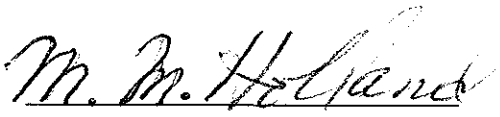
VEGETATION EFFECTS ON RHIZOSPHERE MICROBIALCOMMUNITIES IN COASTAL WETLANDS OF SOUTH MISSISSIPPI

by: Sean Woo Kang

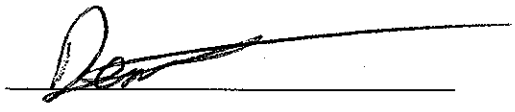
A thesis submitted to the faculty of The University of Mississippi in partial fulfillment of the requirements of the McDonnell-Barksdale Honors College

Oxford
March 2016

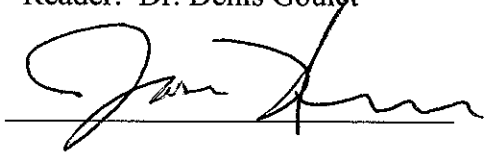
Approved by



Advisor: Dr. Marjorie Holland



Reader: Dr. Denis Goulet



Reader: Dr. Jason Hoeksema

VEGETATION EFFECTS ON RHIZOSPHERE MICROBIALCOMMUNITIES IN COASTAL WETLANDS OF SOUTH MISSISSIPPI

by: Sean Woo Kang

A thesis submitted to the faculty of The University of Mississippi in partial fulfillment of the requirements of the McDonnell-Barksdale Honors College

Oxford
March 2016

Approved by

_____

Advisor: Dr. Marjorie Holland

_____

Reader: Dr. Denis Goulet

_____

Reader: Dr. Jason Hoeksema

Acknowledgements

I would like to thank Dr. Sarah Liljegren for her guidance in search of a thesis project and thesis advisor. I would also like to thank my thesis advisor, Dr. Marge Holland, for her constant advice, support, and motivation. I would like to thank Dr. Denis Goulet and Dr. Jason Hoeksema for taking time out of their day to serve as readers. I would like to thank Emily McCann, Tyler Murphy, Hak Chul Lee, Austin Bosch, and Dr. Holland for serving as readers and giving feedbacks for my Honors Senior thesis. I would like to give a special thanks to Hak Chul Lee for his guidance and patience in our research trips and lab works. I would like to thank Dr. John Samonds for representing the McDonnell-Barksdale Honors College at my thesis defense and helping me through this process. I would like to thank Grand Bay National Estuarine Research Reserve for providing a place to stay close to our research sites and McDonnell-Barksdale Honors College for providing funding for equipment and DNA analysis. I would also like to thank Dr. Holland and Dr. Colin Jackson for allowing me to use their laboratories. Thanks to my family and friends for all their continuous support and encouragement especially Chris Lloyd.

Abstract

The Mississippi vegetated coastal wetlands consist of many salt and brackish marshes. In those marshes, there are two plant species *Spartina alterniflora* and *Juncus roemerianus* that thrive in those environments. This would not be possible without the benefits of microbial communities that live in the portion of the plant's soil called the rhizosphere. The rhizosphere is crucial for plant nutrition, health, and quality. It supports the biomass and activity of microorganisms for carbon sequestration, ecosystem functioning, and nutrient cycling in natural ecosystems. To investigate the vegetation effects on rhizosphere microbial communities in coastal wetlands, plant samples and their rhizosphere soils were collected from two brackish transects and two saltwater transects at Graveline Bayou, Gautier, MS. A number of biotic and abiotic factors were measured, and their impacts on bacterial community composition and diversity were determined via Illumina MiSeq 16S rRNA gene sequence. Overall, the composition of rhizosphere bacterial community in coastal wetlands were dominated by Proteobacteria and Planctomycetes. The effects of seasonal patterns and plant developmental stages had no impacts on rhizosphere microbial communities due to similar pH level, soil moisture, and organic matter content in soil between winter and summer seasons of 2015. Salinity increased bacterial community diversity especially Proteobacteria and Bacteroidetes. There are several contrasting reports that portrayed the dominant factor in determining the diversity of rhizosphere microbial communities as either the plant species itself or the soil type of the site. In this study, the soil type was the major driving force in bacterial community diversity.

Table of Contents

List of Tables

List of Figures

I. Introduction:

A. Mississippi Coastal Wetlands

Vegetated coastal wetlands consist of salt and brackish marshes, tidal freshwater marshes, swamps, and submerged aquatic vegetation beds. Non-vegetated coastal wetlands comprise tidal, open water habitats such as bayous, river channels, the Mississippi Sound along the Gulf Coast, and the Gulf of Mexico. Mississippi's coastal wetlands are not considered federally regulatory wetlands since the substrates of the Mississippi's coastal wetlands do not sustain emergent vegetation. Instead, they are federally classified as deepwater habitats, mudflats, or vegetated shallows. Mississippi's coastal wetlands are part of a large estuarine system. An estuary is created when fresh water from local rivers mixes with the sea water of the Gulf of Mexico. This forms a zone of brackish water that extends from the northern beaches of Mississippi's barrier islands inland to the bays and bayous of the mainland (Mississippi Department of Marine Resources [MDMR], 1999).

B. Habitat Parameters

The type of coastal wetland habitat is determined largely by its location within the landscape, the salinity of the adjacent waters, and the elevation of the site. For example, habitats located on the mouth of a river with low elevation will be unique from those located up the river with higher elevation. Coastal marshes are marked as either "high" or "low" marshes depending on their locations below or above the mark of mean high water. Low marshes are more susceptible to salinity changes since they are often flooded. High marshes are located landward of low marshes and are only flooded during high tidal events. Since coastal wetlands are influenced daily by the rise and fall of the tides, rooted coastal wetland plants have evolved

to live and to compensate for the lack of oxygen in those areas by pumping air through their leaves down to their roots. Salinity levels within the Mississippi coastal wetlands range between from full seawater strength water (35 parts per thousand [ppt]) in open water areas located south of the barrier islands to freshwater levels (0 ppt) in tidal areas located upstream in the rivers leading into the Mississippi Sound (MDMR, 1999). This is important as plants have a specific range of salinity tolerance. If the salinity level changes in an area over time due to saltwater intrusion events or sea-level rise, the physiology of plant species will change and eventually affect the ecosystem structure (Pezeshki *et al*., 1989; McLeod *et al*., 1996; Shirley and Battaglia, 2006).

C. Habitat Types

Coastal salt and brackish marshes have very few plant species especially at lower elevations due to high salinity levels. They can be divided into three main vegetative zones by high, mid, and low elevation. The lowest zone is the outer edge adjacent to open water and is regularly flooded by the tides. It is mostly composed of plants called smooth cordgrass (*Spartina alterniflora*) due to their high salt-tolerance (MDMR, 1999). Smooth cordgrass is a tall, smooth grass that grows from 2 to 7 feet tall. Smooth cordgrass colonies grow parallel to and along shorelines and will tolerate inundations with 0 to 35 ppt salinity and sandy aerobic or anaerobic soils with pH levels from 3.7 to 7.9. *Spartina alterniflora* has a complex root system that strongly binds to the banks which allows the grass to absorb wave energy to prevent the tide from eroding the shoreline (United States Department of Agriculture [USDA], 2002). The intermediate zone is sometimes flooded by higher than average tides and is primarily composed of black needlerush (*Juncus roemerianus*) (MDMR, 1999). Black needlerush is a moderate growing, group forming, grass-like perennial. The plant is very rigid and ranges from 0.5 to 1.5

meters tall, and it has a high tolerance to anaerobic conditions and calcium carbonate. Black needlerush tolerates pH levels from 4.0 to 7.0 (USDA, 2015). The high zone is flooded by high tidal events such as tidal surges and is mostly composed of salt marsh hay and some black needlerush (MDMR, 1999).

D. Plants and Soil Microbes

Each plant species has indigenous microbial populations living in its rhizosphere soil, and specific microbial communities are selected by the plant's root exudates (Berg and Smalla, 2009). Hiltner described a rhizosphere in 1904 as "the portion of soil where microorganisms interact with the plant's root system." In more detail, rhizosphere soil is the narrow region of soil attached to the plant's root system and is directly affected by root secretions and soil microorganisms. The rhizosphere functions to support plant nutrition, health, and quality by being a dynamic and complex interface for chemical, physical, and biological interactions (Berg and Smalla, 2009).

There is also the phenomenon that rhizosphere enhances the biomass and activity of microorganisms due to the secretions from the root exudates (Sørensen, 1997; Raaijmakers *et al*., 2009). Root exudates act as the driving force of selecting specific microbial communities and as messengers that communicate and initiate biological and physical interactions between roots and soil microbes (Berg and Smalla, 2009). Root exudates accomplish this by using ions, free oxygen, water, enzymes, mucilage, and a diverse array of carbon-containing primary and secondary metabolites to attract or to reject specific microorganisms (Uren, 2000; Berg and Smalla, 2009). The composition of root exudates differs from plant to plant and influences the abundance of microorganisms in the vicinity of the root (Somers *et al*., 2004). The root exudates' use of specific compounds recognized by specific microorganisms create a

competitive colonization of the rhizosphere and establishment in the root zone (Bais *et al*., 2002). It is also important to note that microorganisms selected by root exudates can influence or select each other for the composition of microbial communities in the rhizosphere (Rasche *et al*., 2006). In return, microorganisms protect the plant host against pathogens, stimulate plant growth by various mechanisms, decompose and mineralize organic matter, and enhance the bioavailability of mineral nutrients (Ortíz-Castro *et al*., 2009). This makes plant-microorganism interactions in rhizosphere crucial for carbon sequestration, ecosystem function, and nutrient cycling in natural ecosystems (Singh *et al*., 2004).

