Effects of Inundation Regime and Plant Community on Soil Bacterial Communities in an Eastern Shore, VA Salt Marsh

Amanda Lynn Floyd
Havelock, North Carolina

BS, University of North Carolina at Greensboro, 2002

A Thesis presented to the Graduate Faculty
of the University of Virginia in Candidacy for the Degree of
Master of Science

Department of Environmental Sciences

University of Virginia
May, 2007

_____

_____

_____

_____

ABSTRACT

The presence of environmental gradients, high primary productivity and high microbial activity make salt marshes ideal for investigation of plant and location effects on bacterial community structure. In this study, the effects of plant community types and tidal inundation on soil bacteria community structure were examined in Upper Phillips Creek Marsh (UPCM) at the Virginia Coast Reserve LTER. This transgressing mainland valley marsh contains two major plant communities in each of those three regions: stands of *Juncus roemerianus* and a mixed community of *Spartina patens* and *Distichlis spicata*. Transects that bisected these two plant communities were established in low, middle and high marsh regions. Shallow soil cores (3x3x3 cm) were collected at 1.5 m intervals along each 6 m transect in July 2004, February 2005, and July 2005. Total and live bacterial counts and DGGE of 16S-rDNA were done to characterize community structure. Environmental and plant characteristics such as above and below-ground biomass, soil organic matter (SOM) and root OM content, soil and root C:N, and elevation were also measured along experimental transects. Total and live bacterial abundance were high, on average; 4.71×10^{10} cells and 2.54×10^{10} cells g^{-1} dry soil, respectively. Multidimensional scaling of bacterial community similarity revealed differences across marsh locations, but not between plant communities in July 2004 and 2005. No effect of site or plant community was observed in soil bacterial community composition in February 2005. These differences in community structure are correlated with differences in SOM, root OM and elevation, ($r = 0.48$, $p = 0.001$) as determined by Mantel tests of matrix correlation. These results show that environmental gradients in UPCM are stronger than plant community differences in structuring soil bacterial

communities in this marsh. These results also support the presence of an interaction of soil temperature and SOM on bacterial community composition in UPCM soils. Predictions arising from this research are that as marsh transgression occurs, bacterial communities will respond to the accompanying changes in environmental factors such as tidal inundation and SOM.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

I am grateful to have had this chance to carry out my Master's research at the University of Virginia. First, I must thank my advisor, Linda Blum for her patient support, guidance and commitment to my success in this project. Her enthusiasm and dedication towards her students is unparalleled. Many thanks go to my equally patient and encouraging and supportive thesis committee members, Aaron Mills and Howard Epstein; their advice and input on all aspects of my research have been most crucial.

I would like to thank my funding sources for supporting this endeavor: the UVA Department of Environmental Sciences, and the Virginia Coast Long Term Ecological Research Site, via funding by the National Science Foundation. The Nature Conservancy provided access to the field site.

Many thanks to the Laboratory of Microbial Ecology and the supportive lab mates therein: especially Gina Casciano for her collaboration and encouragement in the field and the lab, Sam Flewelling for his helpful insights and MATLAB skills; and Joseph Battistelli for sharing his valuable lab knowledge, laughs, and his love of homemade foodstuffs.

I am eternally grateful to my dear family and friends, your love gives me wings of strength and hope to keep going no matter what. Most of all I thank my husband, Steven Beaty, for his boundless love and understanding. He brings joy to every part of life, even thesis writing.

Dedication

~~~~~~~~~~

For my mom;

Words and images will fade,

But your love endures forever

~~~~~~~~~~

LIST OF FIGURES

LIST OF TABLES

Chapter 1: Introduction

1.1 Research Problem

The relative importance of plant effects verses location differences is a popular area of study in microbial ecology [Costa, et al., 2004] , and the characterization of microbial communities in salt marsh soils is not entirely new [Rooney-Varga, et al., 1997; Hines, et al., 1999; Kuehn, et al., 2000; Burke, et al., 2002a; Franklin, et al., 2002]. What is lacking, however, is the joint investigation of plant and location/inundation effects on bacterial communities in salt marsh soils. Both plant communities and soil properties are projected to change as sea level rises and marshes migrate overland [Brinson, et al., 1995; Brinson and Christian, 1999; Buck, 2001]. How will these changes in the plant community and soil properties alter the interactions between soil bacteria and their environment within a transgressing salt marsh? Knowledge of how bacterial communities in marsh soils vary with differences in plant types and tidal inundation will further our understanding of changes that marsh systems may undergo in the face of rising sea level.

Salt marshes have long been a focus of ecological study [Adams, 1963; Rublee and Dornseif, 1978; Eleuterius and Eleuterius, 1979; Oertel, et al., 1989]. In light of recent events, such as Hurricanes Katrina and Rita in 2005, and the pressing reality of global climate change, recognition of the ecological and economic importance of salt marshes and wetlands has never been more acute. Salt marshes provide many ecosystem services, serving as habitat to a wide range of reptiles, birds, insects and mammals, absorbing the impact of coastal storms, protecting mainland development, and acting as potentially important systems for carbon sequestration [Hussein, et al., 2004]. They are

also highly threatened by sea level rise, sediment and water diversion and encroaching coastal development. Loss rates of coastal marshes in the United States vary widely. In coastal Louisiana, annual loss rates between 1978 and 1990 were estimated to be 9,802 ha yr^{-1} [Johnston, et al., 1995], in Virginia's Nanticoke estuary, the average annual loss rate between 1938 and 1985 was 49.6 ha yr^{-1} [Kearney, et al., 1988]. On Virginia's Eastern Shore, it was observed that 16% of lagoon marshes were lost between 1852 and 1962 [Knowlton, 1971], though marsh expansion has been observed in Eastern Shore mainland marshes [Kastler and Wiberg, 1996].

Though study of salt marshes continues to expand, some aspects of marsh ecology have not been explored in great detail. In particular, the characterization of bacterial communities in salt marsh soils and their dependence on biotic and environmental factors have just recently begun [Franklin, et al., 2002; Buchan, et al., 2003; Blum and Christian, 2004; Blum, et al., 2004]. Little is also known of the variations in microbial processes and community structure among high, mid and low regions within a single marsh, while comparisons of biogeochemistry between marshes (eg. fresh vs. brackish [Neubauer, et al., 2005], high vs. low latitude marshes [Howarth and Giblin, 1983]) are plentiful. The application of molecular genetic techniques to soil bacteria and fungi has greatly advanced the body of knowledge of microbial community structure and diversity, particularly in agricultural or arable soils [Yang and Crowley, 2000; Franklin and Mills, 2003; Girvan, et al., 2003; Lentzsch, et al., 2005] . However, attempts to link bacterial community to ecosystem function are difficult, partly due to the great heterogeneity of soils, horizontal gene transfer among bacteria and the limitations of 16S rDNA and rRNA methods [Cardon and Cage, 2006]. As increasing rates of sea level rise threaten the

survival of coastal marshes, increased study of critical within-marsh processes, i.e. organic matter accumulation and decomposition, plant community changes, and bacterial community function and diversity are essential.

1.2 Salt Marsh Ecology

Salt marshes are some of the most productive ecosystems in the world. Although values greatly vary among plant species, belowground productivity estimates average from to 1500 g dry weight m^{-2} yr^{-1} up to 5000 g m^{-2} yr^{-1} as reviewed in [Blum, 1993], while above ground productivity ranges from 900 g m^{-2} yr^{-1} up to 2200 g m^{-2} yr^{-1} [Edmonds, et al., 1985]. Marshes are also an important source of nutrients for tidal lagoons and estuaries as bacteria and fungi decompose plant material and mineralize plant-derived C, N and P. Obvious spatial heterogeneity exists in salt marsh plant species and conspicuous vegetation zonation has been the focus of decades of research [Adams, 1963; Eleuterius and Eleuterius, 1979; Levine, et al., 1998; Brinson and Christian, 1999; Lejuene, et al., 2002; Silvestri, et al., 2005]. Eutrophication from coastal residential development and agricultural fertilizers could have a lasting impact on marsh plant communities, by affecting plant competition and productivity [Bertness and Ellison, 1987; Levine, et al., 1998; Crain, et al., 2004].

