected. Peroxidase and phenol oxidase each showed much higher activity in locations of warmer water, although phosphatase and β-glucosidase showed slightly lower activity at warmer sites. The large increase in peroxidase and phenol oxidase correlating with warmer water could potentially increase rates of organic carbon metabolism in the wetland. The dual factors of SLR and increased water temperatures may lead to increased carbon release due to enzyme activity. This will cause the further amplification of the conversion of wetlands from a carbon sink to source. Wetland sediment build up will be reduced and salt water migration landward will become even more drastic.

This study shows that wetland microbial extracellular enzyme activity is influenced by salinity, water temperature, and the percentage of soil organic matter. While this study's findings cannot predict how microbial activity in an individual wetland will respond to salt water intrusion, an expectation of what might occur in a higher salinity marsh can be predicted by understanding current wetlands. Wetland soils exposed

to higher salinity are generally higher in extracellular enzyme activity and lower in percent organic matter. Further research is required to understand if these differences correspond to differences in the composition of the sediment microbial community, and the implications of such changes for wetlands loss in the context of global climate change.

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