E. Soil Type and Plant Species

There are contrasting reports (Da Silva *et al.*, 2003; Nunan *et al.*, 2005; Salles *et al.*, 2004) indicating plant species or soil type as dominant factor and also concluding that the rhizosphere bacterial community composition is influenced by a complex interaction between soil type, plant species and root zone location (Marschner *et al*., 2001). Da Silva *et al*. (2003) concluded that soil type instead of maize cultivar type was the overriding determinative factor that affected the rhizosphere microbial community structure of *Paenibacillus*. Salles *et al*. (2004) also found using genus-specific denaturing gradient gel electrophoresis (DDGE) that plant species had less impact than land on rhizosphere microbial community structure of *Burkholderia*. Nunan *et al*. (2005) demonstrated that plant species are the major driver of bacterial community composition by analyzing field-grown root-associated communities of *Agrostis capillaris*, *Agrostis vinealis*, *Deschampsia cespitosa*, *Festuca rubra*, and *Poa pratensis*. Nunan *et al*. (2005) analyzed the plant species using plastid tRNA leucine UAA gene intron and also analyzed plant-related bacterial communities using terminal restriction fragment length polymorphism (T-RFLP) and DGGE.

II. Objectives, Questions, and Hypotheses:

My goal was to collect and analyze *Spartina alterniflora* and *Juncus roemerianus* and their rhizosphere soil from coastal wetlands in Graveline Bayou in south Mississippi. I compared the results to address three main objectives.

The first objective was to investigate the influence of plant species and environmental factors in different coastal wetland conditions on rhizosphere microbial communities. Which factors such as environmental conditions (abiotic) or host plants (biotic) are the dominant factors in influencing the rhizosphere microbial communities? Does each plant species harbor unique microbial community structure? I hypothesize that the dominant effect on the rhizosphere microbial communities will be the plant species itself.

The second objective was to investigate seasonal patterns of coastal wetland rhizosphere microbial community structure of plant species. Do seasonal factors such as temperature influence the rhizosphere microbial communities? I hypothesize that the rhizosphere microbial communities will be different in the summer and the winter because of the seasonal precipitation and temperature.

The third objective was to determine salinity level effects on microbial community structure of rhizosphere soil in coastal marshes. What are the characteristics of rhizosphere microbial communities of halophytes across a salinity gradient? How will the microbial communities react to different salinity levels and plant species? Does salinity level affect the diversity of microbial communities in plants? I hypothesize that rhizosphere microbial communities will vary across the salinity level to adapt the plant's tolerance to salinity.

III. Methods:

Along the Mississippi Gulf Coast, there were two brackish transects (BT3 and BT4) and two saltwater transects (ST6 and ST7) established in 2009 in Graveline Bayou, Gautier, MS (Chen, 2011) (Figures 1 & 2). The two target plant species are *Spartina alterniflora* and *Juncus roemerianus* (Figure 3). Three replicates of a plant sample (consist of the plant, its roots, and bulk soil) of the two plant species were collected in high, mid, and low marsh zones of all four transect. I sampled in February 2015 and August 2015 to determine if the difference in salinity levels between winter and summer conditions had an impact on rhizosphere soil. At each transect, I used markers and an open reel measuring tape to determine the low marsh zone at 0 meter (m) which originates at the targeted plant species closest to the water and low elevation, the mid marsh zone at 20 m, and high marsh zone at 40 m (Figure 4). I measured the salinity and pH using a waterproof portable pH/Salinity Meter. I collected a total of 78 plant samples using a shovel and stored them in zip-lock bags labeled according to the zones, the transect, and the plant species. Before storing the samples in zip-lock bags, I measured ten or more plants' roots in centimeters using a ruler. After collecting my plant samples, I immediately stored the plant samples in coolers and transported them to the laboratory in Shoemaker Hall of the University of Mississippi in Oxford, MS. I then stored the plant samples in freezers at -20°C until DNA extraction.

In the laboratory, I thawed out the plant samples and measured the rhizosphere soil moisture of the best plant sample of each plant species in each zone within the transects. This was accomplished by collecting 10-15 grams from each unique plant sample from the zip-lock bags and placing them in tin foil using a plastic spoon. I measured the original soils first using a Digital Jennings CJ-600 gram scale and then dried them in the convection oven at 70°C for 48

Figure 1. Location of the Graveline Bayou in Gautier, MS (Maphill)

Graveline Bayou

State of Mississippi

Figure 2. Location of the Four Transects at Graveline Bayou, MS (Google Earth)

Figure 3. Two Target Plant Species

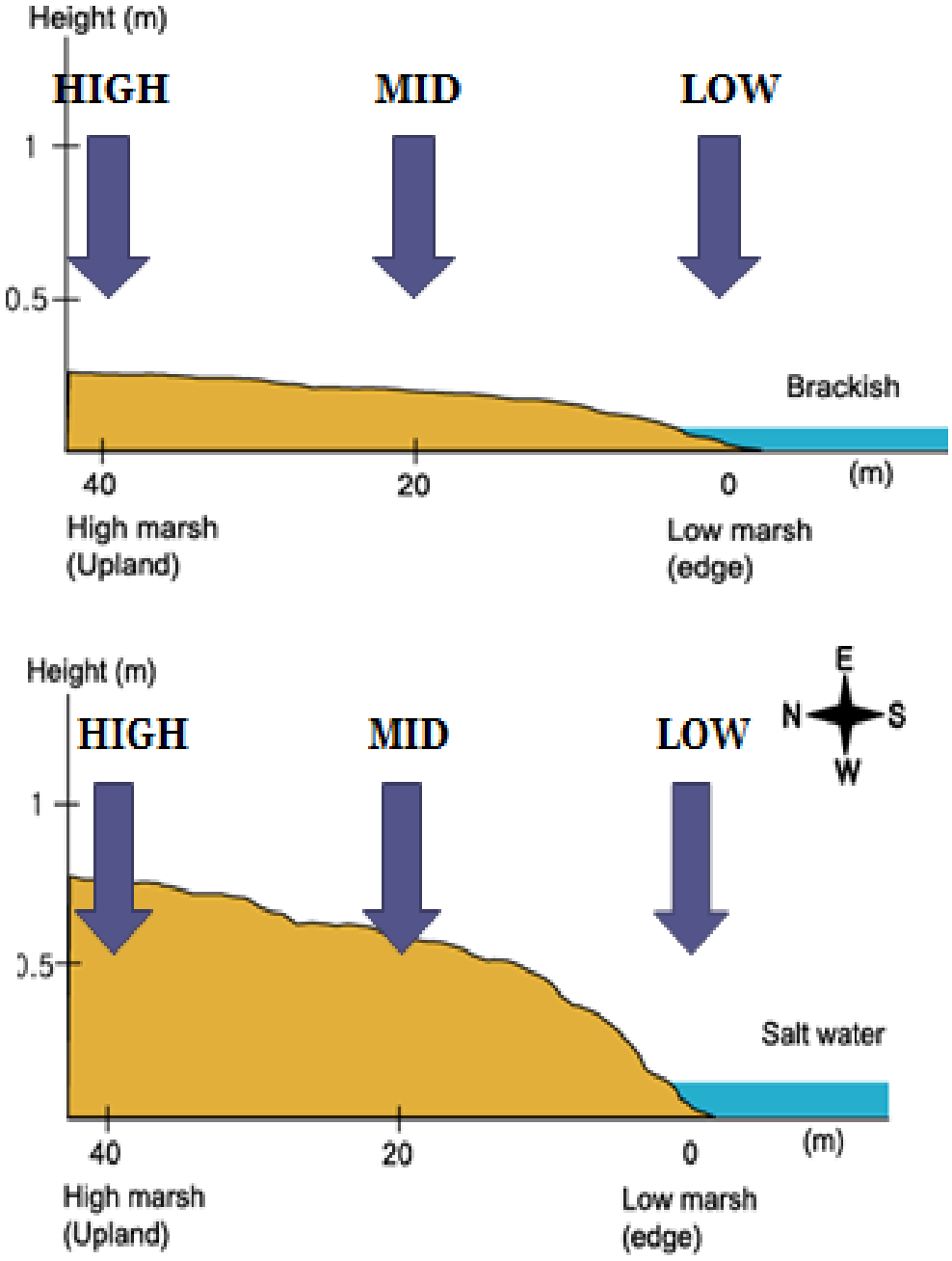
Spartina alterniflora

epod.usra.edu

Juncus roemerianus

regionalconservation.org

Figure 4. Brackish and Coastal Transects Divided into High, Mid, Low Marsh Zones (Chen, 2011)



hours. After samples were dried, I subtracted the dried soils from their original weight to obtain the soil moisture. After I finished measuring the soil moisture, I put the dried soils in small crucibles and ashed them in a Muffle Furnace at 500°C for 4 hours. I subtracted the ashed weight from the weight of the dried soils to obtain the organic matter content.

For DNA extraction and preparation for polymerase chain reaction (PCR) amplification, we used the PowerSoil DNA Isolation Kit. DNA was extracted from 0.25 grams of each original plant soil sample by the process of cell lysis, removing PCR inhibitors, capturing total genomic DNA on a silica membrane in a spin column format, and then washing and eluting DNA from the membrane (MO BIO Laboratories, Inc., 2015). After DNA extraction, the finished samples were sent to the University of Mississippi Medical Center for PCR amplification and Illumnia sequencing using the 16S ribosomal RNA (rRNA) methods. 16S rRNA squencing is a method used to identify the bacteria in a given sample and to study its phylogeny and taxonomy from complex environments (Janda *et al*., 2007).

To analyze the Illumina MiSeq 16S rRNA gene sequence, I used the Mothur processing system and procedures recommended by Kozich *et al*. (2013). I used different programs in the Mothur system in chronological order: to obtain files from the raw fastq data which are the sequence data, to reduce sequence errors and initial processing, to align sequences using SILVA V4, to remove remaining errors, and to classify the sequences using Greengenes. The SILVA V4 is a database specified for the V4 region of 16S rRNA that stretches from position 11,894-25,319 in the SILVA database. This makes the process of aligning sequences faster since SILVA V4 just covers the region of the desired gene. The SILVA database contains 50,000 characters long and accommodates bacteria, archaea, and the eukaryotic 18S rRNA gene. The Greengenes database contains 7,682 characters long and provides over 200,000 reference

bacterial and archaeal sequences (Schloss *et al*., 2009). I used the Mothur processing system to establish operational taxonomic units (OTUs) in the classified sequences for analysis. OTUs are defined as clusters of similar 16S rRNA sequences that are used as basic diversity units in large-scale characterizations of microbial communities (Schmidt *et al*., 2014). This process generates a distance matrix for all sequence combinations and provides similarities to each other.