Tidal marshes are typically divided into three regions, low, mid, and high, relative to their distance from the tidal creek or estuary. Due to regular flooding by tides and precipitation, tidal marshes contain various types of hydric soils. High levels of soil organic matter (hereafter, SOM) and low redox potentials are characteristic of these soils, where anaerobic bacteria thrive in the anoxic conditions and rely on alternative terminal electron acceptors (TEAs) for respiration, and organic matter (OM) for nutrients, energy

and carbon [Rabenhorst, 2001]. Typically, sediment supply is low and SOM accumulation is high in the mid and high marsh regions. In low marsh areas, mineral sediment is delivered by tidal inundation, and occasional aeration occurs from bioturbation and wave energy [Thomas, 2004], causing this region to be lower in SOM and high in mineral content [Christiansen, et al., 2000].

Two natural processes by which marshes increase surface elevation are sediment accumulation, as supplied by tidal inputs, and by SOM accumulation. In addition to SOM accumulation, proliferation of 'aquatic roots' of *Spartina patens* (those which grow above the soil surface into flood waters) has been proposed to be a mechanism of accretion via vegetative growth [Nyman, et al., 2006]. Organic matter build-up is most important to surface accretion in the highly organic high marsh soils, while mineral sediment deposition is more important in the low marsh [Kastler and Wiberg, 1996; Blum and Christian, 2004]. In the low marsh, plant stems act as baffels to decrease the velocity of incoming water and trap fine suspended sediments. A combination of high belowground biomass production, and incomplete decomposition of plant detritus by bacteria and fungi help produce the peat deposits present in high marsh soils.

Rates of relative sea level rise (RSLR) on the Eastern Shore of Virginia range between 2.5 and 3.5 mm/year [Oertel, et al., 1989]. When accretion rates do not compensate for SLR, and the land surface slope toward the upland region is low, mainland salt marshes on the Eastern Shore respond by migrating overland into the upland forest region, a process called transgression. During transgression, marshes can also expand—or prograde—into the estuary if there is a high sediment supply from the tidal creek [Schwimmer and Pizzuto, 2000]. Transgressing salt marshes which have a low

sediment supply will erode at the estuarine margin, converting low marsh to tidal creek, high marsh to low marsh and upland forest into high marsh areas [Brinson, et al., 1995], as is the case in this study. As an example of how these processes can maintain mainland marsh area, in the same 8-year period in which lagoon and island marsh area on Virginia's Eastern Shore decreased by 10.2% and 7.2%, respectively, mainland marsh area increased by 8.2% via transgression into uplands [Kastler and Wiberg, 1996].

Because marshes are recognized to have self-maintaining properties, pulsed events--such as disturbance by wrack or extensive flooding--are usually necessary to initiate these transitions [Brinson, et al., 1995; Buck, 2001]. Depressions in the mid and high marsh can trap tidal water delivered by spring tides or storm surges; the resultant ponding can stress plants enough to cause reduced belowground productivity, plant mortality, and allow for new tidal creek formation [Blum and Christian, 2004]. Also predicted is the likely shift in plant communities as marsh transgression occurs [Brinson, et al., 1995; Buck, 2001]. High marsh plants will be replaced by competition from salt tolerant low marsh plants, or stands of high marsh plants will shrink as invasion of the low marsh species takes place. As agents of decomposition and nutrient cycling, soil bacterial communities could play a large role in the changes that take place during these transitions.

1.3 Bacterial Communities in Soils

Bacteria in soil are extremely abundant and diverse. Direct counts often reach up to 10^9 cells per gram of dry soil and as high as 10^{10} in marsh soils [Rublee, 1982] and estimates of diversity, although fraught with difficulties [Huges, et al., 2001], can have a minimum range from 4,000-7,000 bacterial genomes per gram of dry soil [Torsvik, et al.,

1990]. Interactions among the soil environment, microbes and plants are varied and complex. Basic interactions among these three components are outlined in Fig 1.3.

It is often necessary to make the distinction between bulk soil, and rhizosphere bacteria, as these communities are affected by different processes and environmental conditions. Studies where bacteria from the bulk soil and rhizosphere have been compared show that bulk soil bacteria are often less responsive to plant activities or species differences than rhizosphere bacteria [Smalla, et al., 2001; Kowalchuk, et al., 2002; Costa, et al., 2004].

Because they are so influenced by plant activities, rhizosphere bacteria are often the focus of studies investigating plant species' effects on soil bacterial community structure via molecular methods. Root exudates, lysates, mucilage and secretions provide substantial amounts, and varying types of organic compounds, the microbial utilization of which drives biogeochemical processes, such as nitrate reduction, sulfate reduction and methanogenesis [Whipps and Lynch, 1983; Hines, et al., 1999]. Plants have been found to influence the composition of rhizosphere bacteria not only in salt marsh systems [Hines, et al., 1999; Burke, et al., 2002b], but in agricultural fields [Wieland, et al., 2001], grasslands [Grayston, et al., 2001; Brodie, et al., 2002] and within different crop management zones [Garbeva, et al., 2004; Yao, et al., 2005]. However, much research has focused on microbial community responses to only one plant type or species and associated seasonal or fertilization effects [Rooney-Varga, et al., 1997; Hines, et al., 1999]. Some previous studies of microbe-plant interactions in salt marshes have focused on plant species that do not occur in the same marsh region, such as rhizosphere

communities associated with low marsh *Spartina alterniflora* versus high, fresh-water marsh *Phragmites australis* [Burke, et al., 2002a].

1.4 Molecular Methods for Studying Bacterial Community Structure

Studies of soil bacterial communities have been facilitated by advances in molecular biological techniques and their application to environmental samples. Soil microorganisms are notoriously diverse yet hard to culture in the laboratory. The use of culture-independent techniques allows researchers to capture a greater fraction of the actual community than with culture-dependent methods. The completion of the sequencing of the 16S rRNA (rDNA) gene was instrumental in isolating, amplifying and identifying unculturable bacterial groups in the environment [Ward, et al., 1990]. Such 16S rDNA-based methods as Single-Stranded DNA Conformation Polymorphisms (SSCP) [Stach, et al., 2001], denaturing/temperature gradient gel electrophoresis (DGGE/TGGE) [Muyzer, 1999] and terminal restriction fragment length polymorphism analysis (T-RFLP) [Sakai, et al., 2004] use sequence differences within the 16S rDNA gene to fingerprint bacterial communities. More specific oligonucleotide primers can be used to target certain types of bacteria, such as sulfate-reducers, ammonia-oxidizers or actinomycetes. Methods such as random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) analyses operate on the entire genome, and can yield more information for measuring community similarity among samples than methods based on a single gene [Franklin, 2004].

Starting with DNA extraction, these molecular methods have biases and limitations. Cell lysis efficiency can vary among bacterial groups [Prosser, 2002] and the separation of bacterial cells from soil structures without shearing genomic DNA is a

challenge [Kirk, et al., 2004]. PCR amplification can be biased toward highly abundant DNA templates, or produce chimeric amplification products, which are not found in an actual organism in the environment [Wang and Wang, 1997; Wintzingerode, et al., 1997]. The presence of contaminating chloroplast DNA is common in soil or aquatic DNA extracts, and can greatly overwhelm and inhibit amplification of bacterial DNA in a polymerase chain reaction (PCR), though methods have been developed to minimize this problem [Green and Minz, 2005].

Most importantly, there exists no single primer set or method that can feasibly distinguish the thousands of unique bacterial phylotypes present in the soil environment on one gel or electrophergram. Diversity measures obtained from analyzing a single gene with one primer set are at best a qualitative picture of the 'actual' soil bacterial community. Ideally, combining techniques that evaluate the total community and target functional or genetic subgroups is recommended for comprehensive studies of microbial species richness and diversity [Torsvik and Ovreas, 2002].

1.5 Scaling up: bacterial communities operating on the landscape scale

Preventing loss of coastal marshes depends on the promotion of plant health and productivity in the face of changing environmental conditions, such as increased inundation, eutrophication or physical disturbance. As discussed earlier, bacteria are important agents of decomposition and nutrient cycling in salt marshes, and can also affect plant productivity and physiology [Rovira, 1965]. The genetic variation and precise roles of bacteria in maintaining marsh surface accretion or in processes related to ecosystem state change are just beginning to be understood. The potential of soil microorganisms to be more responsive to environmental changes than to changes in plant

community composition, or vice versa, will affect the ecological outcomes of natural and anthropogenic stress on wetland systems. Understanding how bacterial communities are structured provides a foundation for predicting how environmental changes, including anthropogenic effects, will alter microbial processes and in turn, influence plant productivity at the landscape scales relevant for forecasting wetland losses due to sea-level rise. To further this understanding, this work assesses the relative contributions of plant community type and inundation regime to bacterial community structure in a transgressing mainland marsh on the Eastern Shore of Virginia.

1.6 Hypotheses and Research Objectives

To characterize bacterial community structure, direct counts and community DNA fingerprinting were employed. Shallow soil cores from three marsh sites ('high' 'mid' and 'low') and three dominant plant communities (*Spartina patens-Distichlis spicata* mixture, *Juncus roemerianus* and 'transition') found at each site were examined. The following hypotheses about the soil bacterial communities in Upper Phillips Creek Marsh (Fig 1.1, 1.2), at the Virginia Coast Reserve Long Term Ecological Research (VCR-LTER) site, drove the research involved in this study:

H_{1a}: Bacterial abundance will be highest in the high SOM, high marsh regions. Bacterial abundance in marsh soils has been shown to correlate strongly with soil organic matter content [Blum, et al., 2004].

H_{2a}: Bacterial abundance will vary among plant communities, *Juncus roemerianus*, *Spartina patens-Distichlis spicata* mixed stands and the transition zone between them. Root C:N, and root productivity may vary among these communities, which may affect abundance.

H_{3a}: Bacterial community structure will vary most by marsh site when plants are least active, during winter.

H_{4a}: Plant effects on community structure, if any, are most likely to be observed in summer, when plants are most active. Links between plant phenology and microbial community structure are reported in marsh habitats [Burke, et al., 2002a].

H_{5a}: Greatest differences in bacterial community structure will be observed between sites that differ the most in SOM and inundation regime (high vs. low marsh). Physical-chemical factors are implicated in earlier studies to have an influence over bacterial community diversity and structure [Brodie, et al., 2002; Drenovsky, et al., 2004]

Chapter 2: Materials and Methods

2.1 Site Description

Upper Phillips Creek Marsh (UPCM), located within the Virginia Coast Reserve LTER study site is characteristic of coastal valley marshes of the eastern United States. It is bordered by upland pine forests, as well as active and fallow agricultural fields. The marsh is located near the upper reaches of a semidiurnal tidal creek that has a tidal amplitude of 1.45 m and an average salinity of 22 parts per thousand [Stasavich, 1999]. The soils of the high marsh study sites in this research are characterized as members of the Magotha fine sandy loam series. Low and mid marsh areas qualify as Chincoteage silt loams [Edmonds, et al., 1985]. The four major salt marsh plants, *Spartina alterniflora*, *Spartina patens*, *Distichlis spicata* (C_4 plants), and *Juncus roemerianus* (a C_3 plant) occupy distinct zones across the low, middle and high marsh areas. Within the low marsh, mid- to short-form *S. alterniflora* gives way to small monotypic stands of *J. roemerianus*, embedded in a matrix of *S. patens* or mixed stands of *S. patens* and *D. spicata*. Mid-marsh areas are also mostly populated by *S. patens* or S. *patens-D. spicata* mixture, with distinct patches of *J. roemerianus* of larger size than in the low site. The *S. patens-D. spicata* dominated community finally yields to large, more continuous *J. roemerianus* stands in the high marsh with smaller, intruding *S. patens-D. spicata* stands. At all three sites in Upper Phillips Creek Marsh, there are distinct boundaries between *J. roemerianus* and *S. patens-D. spicata* stands.

Stasavich (1999) explored the hydrodynamics within each of these three zones in a mainland marsh. The average depth of flooding and proportion of flooding from groundwater, precipitation, or tidal inputs was determined for each season from six years

of data logged by water monitors permanently installed in the marsh at various locations, (see fig 1.2). The three sites differed in the level of flooding and the flooding source. For example, the low marsh site received 95% of its flooding from tidal inputs, and roughly 48% of the time the water table was located 5 cm below the sediment surface; 20% of the time between 0 and 5 cm above the surface. Seasonal variability in flooding was not significant in the low or mid marsh. However, in the high marsh site the relative contribution from each water source did vary by season. In the summer, the high marsh received 96% of its above ground flooding, and 100% of its below ground flooding from precipitation, while in the winter, only 81% of the flooding above surface was from precipitation. High marsh flooding data show frequent inundation by fresh water from precipitation during all seasons, but there was considerable drawdown of the water table in summer due to lack of any tidal inputs and high evapotranspiration rates. Water table levels in the high marsh reached as low as -100 cm from soil surface in summer and as high as +25 cm during the winter. The locations of the present study's low, mid and high marsh sites are in the same general area as the water level recorders in Stasavich's study (Fig 1.2).

2.2 Experimental Design

In summer 2004, 3 6-m transects were established in each of the three marsh zones, bisecting a clearly defined *S. patens-D. spicata*, *J. roemerianus* boundary. Transects were placed in areas unaffected by wrack, hummock and hollow terrain, or die-off patches. The center point of each 6 m transect was positioned on the plant boundary and each transect was positioned at least 1.5 m from any other transect. Five sampling

points were positioned along each transect in 1.5 m intervals: two in the *S. patens-D. spicata* area, one in the transition zone, and two in the *J. roemerianus* patch.

To reduce disturbance to these transects, above and belowground biomass samples were collected in proximity to the established transects.

To assess temporal, seasonal differences in bacterial community structure, three identical sampling events took place on July 14, 2004, February 11, 2005 and July 25, 2005. Table 2.1 describes the variables measured at each of these sampling events.

2.3 Field Methods

Marsh elevation was measured along each transect using a surveyor's laser level and referenced to a permanent benchmark previously established in Upper Phillips Creek Marsh by the VCR-LTER (Benchmark "BRNV" latitude: 37.4606 longitude: -75.8347, elevation above MSL: 1.225 m). The accuracy of the elevation of this benchmark relative to sea level was ± 5 cm. However, the surveyor's laser level used to measure the elevations of all the sample points relative to the bench mark is precise to 1 cm.

Shallow soil cores were collected in July 2004, February 2005 and July 2005 for analysis of microbial community structure. All sampling dates fell during the third quarter of the lunar cycle and samples were taken beginning at low tide in the low marsh, to avoid sampling in flooded conditions. Due to the dense root mat of the plant species present, approximately 3-cm deep cores were taken using a 2x2 cm plexiglass template and small knife rather than a hand-held corer. Samples were taken at each of the five sampling points along each of the nine transects for a total of 45 samples per sampling event. After coring, samples were sealed in polyethylene bags and stored on ice. Upon returning to the lab, the samples were kept frozen at -80°C until analysis. Subsamples of

the cores were designated for determination of bacterial abundance, ash-free dry weight (soil organic matter), sediment C and N content, and DNA extraction.

Belowground biomass was sampled in early June 2005 with 6x30 cm PVC pipe cores. Samples were collected for each plant type (*J. roemerianus, S. patens-D. spicata*, and boundary) in areas adjacent to the established transects in each marsh zone. Triplicate cores were taken for each plant type/marsh zone combination, yielding a total of 27 cores. Associated above ground biomass samples were clipped, and sorted live from dead, dried, and weighed. Cores were wrapped in aluminum foil, kept moist and processed upon immediate return to the laboratory. Each 20 cm core was divided into 5 cm depth intervals: 0-5 cm, 5-10 cm, 10-15 cm and 15-20 cm. Each depth section was processed in the following manner: roots were rinsed to remove all visible traces of sediment and sorted live from dead using turgor and color as indicators of viability. Very fine roots were not sorted. Roots were dried to a constant mass and, weighed. Depth sections were combined into live and dead pools and ground in a Wiley mill using a #40 screen. Approximately 0.2 g subsamples of these pooled dry roots were combusted at 450°C for 18 hours and ash free dry weight determined.