In order to run statistical analyses to determine significant differences between the rhizosphere soil and the effects of seasons, sites, and plant species, 3 way design files of seasons, sites, plant species, plant species and sites, season and sites, season and plant species, and season and plant species and sites were created and used in analysis of molecular variance (AMOVA). AMOVA is a statistical method to detect molecular variation in population or individual species.

IV. Results:

In brackish transects 3 and 4, *Spartina alterniflora* was only found in low zone. *Juncus roemerianus* was found in mid and high zones. In saltwater transect 6, *Spartina alterniflora* was only found in low zone. *Juncus roemerianus* was not found in mid zone due to a road but was found in high zone. In saltwater transect 7, *Spartina alterniflora* was only found in low zone. *Juncus roemerianus* was found in mid and high zones.

The salinity levels in all transects (21.1 ppt) in the summer season 2015 were higher than the salinity levels in all transects (15.2 ppt) in the winter season 2015. The highest salinity level (22.8 ppt) was in ST6 in the summer 2015 while the lowest salinity level (14.4 ppt) was in BT3 in the winter 2015 (Figure 5).

The pH levels in all transects in the winter season 2015 (pH: 9.34) were slightly higher than the pH levels in all transects in the summer season 2015 (pH: 8.84). The highest pH level (9.39) was in BT4 in the winter 2015 while the lowest pH level (8.79) was in BT3 in the summer 2015 (Figure 6).

The root length (RL) across the two plant species were slightly longer in the summer 2015 (RL: 9.3 cm) than the root length in the winter 2015 (RL: 9.0 cm). The root length of *Juncus roemerianus* (RL: 10 cm) were longer than the root length of *Spartina alterniflora* (RL: 7.4 cm). The longest root length across the two plant species were in ST7 (RL: 10.6 cm) while the shortest root length across the two plant species were in BT3 (RL: 8.53 cm) (Figure 7). Overall including seasons and plant species, the longest root length of individual plant samples were under *Juncus roemerianus* in the mid zone of ST7 during summer 2015 (RL: 13.4 cm) while the shortest root length of individual plant samples were under *Spartina alterniflora* in the low zone of BT4 over summer 2015 (RL: 4.6 cm) (Appendix B). In the winter 2015, the longest

Figure 5. Salinity Level in Transects Compared between Winter 2015 and Summer 2015 Conditions

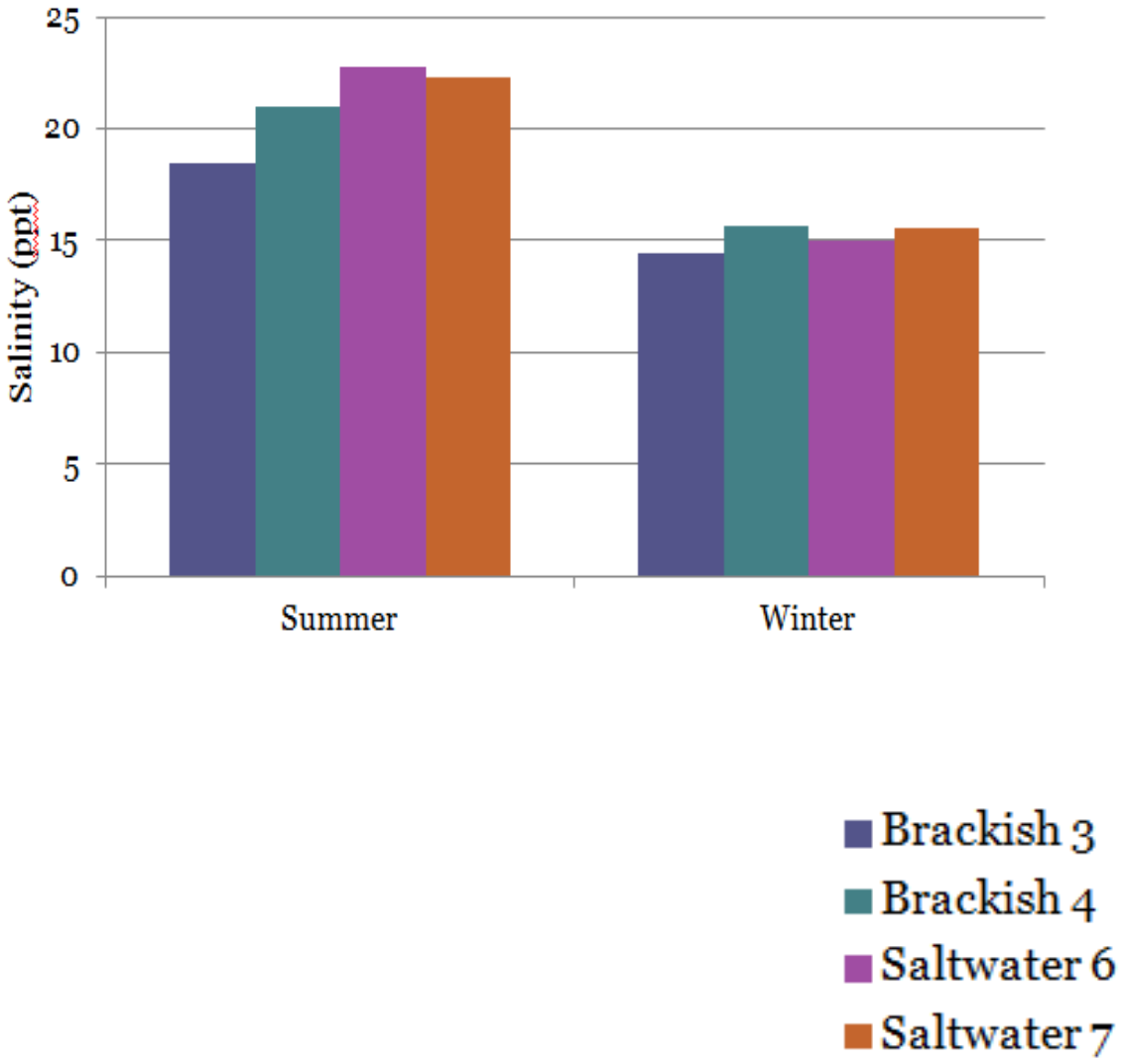


Figure 6. pH Level in Transects Compared between Winter 2015 and Summer 2015 Conditions

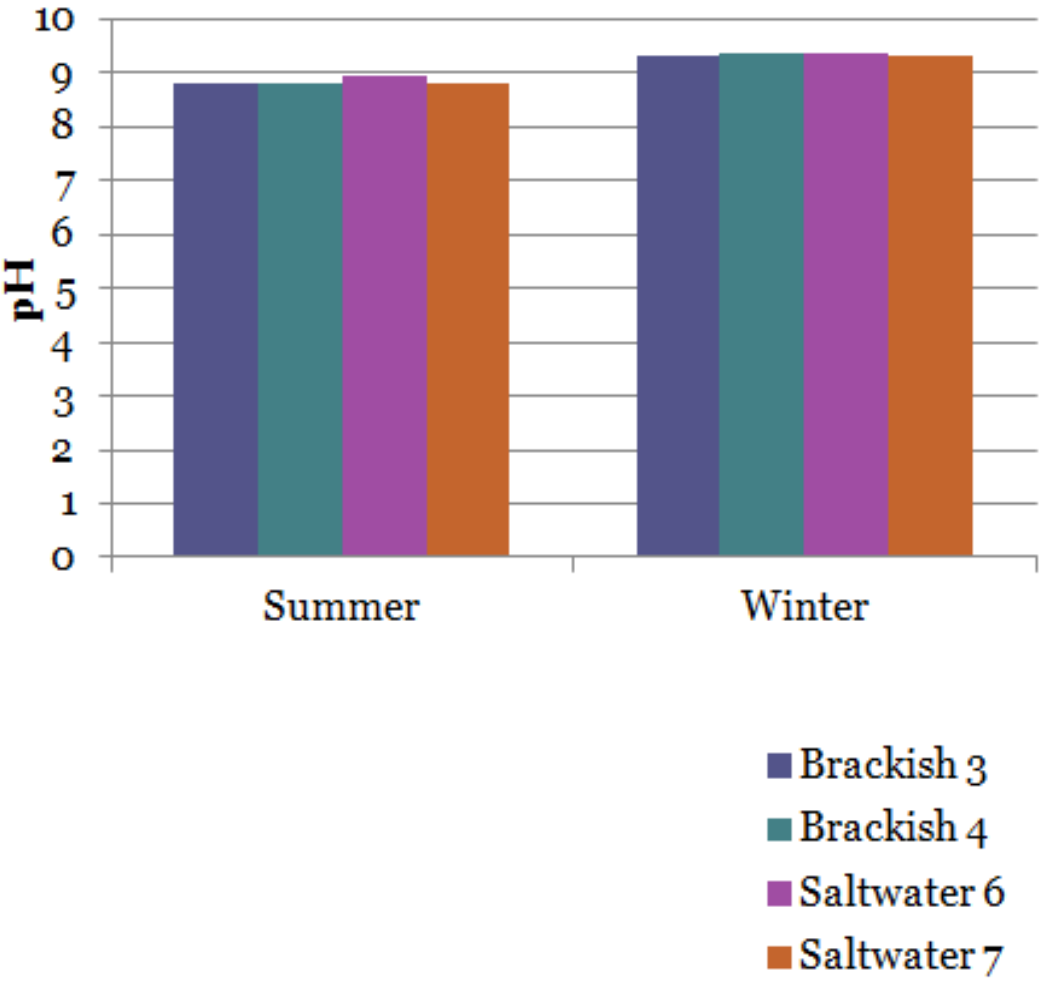
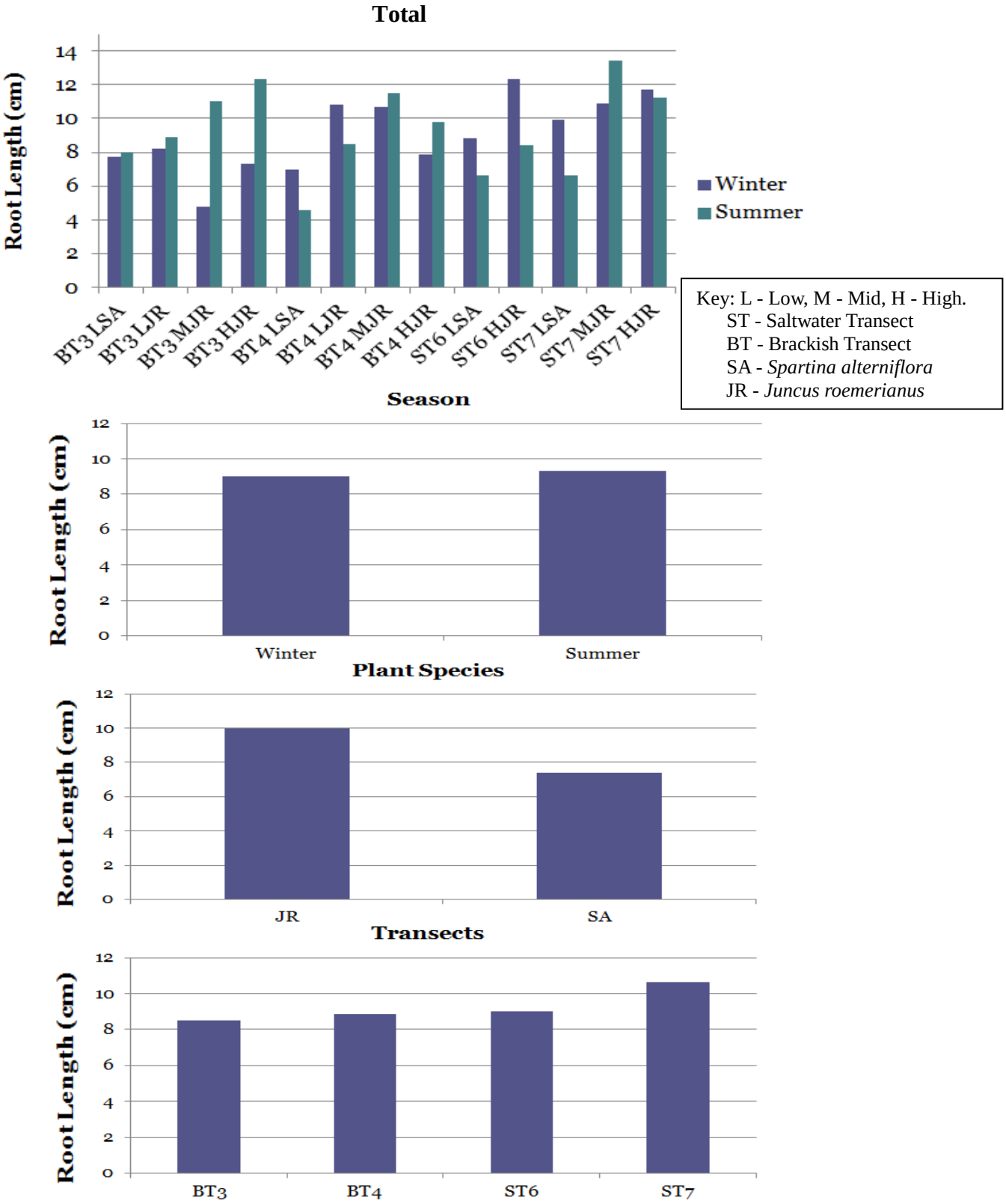


Figure 7. Root Length Compared between Winter 2015 and Summer 2015 Conditions

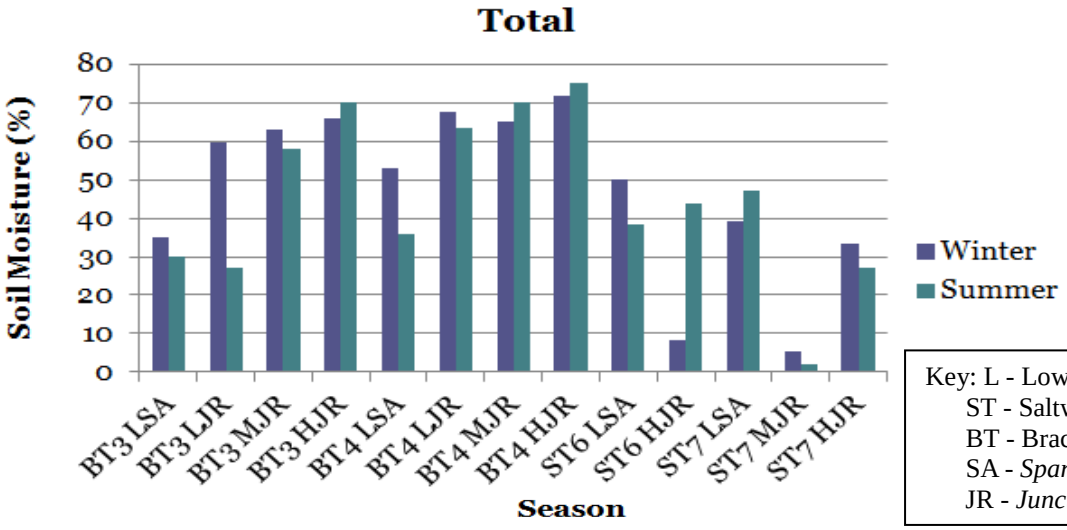


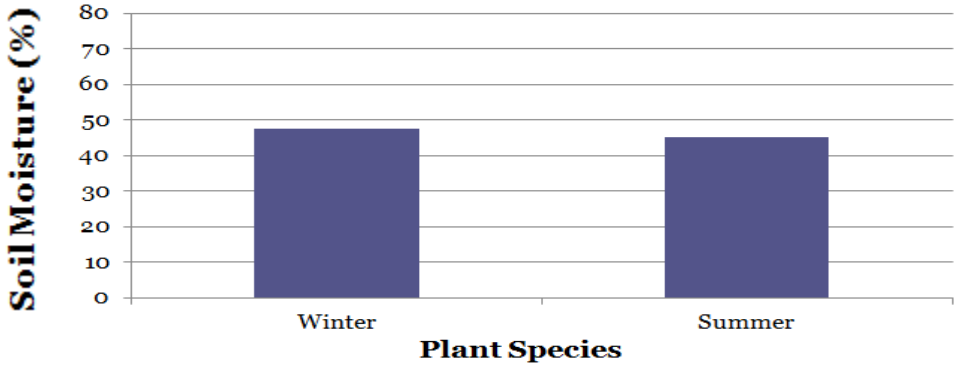
root length of individual plant samples were under *Juncus roemerianus* in the high zone of ST6 (RL: 12.3 cm) while the shortest root length of individual plant samples were under *Juncus roemerianus* in the mid zone of BT3 (RL: 4.8 cm) (Appendix A).

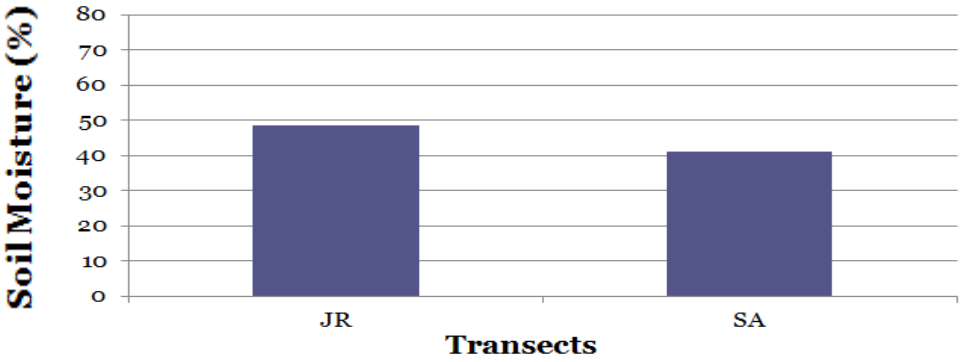
The soil moisture (SM) across the two plant species in the winter 2015 (SM: 47%) was slightly higher than the soil moisture in the summer 2015 (SM: 45%). The soil moisture of *Juncus roemerianus* (SM: 49%) was higher than the soil moisture of *Spartina alterniflora* (SM: 41%). The highest soil moisture across the two plant species was in BT4 (SM: 63%) while the lowest soil moisture across the two plant species was in ST7 (SM: 26%) (Figure 8). Overall including seasons and plant species, *Juncus roemerianus* in the high zone of BT4 during summer 2015 (SM: 75%) had the highest soil moisture while *Juncus roemerianus* in the mid zone of ST7 during summer 2015 (SM: 2.0%) had the lowest soil moisture (Appendix D). In the winter 2015, *Juncus roemerianus* in the high zone of BT4 (SM: 72%) had the highest soil moisture while *Juncus roemerianus* in the mid zone of ST7 (SM: 5%) had the lowest soil moisture (Appendix C).