Aboveground biomass was sampled using an end-of-year-biomass approach in mid-August 2005. The date of peak biomass for these species was previously determined in [Taylor, 1995]. Three replicates for each marsh site/plant zone combination were collected, in areas adjacent to the established transects, yielding 27 plots total. Small quadrats (0.25X0.25 m) were clipped to the sediment surface and stored in a lab freezer until analysis. Plants were sorted by species and then into live and dead categories,

weighed, then dried at 75°C for 24-72 hours until a constant mass was obtained. Representative samples of each species were saved for archival purposes.

For C:N analysis, three replicates of 2-5 mg of dry root material or 15 mg of dry soil was placed into 5X9 mm pressed tin capsules. % C, % N by dry mass, and C:N were determined.

2.4 Laboratory Methods

2.4.1 Direct Bacterial Counts

Bacterial abundance was measured for all samples using the Acridine Orange (AO) direct counting technique developed by [Rublee and Dornseif, 1978]. Briefly, approximately 1 g of wet sediment was added to 99 ml of filtered-sterilized H_2O (fsH_2O) and one drop of Triton X and homogenized in a laboratory blender. 1 ml of this suspension or a dilution thereof, was added to 4.0 ml of fsH_2O and 0.5 ml of 1% AO stain, vortexed to mix, and incubated in the dark for 60 s. The stained suspension was filtered through a black Poretics ™ (GE Osmonics, Minnetonka, MN) 0.2μm membrane filter and mounted for epifluorescence microscopy. The remainder of the sediment suspension was filtered onto pre-ashed Whatman™ (Florham Park, NJ) GF/C filters and dried for 24 hr at 75°C. Bacterial abundance was expressed as cells per gram dry soil using the following formula:

$$\text{Bacteria/g dry soil} = \frac{(\text{total area})/(\text{area/field}) \times (\text{cells/field})}{\text{Volume filtered} \times \text{dilution factor} \times (\text{g dry soil/ml})}$$

Ash-free-dry-weight (AFDW) was determined by combusting the filters at 450°C for 24 hr in a Thermolyne muffle furnace. The following formula was used to calculate % Ash free dry weight, or %SOM:

$$\%SOM = \frac{dry\_weight - ash\_weight}{dry\_weight} \times 100$$

2.4.2 Live Bacterial Counts

To address whether differences in the proportion of live to dead bacteria may contribute to differences in community structure, live-dead bacterial counts were performed using the LIVE/DEAD "BacLight Bacterial Viability kit" #7007 (Molecular Probes/Invitrogen, Carlsbad, CA). Shallow sediment cores were collected in July 2005, and kept in a low temp incubator at 12°C for 72 hours until processing was possible. The live/dead method is similar to AODC, in that a sediment suspension is created, a stain added and incubated, and the entire suspension filtered onto a membrane filter which is mounted for visualization with an epifluoresence microscope. The kit utilizes two fluorescent nucleic acid stains to differentiate between live and dead cells, as defined by membrane integrity. SYTO-9® fluoresces green and can penetrate both intact and damaged membranes, its fluorescence is decreased in the presence of propidium iodide, the counter stain. Propidium iodide fluoresces red and can only penetrate cells with damaged membranes. Thus, with the appropriate mixture of the two stains, bacteria with intact membranes will fluoresce green and bacteria with damaged membranes will fluoresce red (Molecular Probes Product Information, 2004).

For this study, stain components A (3.34 mM SYTO 9 dye in dimethylsulfoxide[DMSO]) and B (20 mM propidium iodide in DMSO) were mixed in a

1:1 ratio and 10 µl were added to a 4 ml suspension of diluted sediment. The formula for calculating cells per gram dry weight of soil is the same as with traditional AODC. Total counts obtained by the LIVE/DEAD kit were higher, on average, than total counts via AODC. However, since we were most interested in relative differences among sites and plant communities, the live cell count data was retained.

2.4.3 DNA Extraction

Community DNA was extracted from all sediment cores using the PowerClean™ Soil DNA kit (MoBio Labs, Solana CA) per the manufacturer's direction, with some modifications. The PowerClean kit includes proprietary reagents to reduce the presence of humic acids and cellular proteins that can inhibit amplification by PCR. Due to the amount of root material present in the soil samples, approximately 1 gram of sample was divided equally among 3 bead-beating tubes and the extraction completed in parallel. To reduce shearing of genomic DNA during initial cell disruption and lysis, the samples underwent two cycles of incubation at 70°C for 5 min, and disruption by vortexing for 5s. To concentrate the DNA extracts for each sample, triplicate extracts were pooled into a single microcentrifuge tube after the final DNA elution step. DNA was then precipitated by adding 2 volumes of 100% ice-cold ethanol and 0.25 volumes of 5M NaCl, centrifuging at 10,000 x g for 8 minutes and carefully decanting the supernatant. Tubes were inverted and air dried. The DNA precipitate was resuspended in 50 µl of sterile Tris buffer, pH 8.0.

Quantitation of genomic DNA was performed using the PicoGreen™ double stranded DNA (dsDNA) Quantitation reagent (Molecular Probes, Invitrogen; Carlsbad, CA) followed by analysis on a Biolume fluorescent plate reader. One microliter of DNA

extract was diluted in 99 µl filter-sterilized H_2O and stained per the manufacturer's instructions. Concentrations of dsDNA were found to be between 5 and 50 ng μl^{-1}. These data were used to standardize the amount of DNA template included in subsequent PCR reactions. Quantitation was also used to confirm successful DNA extraction. For all samples, DNA extraction was successful.

2.4.4 Denaturing Gradient Gel Electrophoresis

Molecular fingerprinting of soil bacterial community DNA was accomplished by denaturing gradient gel electrophoresis (DGGE) of fragments of the16S small ribosomal subunit (16 SSU) gene, which is peculiar only to bacteria and plant chloroplasts. The 16S rDNA gene is highly conserved and is frequently used for genetic analysis of prokaryotes [Ward, et al., 1990; Marchesi, et al., 1998; Watanabe, et al., 2001; Baker, et al., 2003] The technique, now widely used in microbial ecology, is based upon the behavior of DNA fragments in denaturing conditions. Double stranded DNA fragments will melt at specific melting domains, determined by the fragment's guanine + cytosine content. The more G-C bonds a fragment has, the more denaturant is required to sufficiently denature the fragment so that it is immobilized during electrophoresis in a polyacrylamide gel matrix. The technique uses a vertical polyacrylamide gel, in which a linear gradient of denaturants, urea and formamide, is cast. During electrophoresis, PCR products migrate down the gel and stop at a position that is determined by the fragments melting behavior. Thus, fragments of the same length can be separated by sequence differences, and the resulting bands can be recovered for DNA sequencing, if desired. This method has been used to study microbial community structure in a vast spectrum of environments, including soil and sediment habitats [Muyzer, 1999; Nakatsu, et al., 2000;

Duineveld, et al., 2001; Yoshie, et al., 2001; Ibekwe, et al., 2002; Kowalchuk, et al., 2002; Dar, et al., 2005] . For this study, a literature review was done to determine the most appropriate oligonucleotide primers for targeting the 16S rDNA gene by DGGE. Three primer sets were initially selected based upon their usefulness in soil microbial studies, target region, and product length. (See Tables 2.2 and 2.3). The primer pair chosen for DGGE analysis was 357F, 518R (numbering based on the standard *E. coli* 16S rRNA sequence), based on its specificity and quality of DGGE banding pattern it produced (See Appendix D). The primer pair is designed to target an approximately 190 bp fragment, encompassing the highly variable V3 region of the 16S rRNA gene [Heuer, et al., 1999; Hill, et al., 2000; Nakatsu, et al., 2000]. At the 5' end of each forward primer was attached 40 base pairs of 100% GC content, or a 'GC clamp' to prevent PCR products from denaturing completely into two single strands on the DGGE gel [Wu, et al., 1998]. The forward primers were purified via polyacrylamide gel electrophoresis by the manufacturer (Operon, Huntsville, AL) to reduce the likelihood of spurious amplification products.

"Touchdown" PCR was also performed to further protect against spurious priming. Touchdown PCR begins with annealing temperatures above the specified melting temperature (T_m) of the primers, the ideal temperature at which the primer can attach to the corresponding DNA sequence. This forces the primers to anneal stringently to complementary sequences along the DNA strand. Gradually, the annealing temperature is lowered below the determined T_m of the shortest oligonucleotide primer, decreasing specificity of the annealing process, but increasing yield. Thus, during touchdown PCR, the initial cycles ensure strictly that the desired target sequence is

amplified, and in later cycles, as PCR product reaches high concentrations, the primers amplify only those targeted fragments. Both hot-start and touchdown PCR are critical for sensitive applications like DGGE, as this method is theoretically able to distinguish single base pair changes [Meyers, et al., 1987].

2.4.5 PCR Conditions

DGGE PCR conditions were adapted from [Crump, et al., 2003]. Each 50 µl reaction mixture contained 2.25 mM $MgCl_2$, 1X $MgCl_2$-free PCR buffer, 200 µM dNTP's, 1µM each of forward and reverse primer, 1X bovine serum albumin (10mg/ml stock), 1 unit of Amplitaq Gold *Taq* polymerase (Applied Biosystems, Foster City, CA), and 1 µl of a 1:10 dilution of the original DNA extract. DNA extracts contained, on average, 20 ng-40 ng DNA template per µl. High concentrations of primer, as used here, have been shown to prevent heteroduplex formation during PCR, which can result in false bands in DGGE analysis [Crump, et al., 2003]. To safeguard against non-specific priming before thermal cycling commenced, a chemically protected *Taq* polymerase was used in lieu of manual "hot-start" PCR.

For primer pair 357F, 518R the following PCR program was carried out:

Initial denaturation 94° C for 5 min
Then 10 cycles of:
    Denature: 94°C for 15 s
    Anneal: 67°C for 20s (anneal temperature decreased by 0.5 C per cycle)
    Extend: 72°C for 20s
Then 10 cycles of:
    Denature: 94°C for 15s
    Anneal: 57°C for 30s
    Extend: 72°C for 20s
Final extension at 72°C for 35 min
Ramp -1°C/s to 4°C

Seven µl of PCR product was mixed with 6X loading dye and run on a 1.8% agarose gel, stained with ethidium bromide and visualized on a UV transilluminator to verify the success of the PCR reactions in advance of the DGGE runs.

2.4.6 DGGE Set-up

DGGE gels were run on a DGGE-2001 system from CBS Scientific (Del Mar, CA). Briefly, 0.75 mm thick 12% acrylamide (37.5:1 acrylamide to bisacrylamide) gels were poured to contain a linear gradient of 55%-63.3% urea-formamide (100% denaturant is defined as 7M urea plus 40% formamide) [Muyzer, et al., 1993].. A 0% stacking gel was poured over each denaturant gel to ensure comb wells contained no denaturant material. Gels were allowed to polymerize for at least 4 hours. The optimal percentage of acrylamide and denaturant conditions used were determined empirically for this study.

Approximately 40 µl of PCR product was mixed 1:1 with loading dye and loaded into the vertical gel. Instead of a molecular weight marker, 16S rDNA from a single extract of cultured sediment bacteria was loaded into 3 lanes per gel to serve as a migration standard. The extract was chosen for its repeatable fingerprint over successive PCRs and DGGE runs. Electrophoresis conditions were 110V for 15 min, followed by 70V for 16 hours in 60°C 1X TAE buffer (0.004 M Tris-acetate, 0.001 EDTA). DGGE gels were stained by spreading a solution of 1X SYBR Gold solution (1.2 µl 10,000X SYBR Gold ™ (Molecular Probes, Invitrogen; Carlsbad, CA) nucleic acid stain dissolved in 12 ml of 1X TAE) over the surface of the exposed gel, followed by incubation in the dark for 15-20 min. The gels were visualized by UV transillumination and photographed using Polaroid 667 film with a deep yellow, #15 filter.

2.4.7 Gel Image Analysis

All Polaroid images were scanned on a flat bed laser scanner at 800 dpi resolution and saved as grayscale TIFF files. Positive intensities for each image were inverted and levels adjusted to maximize contrast and visibility in Adobe Photoshop CS. Five bands chosen from the migration standard were used as reference bands, to control for warping, smiling or other variations within and among gels compared. R_f values (equivalent to the retardation factor in chromatography) were computed for each band falling between the regions defined by the five reference bands. R_f values were binned into size classes to define a confidence range for distinguishing distinct bands. Size classes were large enough to encompass the variation in R_f measurements of bands observed to be identical upon visual inspection. The binning into size classes also combined closely spaced, but distinct, bands that were within this confidence range. For statistical analysis, bands were scored as either present (1) or absent (0). Band intensity was not considered in this study.

2.4.8 DGGE Gel Effects

One drawback of DGGE is the difficulty in comparing samples across different gels due to differences in gel quality or running conditions. Often, researchers randomize the loading order of samples, or run samples that can be "blocked" on one gel, thereby allowing variation due to a possible gel effect to be removed *post hoc* [Kropf, et al., 2004]. In this case, gels were loaded in order of collection, from low marsh, to high marsh. A preliminary analysis of the full DGGE dataset suggested the possibility of a gel effect in Summer 2005 samples, and to a lesser extent in the Summer 2004, and Winter 2005 data sets. To eliminate the effects of gel quality on community identity, subsets of 9 samples from each sampling event were run on three separate gels. This allowed for

testing of plant and location effects, but due to the limitation of gel size, did not allow for replication.

2.5 Statistical Methods for Continuous Empirical Variables

2.5.1 ANOVA

Group differences were tested by 2-way and 3-way ANOVA tests in SAS 8.2 (Cary, NC) using PROC GLM with Tukey adjustment of experiment-wise error rate when multiple comparisons were employed. When necessary to meet the assumptions of homoscedasticity and normality, data were log-transformed prior to ANOVA and back transformed for reporting of means and 95% confidence limits. A multiple analysis of variance (MANOVA) also was performed using PROC GLM.

2.5.2 Pearson Correlation Test

A Pearson correlation matrix was constructed using PROC CORR in SAS 8.2. The matrix contained the following variables: aboveground biomass, bacterial abundance, live bacterial cells, %SOM, belowground biomass, root C:N, root OM content, elevation, live bacterial abundance, and soil C, soil N, and soil C:N.

2.6 Statistical Analysis for Community Fingerprinting

2.6.1 Non-metric Multidimensional Scaling

Multidimensional scaling (MDS) is a distance-based ordination method that estimates the location of objects in a space of specified dimensions based upon the distances or dissimilarities between pairs of objects [Kruskal, 1964a; Kruskal, 1964b] Most data must be transformed by some function in order to relate dissimilarity between objects to Euclidian distances in n-dimensions. The loss of information (distortion) that occurs during the transformation from dissimilarity to distance is expressed as the

"stress" value. Intuitively, as dimensionality increases, stress decreases; S values of 0.01 or less are generally considered ideal [Johnson and Wichern, 1992]. In highly dimensional datasets, the stress value may never reach an "acceptable" level, or does so only at a high number of dimensions, at which visualization is confusing or uninformative [Rodrigues, et al., 2002]. The number of axes to use in a specific ordination must be determined by the researcher by weighing all the factors of stress, interpretability and accurate representation of the dissimilarities [Johnson and Wichern, 1992; Legendre and Legendre, 1998]. For the analysis of bacterial community dissimilarity in this thesis, three dimensions were used to reach an acceptable level of stress and clear representation of the data.

MDS can perform either a monotonic, power, or affine function to convert dissimilarity coefficients to distances in n-dimensional space that best retains the original dissimilarities of the data matrix. (An affine transformation is one which preserves collinearity, i.e. all points lying on a line initially will lie on a line after the transformation, and the ratios of the distances between the points are preserved.) When employing non-metric multidimensional scaling (NMDS), it is only necessary to use a monotonic function that preserves the rank dissimilarities of all pairs of observations. When the configuration values of the scaling are plotted, the physical distances between objects in the specified dimensions represent the dissimilarity of those objects. Orientation of axes and their "meaning" are arbitrary and can only be interpreted by visual inspection.