There was basically no difference in organic matter content (OM) across the two plant species in the winter 2015 (OM: 8.4%) and in the summer 2015 (OM: 8.2%). The organic matter content of *Juncus roemerianus* (OM: 11%) was higher than the organic matter content of *Spartina alterniflora* (OM: 3%). The highest organic matter content across the two plant species was in BT4 (OM: 14%) while the lowest organic matter content across the two plant species was in ST7 (OM: 3%) (Figure 9). Overall including seasons and plant species, *Juncus roemerianus* in the high zone of BT4 during summer 2015 (OM: 23%) had the highest organic matter content while *Juncus roemerianus* in the mid zone of ST7 (OM: 1%) during summer 2015 and *Spartina alterniflora* in the low zone of BT3 (OM: 1%) during winter 2015 had the lowest soil moisture

Figure 8. Soil Moisture Compared between Winter 2015 and Summer 2015 Conditions







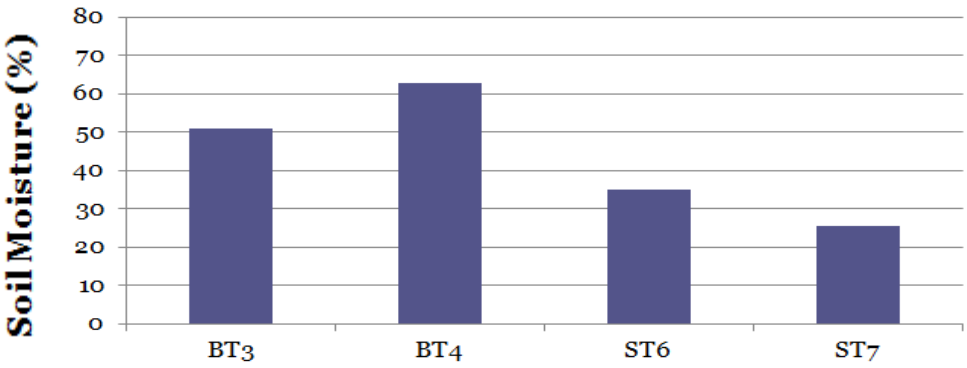
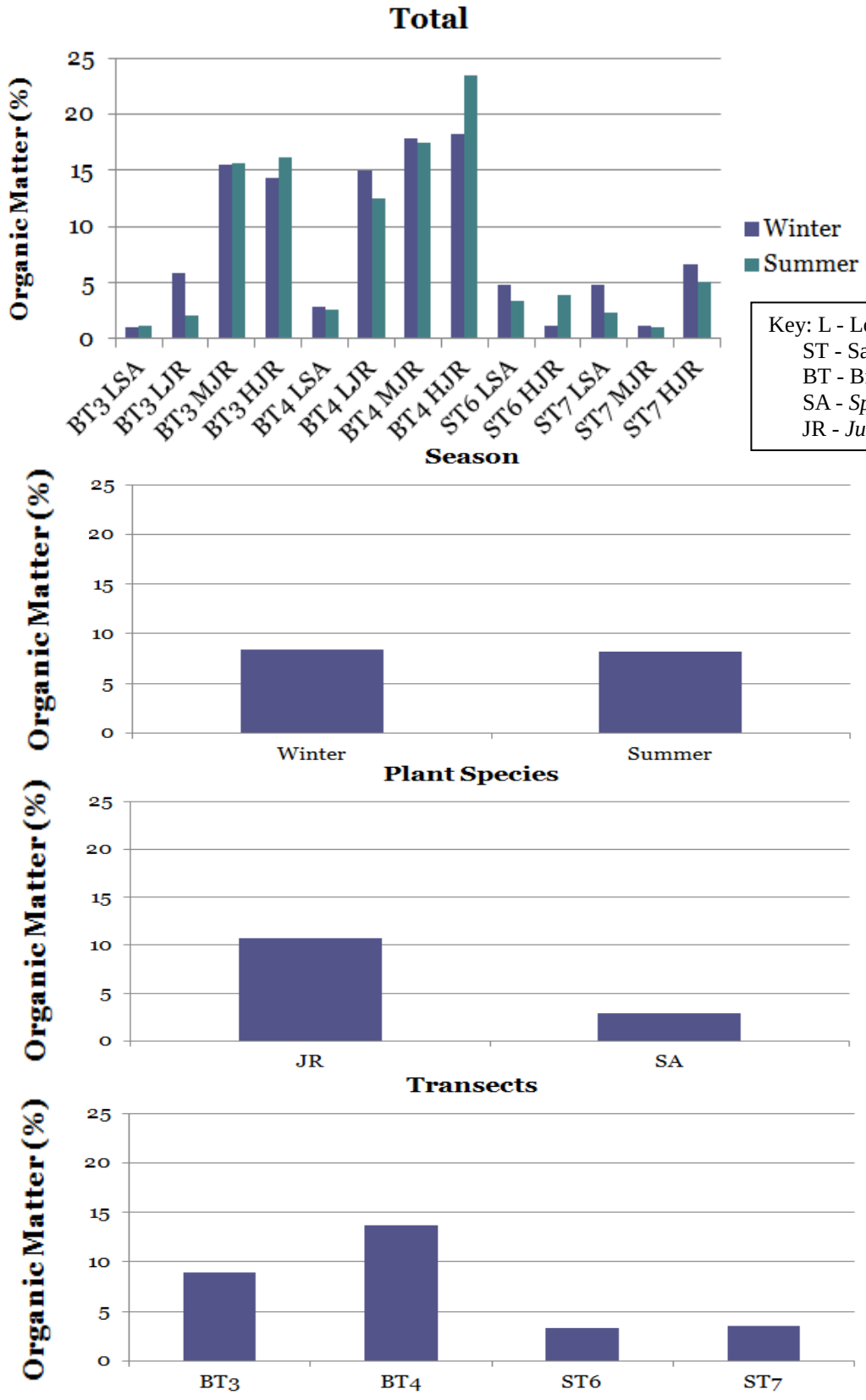


Figure 9. Organic Matter Compared between Winter 2015 and Summer 2015 Conditions



contents (Appendix E & F). In winter 2015, *Juncus roemerianus* in the high zone of BT4 had the highest organic matter content (Appendix E).

A total of 1,855,732 sequences with a mean length of 253.059 base pairs from 78 samples were identified using Illumina 16S rRNA gene sequencing. After removing repetitive sequences such as chimeras which are sequences that originated from more than one initial sequence, 880,689 sequences with 122,829 unique sequences remained from the total sequences. They were then classified into OTUs with a 0.03 cutoff (grouped sequences with greater than 97% similarities into a single OTU) to create a taxonomy file with 41,084 OTUs.

In the whole data set of sequences, the most classified dominant bacterial phylum was Proteobacteria (25.0%), followed by Planctomycetes (13.9%) and Chloroflexi (7.90%) (Figure 10). The percentages of the phyla varied among the two plant species, seasons, and sites.

The percentage of Proteobacteria was the lowest in *Spartina alterniflora* in the low zone of ST6 during winter 2015 (18.7%) while the percentage of Proteobacteria was the highest in *Juncus roemerianus* in the high zone of ST7 during summer 2015 (29.8%). There were significant differences in percentages of Proteobacteria between winter 2015 and summer 2015 in *Juncus roemerianus* in the high zone of BT3 (19.5% and 27.2% respectively) and *Spartina alterniflora* in the low zone of ST6 (18.7% and 27.5% respectively). There were no differences in percentages of Proteobacteria between *Spartina alterniflora* and *Juncus roemerianus* in the low zone of BT3 and BT4 in both winter 2015 and summer 2015 (Tables 1 & 2).

The percentage of Planctomycetes was lowest in *Juncus roemerianus* in the high zone of ST6 during summer 2015 (12.3%) while the percentage of Planctomycetes was the highest in *Juncus roemerianus* in the low zone of BT3 during winter 2015 (17%). There were no significant differences in percentages of Planctomycetes between winter 2015 and summer 2015 including

Figure 10. Overall Composition of Rhizosphere Bacterial Communities in Coastal Wetlands of South Mississippi

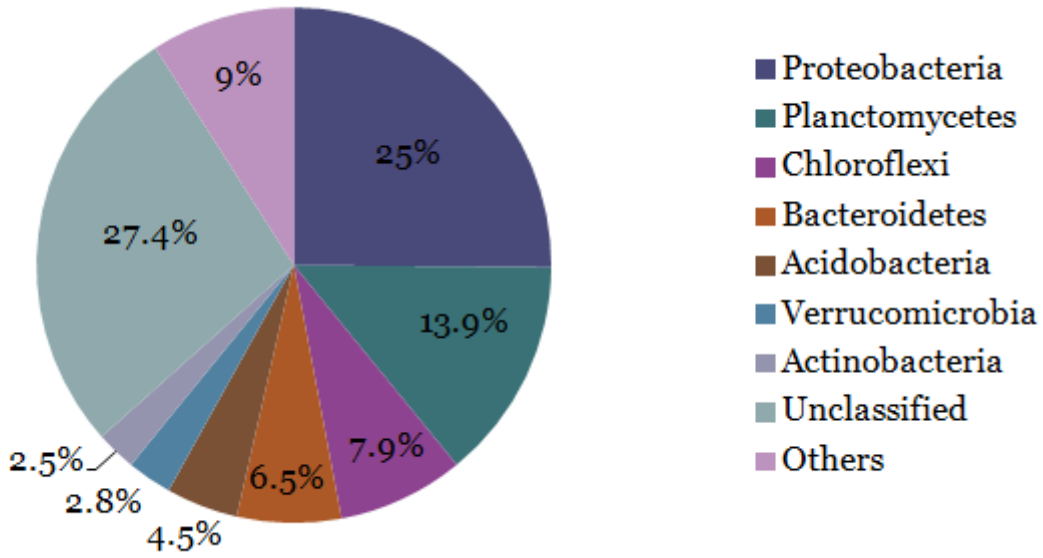


Table 1. The list of phyla from sequences of collected plant samples in each transect in February 2015.