Ordination of binary community fingerprint data is frequently performed by NMDS [Fromin, et al., 2002; Rodrigues, et al., 2002]. Since variables in community

fingerprints are bands/ribotypes/populations, extraction of eigenvalues from a PCA or DFA of this data would be meaningless. As NMDS is not an eigenvalue-based analysis, its use is more appropriate in ordination of DNA fingerprints. NMDS does not require assumptions of normality or homoscedasticity, and as such is more appropriate for binary data sets [Fromin, et al., 2002]. There are conflicting data concerning the agreement of ordinations by PCA versus NMDS on binary data (Kang and Mills, in press;). Likely, the agreement of PCA and NMDS depends on the structure of the particular dataset in question.

2.6.2 Dissimilarity Matrices and Ordination by NMDS

To work with the binary DNA fingerprint data, a dissimilarity matrix was constructed for ordination by nonmetric multidimensional scaling for each sampling (July 2004, February 2005, July 2005). Using PROC DISTANCE in SAS 9.1, dissimilarity matrices were constructed using Jaccard's dissimiliarity coefficient (1-similarity). Absent-absent (0-0) pairs were omitted as they contain no information, unlike present-absent (1-0) or present-present (1-1) pairs; variables were specified as asymmetric nominal variables, and the following formula was used to compute the Jaccard dissimilarity coefficient:

$$J_d = 1 - (M / (M+N))$$

Where M= the number of positive (1-1) matches and N= number of mismatches (1-0 or 0-1).

To compute dissimilarity matrices for the other environmental and biotic variables, Gower's dissimilarity coefficient was computed in PROC DISTANCE. Gower's

coefficient is useful for mixed data types and includes a standardization of the data ranges.

$$S_{ij} = \frac{\sum_k W_{ijk} S_{ijk}}{\sum_k W_{ijk}}$$

Where W_{ijk} is 1 or 0 depending on whether the comparison for ij is valid. K is the number of variables, i and j are the two samples to be compared and S_{ijk} is the similarity of i and j at the Kth variable.

Once similarity matrices were constructed for each sampling period and an overall matrix for all samples, PROC MDS (SAS 9.1) was used to ordinate the data. To specify a non-metric analysis, a monotone transformation of the data with no explicit parameters was used. Thus, only the rank orders of dissimilarities among the samples were considered. The 3D scatterplots of the PROC MDS configurations were produced in Minitab 14.1.

2.6.3 Mantel Tests

The Mantel test is one way of comparing two similarity or distance matrices that are computed around the same set of objects or observations [Legendre and Legendre, 1998]. The Mantel Test involves three basic steps: 1.) the determination of the correlation of two matrices, A and B, as defined by the Mantel statistic, Z_{AB}, or its standardized value, r_{AB}, 2.) a series of random permutations of the rows and columns of one matrix, and a re-calculation of the test statistic for each permutation, and 3.) determining the significance of the correlation by computing how many times the actual test statistic was

exceeded in the random permutations. It is important to remember that the matrix correlation coefficient Z_{AB}, or r_{AB}, describes the extent to which the *distances* in matrix A correspond to the variations of matrix B, not the correlation of the *variables* represented in the two matrices [Legendre, 1993].

For ecological studies, the Mantel test is often used to compare a matrix of genetic dissimilarity and a distance matrix of some measured environmental variable (continuous values). The Mantel test can also be used for *a priori* hypothesis testing by building discrete matrices of 1's and 0's that describe whether samples are "linked" or not within the model that matrix represents [Rodrigues, et al., 2002]. The correlation of an empirical dissimilarity matrix with this discrete "model" matrix (if it represents group classifications), will be equivalent to a nonparametric multivariate analysis of variance [Legendre and Legendre, 1998]. Both the *a priori* and empirical applications were used in this research.

In this study, the same community dissimilarity matrices used in the NMDS were used in the Mantel tests. Empirical distance matrices were constructed using Gower's dissimilarity coefficient in SAS 9.1, as described above. For the *a priori* tests, model matrices were constructed for "site" and "plant" to test hypotheses that soil bacteria communities were different among sites and among plant types. Matrices describing the gel on which each sample was run were constructed to determine the effect of gel differences on DGGE community fingerprints.

To simplify the Mantel tests, environmental and biotic explanatory variables were divided into 5 groups of correlated variables. A Pearson's Correlation test was performed in SAS 9.1 to find which environmental variables were most correlated with one another.

A dissimilarity matrix of "environment" included %OM, and elevation. The following variables were not significantly correlated and separate dissimilarity matrices were computed for each: aboveground biomass, belowground biomass, root C:N, sediment C:N, bacterial abundance.

The Mantel tests were carried out in PopTools, a free Excel add-in program. In most cases, 999 permutations were carried out for significance testing. The p value is computed by hand as the number of times the random permutations returned a r_{AB} greater than the test statistic, divided by the total number of permutations. Thus, the Mantel test is usually one tailed, where the alternative hypothesis is that the two matrices being compared are positively correlated. The alpha level was adjusted for multiple comparisons via a Bonferroni correction:

$$\alpha = \frac{p}{n}$$

where α = adjusted alpha level, p= 0.05 and n= number of comparisons.

Chapter 3: Results

To begin this chapter, I briefly summarize significant differences in continuous variables and bacterial community structure observed among Upper Phillips Creek Marsh (UPCM) locations and plant communities in sections 3.1 through 3.3. Detailed results are reported in subsequent sections corresponding to each variable measured or analysis. Within each section, results are presented focusing on differences between plant communities, and marsh locations. All means are reported with their standard errors, except in cases of transformed data, where 95% confidence limits are given. See Appendix M for tables of all means and errors for data presented herein. ANOVA tables for each section describing ANOVA results can be found in Appendix A.

3.1 Continuous variables: differences among marsh locations

Significant differences across marsh sites were observed in soil organic matter (SOM), root organic matter, sediment and root C and N content. These observed differences reflect the unique inundation regime, sediment supply, root production, and microbial activity at each marsh site. SOM, root OM, soil and root C and N increased from the low to high marsh (Fig 3.2, 3.4 and 3.6). There were no significant site differences in soil C:N, or root C:N.

3.2 Continuous variables: differences among plant communities

Indicative of variability in plant physiology among different marsh plant species, differences were seen in belowground and aboveground biomass, and sediment and root C:N contents between the *J. roemerianus* and *S. patens-D. spicata* communities. Highest belowground biomass was observed in the transition zone between the *J. roemerianus* and *S. patens-D. spicata* communities, suggesting that competition for nutrients and

space in this zone may lead to increased root production. Clip plots from the *J. roemerianus* stands and the transition zone had considerably higher aboveground biomass than the *S. patens-D. spicata* plots, due to the tall, thick blades of the *J. roemerianus* plant. Soil C:N ratio varied by plant community also. Highest soil C:N ratios were found in the *S.patens-D.spicata* communities, and the lowest in the *J. roemerianus* community. These are consistent with published C:N values for these plant species in the literature for this marsh [Blum and Christian, 2004]. The plant community differences in C:N ratios of roots and soil were small, and likely not nutritionally different to soil bacteria [Drenovsky, et al., 2004]. There were some plant community differences in bacterial abundance in July 2004 and February 2005 but the trends were not consistent among sampling dates.

3.3 Bacterial Community Structure

The differences among soil bacteria communities in Upper Phillips Creek Marsh are correlated to differences in variables that vary consistently with marsh site--SOM, elevation (inundation regime), and root organic matter. *A priori* tests of correlation between model and bacterial community dissimilarity matrices from both summer sampling events showed significantly high correlation of community structure with the site model matrix, and low, insignificant correlation with the plant model matrix. This pattern is supported by the observation that in both summers, dissimilarity of bacterial community structure was most correlated with differences in root OM, and/or elevation, and SOM which are closely linked to marsh location (Tables 3.4a, 3.4c). In the February 2005 DGGE subset, no significant bacterial community structure differences were found in the comparison with either site or plant model matrices, nor across any continuous

variables examined, suggesting low temperatures have a greater effect on bacteria than plant communities, inundation, or SOM.