Total Including Classified and Others (63700)	BT3 LSA (6453)	BT3 LJR (4057)	BT3 MJR (5449)	BT3 HJR (5999)	BT4 LSA (7029)	BT4 LJR (5889)	BT4 MJR (3542)	BT4 HJR (4785)	ST6 LSA (4911)	ST6 HJR (3335)	ST7 LSA (5304)	ST7 MJR (1837)	ST7 HJR (5060)
Proteobacteria	1501 (23%)	945 (23%)	1300 (23.9%)	1169 (19.5%)	1572 (22.4%)	1579 (26.8%)	799 (22.6%)	1167 (24.4%)	919* (18.7%)	977 (29.3%)	1304 (24.6%)	542 (29.5%)	1397 (27.6%)
Planctomycetes	877 (13.6%)	691* (17%)	730 (13.4%)	803 (13.4%)	1014 (14.4%)	848 (14.4%)	455 (12.8%)	661 (13.8%)	689 (14%)	475 (14.2%)	806 (15.2%)	265 (14.4%)	725 (14.3%)
Chloroflexi	614 (9.5%)	366 (9.0%)	392 (7.2%)	522 (8.7%)	619 (8.8%)	425 (7.2%)	281 (7.9%)	349 (7.3%)	593* (12.1%)	137 (4.1%)	402 (7.6%)	64 (3.5%)	259 (5.1%)
Bacteroidetes	398 (6.2%)	231 (5.7%)	272 (5.0%)	215* (3.6%)	469 (6.7%)	463 (7.9%)	189 (5.3%)	295 (6.2%)	243 (4.9%)	257 (7.7%)	466 (8.8%)	112 (6.1%)	288 (5.7%)
Acidobacteria	248 (4.0%)	206 (5.1%)	250 (4.6%)	208 (3.5%)	255 (3.6%)	249 (4.2%)	167 (4.7%)	183 (3.8%)	156 (3.2%)	200 (6.0%)	276 (5.2%)	126 (6.9%)	277 (5.5%)
Verrucomicrobia	116 (1.8%)	118 (2.9%)	132 (2.4%)	86 (1.4%)	203 (2.9%)	168 (2.9%)	88 (2.5%)	154 (3.2%)	109 (2.2%)	142 (4.3%)	229 (4.3%)	95 (5.2%)	201 (4.0%)
Actinobacteria	111 (1.7%)	84 (2.1%)	127 (2.3%)	93 (1.6%)	147 (2.1%)	125 (2.1%)	67 (1.9%)	103 (2.2%)	154 (3.1%)	259* (7.8%)	251 (4.7%)	147* (8.0%)	204 (4.0%)

The percentage was found by dividing the bacterial phylum by the total sequences of the specific plant sample.
* means significant difference from the norm.

Table 2. The list of phyla from sequences of collected plant samples in each transect in August 2015.

Total Including Classified and Others (53384)	BT3 LSA (4844)	BT3 LJR (5760)	BT3 MJR (4322)	BT3 HJR (3089)	BT4 LSA (6023)	BT4 LJR (4707)	BT4 MJR (3900)	BT4 HJR (2427)	ST6 LSA (3969)	ST6 HJR (3741)	ST7 LSA (5280)	ST7 MJR (2084)	ST7 HJR (3239)
Proteobacteria	1225 (25.3%)	1455 (25.3%)	1031 (23.9%)	841 (27.2%)	1519 (25.2%)	1316 (28.0%)	1041 (26.7%)	593 (24.4%)	1091 (27.5%)	1024 (27.4%)	1424 (27.0%)	605 (29.0%)	964 (29.8%)
Planctomycetes	604 (12.5%)	853 (14.8%)	603 (14.0%)	396 (12.8%)	802 (13.3%)	634 (13.5%)	516 (13.2%)	358 (14.8%)	559 (14.1%)	461 (12.3%)	763 (14.5%)	309 (14.8%)	404 (12.5%)
Chloroflexi	409 (8.4%)	407 (7.1%)	340 (7.9%)	227 (7.3%)	584 (9.7%)	336 (7.1%)	279 (7.2%)	202 (8.3%)	410* (10.3%)	241 (6.4%)	486 (9.2%)	89 (4.3%)	242 (7.5%)
Bacteroidetes	314 (6.5%)	270 (4.7%)	218 (5.0%)	229 (7.4%)	565* (9.4%)	339 (7.2%)	208 (5.3%)	152 (6.3%)	368* (9.3%)	188 (5.0%)	452 (8.6%)	119 (5.7%)	150 (4.6%)
Acidobacteria	188 (3.9%)	294 (5.1%)	180 (2.5%)	138 (4.5%)	186 (3.1%)	242 (5.1%)	176 (4.5%)	124 (5.15)	173 (4.4%)	247 (6.4%)	214 (4.1%)	135 (6.5%)	159 (4.9%)
Verrucomicrobia	85 (1.8%)	104 (1.8%)	106 (2.5%)	90 (2.9%)	162 (2.7%)	132 (2.8%)	111 (2.8%)	65 (2.7%)	119 (3.0%)	164* (4.4%)	134 (2.5%)	65 (3.1%)	87 (2.7%)
Actinobacteria	60 (1.2%)	93 (1.6%)	54 (1.2%)	51 (1.6%)	81 (1.3%)	50 (1.1%)	51 (1.3%)	28 (1.2%)	77 (1.9%)	65 (1.7%)	116 (2.2%)	203* (9.7%)	85 (2.6%)

The percentage was found by dividing the bacterial phylum by the total sequences of the specific plant sample.
* means significant difference from the norm.

Spartina alterniflora and *Juncus roemerianus* in the low zone of BT3 and BT4 (Tables 1 & 2).

The percentage of Chloroflexi was lowest in *Juncus roemerianus* in the mid zone of ST7 during winter 2015 (3.5%) while the percentage of Chloroflexi was the highest in *Spartina alterniflora* in the low zone of ST6 (12.1%). There were no differences in percentage of Chloroflexi between winter 2015 and summer 2015 including *Spartina alterniflora* and *Juncus roemerianus* in the low zone of BT3 and BT4. *Spartina alterniflora* in the low zone of ST6 during winter 2015 and summer 2015 (12.1% and 10.3%) had higher percentages of Chloroflexi than the total percentage (7.9%) of the data set (Tables 1 & 2).

The percentage of Bacteroidetes was lowest in *Juncus roemerianus* in the high zone of BT3 during winter 2015 (3.6%) while the percentage of Bacteroidetes was highest in *Spartina alterniflora* in the low zone of BT4 during summer 2015 (9.4%). There were differences in percentages of Bacteroidetes between winter 2015 and summer 2015 in *Juncus roemerianus* in the high zone of BT3 (3.6% and 7.4% respectively) and in *Spartina alterniflora* in the low zone of ST6 (4.9% and 9.3% respectively). There were no differences in percentages of Bacteroidetes between *Spartina alterniflora* and *Juncus roemerianus* in the low zone of BT3 and BT4 in both winter 2015 and summer 2015 (Tables 1 & 2).

The percentage of Acidobacteria was lowest in *Juncus roemerianus* in the mid zone of BT3 during summer 2015 (2.5%) while the percentage of Acidobacteria was highest in *Juncus roemerianus* in the mid zone of ST7 during winter 2015 (6.9%). There was a difference in percentage of Acidobacteria between winter 2015 and summer 2015 in *Juncus roemerianus* in the mid zone of BT3 (4.6% and 2.5% respectively). There was also a difference in percentage of Acidobacteria between *Spartina alterniflora* (3.1%) and *Juncus roemerianus* (5.1%) in the low zone of BT4 during summer 2015 (Tables 1 & 2).

The percentage of Verrucomicrobia was lowest in *Juncus roemerianus* in the high zone of BT3 during winter 2015 (1.4%) while the percentage of Verrucomicrobia was highest in *Juncus roemerianus* in the mid zone of ST7 during winter 2015 (5.2%). There was a difference in percentage of Verrucomicrobia between winter 2015 and summer 2015 in *Juncus roemerianus* in the mid zone of ST7 (5.2% and 3.1% respectively). There were no differences in percentages of Verrucomicrobia between *Spartina alterniflora* and *Juncus roemerianus* in the low zone of BT3 and BT4 in both winter 2015 and summer 2015 (Tables 1 & 2).

The percentage of Actinobacteria was lowest in *Juncus roemerianus* in the low zone of BT4 during summer 2015 (1.1%) while the percentage of Actinobacteria was highest in *Juncus roemerianus* in the mid zone of ST7 during summer 2015 (9.7%). The *Juncus roemerianus* in the mid zone of ST7 during winter 2015 and summer 2015 (8.0% and 9.7% respectively) and *Juncus roemerianus* in the high zone of ST6 during winter 2015 (7.8%) had higher percentages of Actinobacteria than the total percentage of the data set of 2.5%. There was also a difference between winter 2015 and summer 2015 in *Juncus roemerianus* in the high zone of ST6 (7.8% and 1.7% respectively). There were no differences in percentages of Actinobacteria between *Spartina alterniflora* and *Juncus roemerianus* in the low zone of BT3 and BT4 in both winter 2015 and summer 2015 (Tables 1 & 2).

Overall, seasons had no significant effect on rhizosphere soil (AMOVA, $p>0.005$). Sites and plant species had significant effects on rhizosphere soil (AMOVA, $p<0.001$ and $p=0.001$ respectively). In AMOVA of two factors plant species and sites, there were significant differences between *Juncus roemerianus* in brackish sites and *Juncus roemerianus* in saltwater sites ($p<0.001$), *Juncus roemerianus* in brackish sites and *Spartina alterniflora* in saltwater sites ($p=0.001$), *Juncus roemerianus* in brackish sites and *Spartina alterniflora* in saltwater sites

(p=0.002), and *Juncus roemerianus* in saltwater sites and *Spartina alterniflora* in brackish sites (p=0.004) on rhizosphere soil. In AMOVA of two factors seasons and sites, there were significant differences between brackish sites in the summer and saltwater sites in the winter (p=0.002), saltwater sites in the summer and brackish sites in the winter (p=0.003), and brackish sites in the winter and saltwater sites in the winter (p<0.001) on rhizosphere soil. In AMOVA of two factors seasons and plant species, there were significant differences between *Juncus roemerianus* in the summer and *Spartina alterniflora* in the summer (p<0.001) and between *Juncus roemerianus* in the summer and *Spartina alterniflora* in the winter (p=0.005) on rhizosphere soil. In AMOVA of three factors seasons, plant species, and sites, there were no significant differences in any of the 28 combinations on rhizosphere soil (Table 3).

Table 3. Analysis of Molecular Variance (AMOVA) of plant species, sites, and seasons and their effects on rhizosphere soil.