3.4 Elevation

Marsh elevation varied significantly across marsh sites. Elevation ranged from an average of 0.6 m above msl to 0.78 m above msl in the low and high marsh respectively (Fig 3.1). There was more variation in elevation in the high marsh than in the low or mid marsh, due to the higher presence of hummock and hollow terrain in the high marsh.

3.5 Soil Organic Matter

Soil organic matter content (SOM) varied significantly by marsh site, but not by plant community (Fig 3.2). A 2-way ANOVA revealed high marsh (69.33% ± 7.29) and mid-marsh SOM (60.60 0% ± 6.52) were significantly different from low marsh SOM (46.22% ± 6.68) (Fig 3.2). Percent SOM did not vary significantly by plant community, and there was no significant plant-site interaction (data not shown).

3.6 Live Root Organic Matter

To account for any salt or silica stored in plant tissues, live and dead belowground organic matter were analyzed for site and plant differences by 2-way ANOVA. Live roots contained significantly greater organic matter than dead roots (Fig 3.3). Live root OM content was significantly higher in roots from the high marsh (92.02%, ±1.11) than roots from the low marsh (87.92%, ±0.87) and echoed significant differences in SOM observed across marsh locations (Fig 3.4). Dead roots from the high marsh were also significantly greater in OM than roots from the low marsh (88.04% +/- 1.32 vs. 80.08% +/- 1.67, respectively) (Fig 3.4). Though it was not statistically significant, highest live root organic matter was found in the *S. patens-D. spicata* roots (91.28, ± 1.15) and the

lowest was found in the *J. roemerianus* roots (88.26, ± 0.88). However, dead *J. roemerianus* roots had significantly lower OM than dead roots from the transition zone communities (Fig 3.4).

3.7 Soil Carbon and Nitrogen Content

Total C and N (TC and TN) content of the marsh soils did not vary significantly by plant type (Fig 3.5) but did by marsh location ($p < 0.0001$) (Fig 3.6) in either winter or summer. Soil nutrient content was highest in the high marsh (33.9%TC ± 0.89, 1.83%TN ± 0.04) and lowest in the low marsh (20.08%TC ± 20.16, 1.16%N ± 0.04), following patterns in %SOM seen across the marsh. There was not a significant plant-site interaction for either TC or TN content. Alternatively, a 3-way ANOVA showed C:N varied by plant type and sampling date but not by marsh site (see Appendix A). Mean soil C:N was highest in the *S. patens-D. spicata* community (19.35) and lowest in the *J. roemerianus* community (17.40) (Fig 3.5).

3.8 Live Root Carbon and Nitrogen Content

Two-way ANOVA for plant and site effects on live root C:N was not significant. (See Appendix A). Due to the inability to reliably differentiate plant roots for these communities, C:N of individual plant species could not be compared. However, differences in C:N of roots from the *S. patens-D. spicata* communities versus the *J. roemerianus* communities were not significant. Live root carbon content was significantly highest in roots from the high marsh and the *S. patens-D. spicata* communities (See Appendix M).

3.9 Aboveground Biomass

Total above ground biomass differed significantly by marsh site and plant type, with no site-plant interaction in a 2-way ANOVA. Composition of the plant communities was similar across sites, with the exception of the presence of *S. alterniflora* in the *S.patens-D.spicata* community only in the low marsh (Fig 3.7). Aboveground biomass values for *J. roemerianus* communities and the transition communities were similar, (291.24 and 298.44 g m^{-2} respectively, as those zones are dominated by the tall stalks of *J. roemerianus* (Fig 3.8). Aboveground biomass was highest in the low marsh (296.01g m^{-2}), and lowest in high marsh (211.06 g m^{-2}) (Fig 3.9).

3.10 Belowground Biomass

Although the sampling scheme did not allow for a large degree of replication (three replicates per plant/site combination), significant differences in belowground biomass by plant type were observed. Among all cores, the amount of root biomass varied with depth; the 0-5 cm section had the most live roots (482.23g m^{-2} ± 51.53), and the 15-20 cm section had the least (43.71 g m^{-2}; ± 8.05) (Fig. 3.10). The greatest amount of dead root material was found in the 10-15 cm core section (954.12 g m^{-2}, ± 91.46). The mineral clay layer was always found within the 20 cm long core, and often comprised part or all of the lowest two depth sections. Results from the upper 5 cm were used for all subsequent belowground biomass comparisons, as it coincided with the depth of the cores used for community analysis. Belowground biomass from the transition zone was significantly higher than in the *S. patens-D. spicata* and *J. roemerianus* communities (688.99, ±73.57 vs. 403.97g, ±84.85 and 353.72g, ±71.45, respectively) (Fig 3.9). Belowground biomass was not significantly different among marsh sites (Fig 3.10).

3.11 Acridine Orange Direct Counts

Total bacterial abundance (mean = 4.71×10^{10} cells g^{-1} dry soil) was on the same order of magnitude to previously reported numbers in marsh soils or sediments (10^{10} cells g^{-1} dry soil) [Rublee and Dornseif, 1978]. In July 2004, bacteria were most abundant in the *J. roemerianus* communities and lowest in *S. patens-D. spicata* communities (Fig 3.11). In February 2005 and July 2005, the transition zone had higher bacterial abundance than either *J. roemerianus* sites or *S. patens-D. spicata* sites, but the differences were only significant in February 2005 (Fig. 3.11).

Bacterial counts were consistently highest in the mid marsh and lower in the low and high marsh for each of the three sampling periods, although this relationship was not significant in the February 2005 samples (Fig 3.12) The mid marsh may provide an optimal habitat for microbial growth, where soil OM is relatively high and regular tidal flushing removes buildup of metabolic waste products. High belowground root production in the mid marsh of Upper Phillips Creek, as observed by Blum and Christian, [2004], could also be a contributing factor to increased bacterial abundance at that site.

3.12 Live Bacterial Abundance

Live bacteria, on average, comprised 65.5% of the total bacterial cell count. This percentage did not vary significantly across the plant communities or sites compared. The actual number of live bacteria did significantly vary across marsh sites, with the mid marsh having the most (2.88×10^{10} cells g^{-1} dry soil), and the low marsh having the least (2.02×10^{10} cells g^{-1} dry soil) (Fig. 3.13). There was no significant difference across the plant community types compared (Fig 3.14).

Although few data are available for comparison, the ratio of live to dead bacteria in this study were high compared to those reported for limnetic (<20% to 70%, average 38%) [Tietjen and Wetzel, 2003] and sediment systems (24-30%) [Luna, et al., 2002], suggesting a highly active and productive bacterial community in UPCM. Live bacterial abundance was only measured once in this study, such that seasonal differences in live cells can not be ascertained.

3.13 MANOVA and Pearson correlation analysis

The following continuous variables from the July 2005 sampling event were analyzed by a multiple analysis of variance: aboveground biomass, belowground biomass, total bacterial abundance, live bacterial abundance, soil organic matter, root organic matter, C:N, elevation, , soil C, and soil N content. The MANOVA test rejected the null hypothesis of no overall plant and site effect (Table 3.1b, 3.1c). There was an overall significant site, plant and site-plant interaction; however, these were not found for every variable included in the MANOVA (Table 3.1a, 3.1d). Significant site-plant interactions were observed in belowground biomass--a result of very low root biomass from *S. patens-D. spicata* communities in the low marsh compared to other regions--and root OM—due to much lower root OM in the transition or *S. patens-D. spicata* communities in low marsh than other marsh regions (data not shown).

The following data from summer 2005 were used in the Pearson correlation: aboveground and belowground biomass, soil organic matter, live root organic matter, total bacterial abundance and live bacterial counts, elevation, soil C, soil N, soil C:N and soil moisture. Soil moisture content data of replicate cores were obtained from Gina Casciano. Aboveground biomass was negatively correlated with elevation and soil C:N,

while live root OM was positively correlated with elevation and soil C:N and belowground biomass; total bacterial abundance was positively correlated with %SOM, and soil moisture; %SOM was positively correlated with elevation, belowground biomass, soil C, soil N, and live root OM. (Table 3.2). These data were used to help group variables for Mantel tests of matrix correlation with the bacterial community fingerprints.