Source	P-Value
Seasons: S-W	NS
Sites: B-S	<0.001
Plant Species: J-S	0.001

Plant Species and Sites	P-Value
JB-JS-SB-SS	<0.001
JB-JS	<0.001
JB-SB	0.001
JB-SS	0.002
JS-SB	0.004
JS-SS	NS
SB-SS	NS

Season and Sites	P-Value
SB-SS-WB-WS	<0.001
SB-SS	NS
SB-WB	NS
SB-WS	0.002
SS-WB	0.003
SS-WS	NS
WB-WS	<0.001

Key: S - Summer, W- Winter, B - Brackish, S - Saltwater, J - *Juncus roemerianus*, S - *Spartina alterniflora*, SS - Summer/Saltwater, SS- Summer/*Spartina alterniflora*, SS- *Spartina alterniflora*/Saltwater depending on the source or title. P-values indicate significant effects, and NS means no significant effects ($p>0.005$).

Table 3 cont.

Season and Plant Species	P-Value
SJ-SS-WJ-WS	0.003
SJ-SS	<0.001
SJ-WJ	NS
SJ-WS	0.005
SS-WJ	NS
SS-WS	NS
WJ-WS	NS

Season and Plant Species and Sites	P-Value
JBS-JBW-JSS-JSW-SBS-SBW-SSS-SSW	<0.001
All 28 Combinations	NS

Key: S - Summer, W- Winter, B - Brackish, S - Saltwater, J - *Juncus roemerianus*, S - *Spartina alterniflora*, SS - Summer/Saltwater, SS- Summer/*Spartina alterniflora*, SS- *Spartina alterniflora*/Saltwater depending on the source or title. P-values indicate significant effects, and NS means no significant effects ($p>0.005$).

V. Discussion:

This study investigated the effects of seasons, sites, and two plant species (*Spartina alterniflora* and *Juncus roemerianus*) on rhizosphere microbial communities in coastal wetland located at Graveline Bayou, Gautier, MS. Overall, the results showed that there was no significant effect of seasonal patterns alone in coastal wetlands on rhizosphere microbial communities (AMOVA, $p>0.005$). In AMOVA of two factors (sites and seasons), there were also no significant differences between brackish transects during the summer 2015 and winter 2015 ($p>0.005$) and between saltwater transects during the summer 2015 and winter 2015 ($p>0.005$) on rhizosphere microbial communities. This could be due to the fact that there were similar pH levels (8.84, 9.34 respectively), soil moistures (45%, 47% respectively), and organic matter contents (8.2%, 8.4% respectively) of rhizosphere soils between the summer 2015 and winter 2015 seasons. The relationship between both seasons having a similar pH level and the lack of diversity of microbial communities is supported by this study and in the continental-scale study of soil bacterial communities by Fierer & Jackson (2006). The authors discussed that microbial biogeography and diversity are controlled primarily by edaphic variables, especially pH level (Fierer and Jackson, 2006).

The results also suggested that plant developmental stages have little effect on microbial communities. In AMOVA of two factors (seasons and plant species), there were no significant differences between *Juncus roemerianus* in the winter 2015 and summer 2015 ($p>0.005$) and between *Spartina alterniflora* in the winter 2015 and summer 2015 ($p>0.005$) on rhizosphere microbial communities.

It is still important to note that seasonal effects combined with sites and plant species had significant differences on the microbial communities (AMOVA, $p<0.001$), for the salinity

levels in transects during the winter 2015 (15.2 ppt) and summer 2015 (21.1 ppt) differed drastically from each other. It makes sense that the salinity level is higher in the summer since there is more water evaporation from the soil due to higher temperature. The increase in salinity level of the soil type forced the two plant species (*Spartina alterniflora* and *Juncus roemerianus*) to adapt by harboring specific microbial communities explained earlier in Fierer and Jackson's study (2006). A previous study evaluating the effects of saltwater intrusion on wetland microbial communities discussed that the increase in salinity promoted bacterial diversity (Jackson and Vallaire, 2009). Their results showed that salinity increased the proportion of Betaproteobacteria while my results showed that the salinity increased the proportions of Proteobacteria and Bacteroidetes. For instance, there were differences in percentages of Proteobacteria between winter 2015 and summer 2015 in *Juncus roemerianus* in the high zone of BT3 (19.5% and 27.2% respectively) and *Spartina alterniflora* in the low zone of ST6 (18.7% and 27.5% respectively). There were differences in percentages of Bacteroidetes between winter 2015 and summer 2015 in *Juncus roemerianus* in the high zone of BT3 (3.6% and 7.4% respectively) and in *Spartina alterniflora* in the low zone of ST6 (4.9% and 9.3% respectively).

The results supported significant effects of sites and plant species on rhizosphere microbial communities in coastal wetlands. AMOVA showed that sites ($p<0.001$) had a bigger impact on rhizosphere microbial communities than the host plant species ($p=0.001$). In AMOVA of two factors sites and plant species, rhizosphere microbial communities in *Juncus roemerianus* in brackish transects were significantly different from those in *Juncus roemerianus* in saltwater transects ($p<0.001$). There were also significant differences but not as high in *Juncus roemerianus* and *Spartina alterniflora* in brackish transects (AMOVA, $p=0.001$). This proved that the major driving force for the diversity of rhizosphere microbial communities is the soil

type of the sites. This supports other studies such as Da Silva's experiment which determined that soil type instead of maize cultivar type was the dominant factor influencing the composition of the *Paenibacillus* communities in the rhizosphere (Da Silva *et al.*, 2003). The p values for sites and plant species were very close, so it is still important to note that the effects of plant species and their root exudates are just as essential as the soil type in influencing the composition and diversity of the microbial communities in the rhizosphere.

There are not many studies on rhizosphere microbial communities and their interactions in Mississippi coastal wetlands. This study is helpful in understanding the microorganisms to soil types, seasonal patterns, and host plant species, for they provide key processes in organic matter decomposition and nutrient cycling in wetlands (Brinson *et al.*, 1981; Wetzel, 1992). Thus, by understanding these patterns in rhizosphere microbial communities, they can be useful as bioindicators of degradation in wetlands (Merkley *et al*., 2004).

VI. References

Bais HP, Weir TL, Perry LG, Gilroy S and Vivanco JM. 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. Annu Rev Plant Biol 57: 234-266.

Berg G and Smalla K. 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiol Ecol 68.1: 1-13.

Brinson MM, Lugo AE, and Brown S. 1981. Primary productivity, decomposition, and consumer activity in freshwater wetlands. Annu Rev of Ecol Syst 12: 123-61.

Chen, Y. 2011. Relationship between coastal vegetation biomass with elevation and salinity gradients. Master of Science Thesis, Department of Biology, The University of Mississippi.

Da Silva KRS, Salles JF, Seldin L and van Elsas JD. 2003. Application of a novel *Paenibacillus*-specific PCR-DGGE method and sequence analysis to assess the diversity of *Paenibacillus* spp. in the maize rhizosphere. J Microbiol Meth 54: 213-231.

Fierer N and Jackson RB. 2006. The diversity and biogeography of soil bacterial communities. P Natl Acad Sci USA 103: 626-631.

Jackson CR and Vallaire SC. 2009. Effects of salinity and nutrients on microbial assemblages in Louisiana wetland sediments. Wetlands 29: 277-287.

Janda JM and Abbott SL. 2007. 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls. Clin Microbial 45: 2761-2764.

Kozich JJ, Westcott SL, Baxter NT, Highlander SK and Schlossa PD. 2013. Development of a dual-Index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina Sequencing Platform. Appl Environ Microbiol 79: 5112-5120.

Marschner P, Yang CH, Lieberei R and Crowley DE. 2001. Soil and plant specific effects on bacterial community composition in the rhizosphere. Soil Biol Biochem 33: 1437-1445.

McLeod KW, McCarron JK and Conner WH. 1996. Effects of flooding and salinity on photosynthesis and water relations of four Southeastern Coastal Plain forest species. Wetl Ecol and Manage 4: 31-42.

Merkley M, Rader RB, McArthur JV and Eggett D. 2004. Bacteria as bioindicators in wetlands: bioassessment in the Bonneville Basin of Utah, USA. Wetlands 24: 600-607.

Mississippi Department of Marine Resources. 1999. Mississippi's coastal wetlands. http://www.dmr.state.ms.us/joomla16/images/publications/mississippi-coastal-wetlands.pdf [accessed 28 March 2016].

MO BIO Laboratories, Inc. 2015. Instruction Manual for PowerSoil® DNA Isolation Kit.

Nunan N, Daniell TJ, Singh BK, Papert A, Mc Nicol JW and Prosser JI. 2005. Links between plant and rhizoplane bacterial communities in grassland soils, characterized using molecular techniques. Appl Environ Microbiol 71: 6784-6792.

Ortíz-Castro R, Contreras-Cornejo HA, Macías-Rodríguez L and López-Bucio J. 2009. The role of microbial signals in plant growth and development. Plant Signaling and Behav 4.8: 701-12.

Pezeshki SR, DeLaune RD and Patrick, Jr., WH. 1989. Assessment of saltwater intrusion impact on gas exchange behavior of Louisiana Gulf Coast wetland species. Wetl Ecol and Manage 1:21-30.

Raaijmakers JM, Paulitz CT, Steinberg C, Alabouvette C and Moënne-Locooz Y. 2009. The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. Plant Soil.

Rasche F, Velvis H, Zachow C, Berg G, van Elsas JD and Sessitsch A. 2006. Impact of transgenic potatoes expressing antibacterial agents on bacterial endophytes is comparable to effects of soil, wildtype potatoes, vegetation stage and pathogen exposure. Can J Microbiol 43:555-566.

Salles JF, van Veen JA and van Elsas JD. 2004. Multivariate analyses of *Burkholderia* species in soil: effect of crop and land use history. Appl Environ Microbiol 70: 4012-4020.

Schloss PD, Westcott SL, and Ryabin T. 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75: 7537-7541.

Schmidt TSB, Rodrigues JFM and Mering CV. 2014. Ecological consistency of SSU rRNA-based operational taxonomic units at a global scale. PLoS Comput Biol 10(4):e1003594.

Shirley LJ and Battaglia LL. 2006. Assessing vegetation changes in coastal landscapes of the northern Gulf of Mexico. Wetlands 26:1057-70.

Singh BK, Milard P, Whitely AS and Murrell JC. 2004. Unravelling rhizosphere-microbial interactions: opportunities and limitations. Trends Microbiol 12: 386-393.

Somers E, Vanderleyden J and Srinivisam M. 2004. Rhizosphere bacterial signaling: a love parade beneath our feet. Crit Rev Microbiol 30: 205-240.

Sørensen J. 1997. The rhizosphere as a habitat for soil microorganisms. Modern Soil Microbiology (Van Elsas JD, Trevors JT and Wellington EMH, eds), pp. 21-45. Marcel Dekker Inc., New York.

United States Department of Agriculture. 2002. Plants profile for *Spartina alterniflora* (smooth cordgrass). http://plants.usda.gov/core/profile?symbol=spal [accessed 28 March 2016].

United States Department of Agriculture. 2015. Plants profile for *Juncus roemerianus* (needlegrass rush). http://plants.usda.gov/core/profile?symbol=juro [accessed 28 March 2016].

Uren NC. 2000. Types, amounts and possible functions of compounds released into the rhizosphere by soil grown plants. The Rhizosphere: Biochemistry, and Organic Substances at the Soil Interface (Pinton R, Varani Z and Nanniperi P, eds), pp.19-40. Marcel Dekker Inc., New York.

Wetzel RG. 1992. Wetlands as metabolic gates. Great Lakes Res 18: 529-32.

VII. Appendices

Appendix A. Root length of the collected plant samples on each transect in February 2015.

Transect	Zones/Plant Species	Mean of Root Lengths (cm)	Standard Error
BT3	LSA	7.7	1.0
BT3	LJR	8.2	0.7
BT3	MJR	4.8	0.5
BT3	HJR	7.3	0.4
BT4	LSA	7.0	0.9
BT4	LJR	10.8	1.2
BT4	MJR	10.7	0.6
BT4	HJR	7.9	0.9
ST6	LSA	8.8	0.9
ST6	HJR	12.3	1.1
ST7	LSA	9.9	0.8
ST7	MJR	10.9	0.8
ST7	HJR	11.7	1.3

There are four transects as follows: BT3 - Brackish Transect 3, BT4 - Brackish Transect 4, ST6 - Saltwater Transect 6, ST7 - Saltwater Transect 7. There are four plant samples collected in each transect and are as follows: LSA - Low *Spartina alterniflora*, LJR - Low *Juncus roemerianus*, MJR - Mid *Juncus roemerianus*, HJR - High *Juncus roemerianus*. The MJR on transect ST6 was not found due to a road. Note that low, mid, and high are the marsh zones.

Appendix B. Root lengths of the collected plant samples on each transect in August 2015.

Transect	Zones/ Plant Species	Mean of Root Length (cm)	Standard Error
BT3	LSA	8.0	0.8
BT3	LJR	8.9	1.0
BT3	MJR	11.0	1.1
BT3	HJR	12.3	1.0
BT4	LSA	4.6	0.5
BT4	LJR	8.5	0.8
BT4	MJR	11.5	1.0
BT4	HJR	9.8	0.8
ST6	LSA	6.6	0.9
ST6	HJR	8.4	0.8
ST7	LSA	6.6	0.8
ST7	MJR	13.4	1.9
ST7	HJR	11.2	1.1

There are four transects as follows: BT3 - Brackish Transect 3, BT4 - Brackish Transect 4, ST6 - Saltwater Transect 6, ST7 - Saltwater Transect 7. There are four plant samples collected in each transect and are as follows: LSA - Low *Spartina alterniflora*, LJR - Low *Juncus roemerianus*, MJR - Mid *Juncus roemerianus*, HJR - High *Juncus roemerianus*. The MJR on transect ST6 was not found due to a road. Note that low, mid, and high are the marsh zones.

Appendix C. Soil moisture of the collected plant soil samples on each transect in February 2015.

Transect	Zones/Plant Species	Before Oven/ After Oven (g)	Water Volume (g)	Soil Moisture (%)
BT3	LSA	19.693/ 12.788	6.905	35.06
BT3	LJR	9.366/ 3.763	5.603	59.82
BT3	MJR	18.308/ 6.781	11.527	62.96
BT3	HJR	17.097/ 5.852	11.245	65.77
BT4	LSA	19.122/ 9.022	10.1	52.82
BT4	LJR	18.666/ 6.067	12.599	67.50
BT4	MJR	23.880/ 8.331	15.549	65.11
BT4	HJR	15.895/ 4.468	11.427	71.89
ST6	LSA	16.135/ 8.028	8.107	50.24
ST6	HJR	17.132/ 15.736	1.396	8.15
ST7	LSA	25.353/ 15.456	9.897	39.04
ST7	MJR	8.286/ 7.839	0.447	5.39
ST7	HJR	13.312/ 8.878	4.434	33.31

There are four transects as follows: BT3 - Brackish Transect 3, BT4 - Brackish Transect 4, ST6 - Saltwater Transect 6, ST7 - Saltwater Transect 7. There are four plant samples collected in each transect and are as follows: LSA - Low *Spartina alterniflora*, LJR - Low *Juncus roemerianus*, MJR - Mid *Juncus roemerianus*, HJR - High *Juncus roemerianus*. The MJR on transect ST6 was not found due to a road. Note that low, mid, and high are the marsh zones. The water volume was calculated by subtracting the weight before oven to the weight after oven. The soil moisture was calculated by dividing the water volume to the weight after oven and multiplying it by hundred.

Appendix D. Soil moisture of the collected plant soil samples on each transect in August 2015.

Transect	Zones/ Plant Species	Before Oven/ After Oven (g)	Water Volume (g)	Soil Moisture (%)
BT3	LSA	15.757/ 11.03	4.727	30.00
BT3	LJR	28.561/ 20.791	7.77	27.20
BT3	MJR	20.847/ 8.766	12.081	57.95
BT3	HJR	43.77/ 13.124	30.646	70.02
BT4	LSA	30.946/ 19.824	11.122	35.94
BT4	LJR	19.57/ 7.142	12.428	63.51
BT4	MJR	30.605/ 9.146	21.459	70.12
BT4	HJR	32.741/ 8.121	24.62	75.20
ST6	LSA	36.421/ 22.498	13.923	38.23
ST6	HJR	31.699/ 17.8	13.899	43.85
ST7	LSA	34.343/ 18.182	16.161	47.06
ST7	MJR	23.606/ 23.166	0.44	1.86
ST7	HJR	40.108/ 29.194	10.914	27.21

There are four transects as follows: BT3 - Brackish Transect 3, BT4 - Brackish Transect 4, ST6 - Saltwater Transect 6, ST7 - Saltwater Transect 7. There are four plant samples collected in each transect and are as follows: LSA - Low *Spartina alterniflora*, LJR - Low *Juncus roemerianus*, MJR - Mid *Juncus roemerianus*, HJR - High *Juncus roemerianus*. The MJR on transect ST6 was not found due to a road. Note that low, mid, and high are the marsh zones. The water volume was calculated by subtracting the weight before oven to the weight after oven. The soil moisture was calculated by dividing the water volume to the weight after oven and multiplying it by hundred.

Appendix E. Organic matter content of the collected plant samples on each transect in February 2015.

Transects	**Zones/Plant Species**	**Before/After Ashing (g)**	**Organic Matter Content (%)**
BT3	LSA	9.7/ 9.6	1.0
BT3	LJR	5.2/ 4.9	5.8
BT3	MJR	5.8/ 4.9	15.5
BT3	HJR	6.3/ 5.4	14.3
BT4	LSA	7.2/ 7	2.80
BT4	LJR	2/ 1.7	15.0
BT4	MJR	3.9/ 3.2	17.9
BT4	HJR	3.3/ 2.7	18.2
ST6	LSA	6.3/ 6	4.8
ST6	HJR	8.5/ 8.4	1.2
ST7	LSA	6.3/ 6	4.8
ST7	MJR	9.3/ 9.2	1.1
ST7	HJR	6/ 5.6	6.7

There are four transects as follows: BT3 - Brackish Transect 3, BT4 - Brackish Transect 4, ST6 - Saltwater Transect 6, ST7 - Saltwater Transect 7. There are four plant samples collected in each transect and are as follows: LSA - Low *Spartina alterniflora*, LJR - Low *Juncus roemerianus*, MJR - Mid *Juncus roemerianus*, HJR - High *Juncus roemerianus*. The MJR on transect ST6 was not found due to a road. Note that low, mid, and high are the marsh zones. The organic matter content was calculated by subtracting the before ashing of the soil from the after ashing of the soil and then multiplying the difference by hundred.

Appendix F. Organic matter content of the collected plant samples on each transect in August 2015.

Transects	**Zones/Plant Species**	**Before/After Ashing (g)**	**Organic Matter Content (%)**
BT3	LSA	9.4/ 9.3	1.1
BT3	LJR	9.6/ 9.4	2.1
BT3	MJR	6.4/ 5.4	15.6
BT3	HJR	6.8/ 5.7	16.2
BT4	LSA	7.6/ 7.4	2.6
BT4	LJR	4.8/ 4.2	12.5
BT4	MJR	6.3/ 5.2	17.5
BT4	HJR	4.7/ 3.6	23.4
ST6	LSA	8.7/ 8.4	3.4
ST6	HJR	7.6/ 7.3	3.9
ST7	LSA	8.7/ 8.5	2.3
ST7	MJR	10.2/ 10.1	1.0
ST7	HJR	9.9/ 9.4	5.1

There are four transects as follows: BT3 - Brackish Transect 3, BT4 - Brackish Transect 4, ST6 - Saltwater Transect 6, ST7 - Saltwater Transect 7. There are four plant samples collected in each transect and are as follows: LSA - Low *Spartina alterniflora*, LJR - Low *Juncus roemerianus*, MJR - Mid *Juncus roemerianus*, HJR - High *Juncus roemerianus*. The MJR on transect ST6 was not found due to a road. Note that low, mid, and high are the marsh zones. The organic matter content was calculated by subtracting the before ashing of the soil from the after ashing of the soil and then multiplying the difference by hundred.