3.14 Bacterial Community Analysis

3.14.1 Bacterial community structure

Bacterial community structure was measured by analysis of denaturing gradient gel electrophoresis of 16S-rDNA fragments (16S-DGGE). Gel fingerprints were analyzed for dissimilarity in band pattern, as well as band richness, or the number of different bands in a given sample. On average, 13 bands were seen per sample on the DGGE gels. Lowest band richness occurred in the February samples (average 7.3 bands/sample). Band richness did not consistently correlate, either positively or negatively, with bacterial abundance, plant community, and only weakly, with one exception, with SOM. The highest r^2 value was 0.65, between band richness and SOM in the July 2005 subset (Table 3.3). It is probable that with increased sample size and replication, DGGE analysis could provide richness data that are more reliable, although it will always provide a profound underestimation of the actual phylotype richness present. One recent study used band richness from DGGE fingerprints as the sole measure of bacterial community structure [Wertz, et al., 2006]. Obviously, comparisons of band richness across gels are subject to the same caveats as comparing band patterns across gels. Although every effort was made here to ensure consistency among gels, variations

in any component of the gel casting process can compromise gel quality, causing fewer observable or distinguishable bands. Because gel assignment was found to have such a confounding effect on fingerprints in this study, comparison of communities from different sampling dates was not feasible.

As mentioned above, the full DGGE dataset--12 gels, 135 samples total--were not used in the following analyses due to autocorrelation between a given sample's location in the marsh and the gel on which the sample was run. To correct this problem, subsets of 9 samples (1 sample per plant-site combination) were run on a single gel. (DGGE gels cast in the CBS 2000 system can hold up to 11 samples after excluding outside lanes and allowing for migration standards.) For optimal data analysis, a randomization of the sample loading order would have been performed before running any gels. A few statistical approaches have been developed to address analysis of large numbers of DGGE fingerprints, and should be explored in future experiments [Kropf, et al., 2004].

3.14.2 Ordination Results by NMDS

Non-metric multidimensional scaling of July 2004, February 2005, and July 2005 bacterial community dissimilarity were performed in 2, 3 and 4 dimensions. For all NMDS models run, the 3 dimensional configurations attained acceptable stress values while allowing for easy visualization of community differences (Table 3.4). In the full dataset for each sampling event (45 samples over 5 gels), NMDS analysis showed tight groups relating to marsh location, and no groups correlated with plant community (data not shown). Due to the correlation between gel assignment and marsh site, NMDS configurations of the full datasets also showed groupings by gel assignment (Fig 3.15). Both NMDS configurations of the full data set (e.g., Fig 3.15) and results of Mantel tests

comparing community structure, and the 'site' and 'gel' model matrices (Tables 3.5a-c) demonstrate this confounding effect of gel assignment.

Ordination of the dissimilarity matrices of a subset of the samples for each sampling event (2 subsets for July 2004, 1 each for February 2005 and July 2005) eliminated the effect of gel assignment, since all samples included in a given matrix originated from the same gel. Groups of samples by marsh location but not by plant community type were observed in some of these subsets, suggesting that the site effect observed for the entire data set may be a valid effect (Figures 3.16A-3.19A,). No clear groups related to plant community were observed in any of the NMDS plots from any sampling event (Fig 3.16B-3.19B).

3.14.3 Mantel Tests

Mantel tests were used to compare the following variables with community structure of each of the three sampling dates: "environment" (%SOM and elevation) and soil C:N. "Site" and "plant" model matrices were also compared with the dissimilarity matrices of bacterial community structure to test *a priori* hypotheses regarding differences in bacterial communities among marsh sites and plant communities.

For the July 2005 samples, additional comparisons were made between the community structure matrix and aboveground and belowground biomass, since these data were collected only in the summer of 2005 and values of these properties can exhibit high interannual variability, depending on variation in other variables such as rainfall or inundation frequency [Blum and Christian, 2004]. The "environment" matrix included root organic matter content along with SOM and elevation.

Mantel tests showed significant correlations in some comparisons after the Bonferroni correction for multiple comparisons (Tables 3.6a-c). In one of the bacterial community matrices from July 2004 (subset "B"), the bacterial community (hereafter simply, "community") vs. "site" comparison was highly significant, ($p<0.0001$). In February 2005, none of the Mantel comparisons were significant at an adjusted α level of 0.013 (Table 3.6b). In July 2005, three comparisons were significant at an adjusted α-level of 0.007 (Table 3.6c): Community vs. Environment (%SOM, root OM, elevation) ($r = 0.46$ $p = 0.006$), community vs. "site" model matrix ($r = 0.48$ $p = 0.001$), and community vs. aboveground biomass $r = -0.42$, $p = 0.003$). The comparison of the "site" model matrix vs. environment was also significant. It is important to note that the "site" model matrix is not independent from the environment matrix; the model matrices are used here for *a priori* hypothesis testing only.

Chapter 4: Discussion

Bacterial communities in Upper Phillips Creek Marsh (UPCM) are primarily structured by environmental factors: temperature, and the combined effects of elevation, inundation and soil organic matter (SOM). Plant community effects were not observed here; yet healthy, productive plant communities provide belowground root stocks for microbial decomposition, and are therefore a critical resource for bacteria. The patterns seen in soil bacterial community structure in this study support the results of others in wetlands and agricultural soil, [e.g. Burke, et al., 2002a; Kang and Mills, 2004] but counter to other studies, [e.g. Kowalchuk et al., 2002].

4.1 Environmental Factors: temperature, inundation/elevation/SOM

The lack of site or plant community effects on bacterial community structure in the winter sampling demonstrates a temperature (or seasonal) dependence of salt marsh soil bacterial communities (Fig 3.18A,B and Table 3.6b). This lack of observed site effect may be a result of temperature-related factors causing decreased variance among communities, such that they are not distinct across marsh sites. Conversely, the variance in the winter sampling could be so large that a site effect could not be observed. (Comparisons of the variance among the three sampling dates were not performed due to problems comparing data among gels.) Temperature-related effects on bacterial community structure have been demonstrated previously in aquatic systems [Poremba, et al., 1999; Pinhassi and Hagstrom, 2000; Schauer, et al., 2003]. The results reported here also provide further evidence of bacterial communities' complex responses to temperature and substrate availability, as changes in community structure with varying SOM were observed in summer, but not in winter (Tables 3.6a-c). [Shia and Ducklow,

1994] found that bacterial productivity in an estuarine water column was temperature-limited below water temperatures of 20°C, but substrate limited at higher temperatures. Apple [2006] observed that bacterioplankton production and growth efficiency in a temperate estuary were influenced by both temperature and organic matter, though the relative importance of each variable changed over the course of the year. Similar mechanisms of bacterial productivity and growth efficiency may be at work in marsh soils, and could be reflected in this study's bacterial community structure results. Also, temperature may indirectly affect chemical or biological variables that have a direct effect on bacterial community composition, such as pore water chemistry, supply of OM or substrate by plants or grazing by protozoans and interactions with viruses [Ram, et al., 2005; Liu, et al., 2006]. Temperature-induced changes in the relative abundance of bacterial groups, via the selection of cold-tolerant, or cold-adapted, organisms, could also contribute to the lack of site or plant effects in the February 2005 data. For example, although sulfate reduction rates (SRR) decrease greatly in the cooler months, one study found an *increase* in relative abundance of sulfate reducing bacteria (SRB) in winter [Hines, et al., 1999], suggesting that SRB may be better at surviving cold conditions than other members of the community.

Seasonal variability in inundation regime found in UPCM [Stasavich, 1999], and therefore porewater chemistry, could cause a change in the strength of site effects on bacterial communities in a marsh, and partly explain the absence of a site effect in winter. Increased anoxia in the high marsh in winter due to prolonged flooding could override—from a bacterial community perspective--the differences that exist in porewater chemistry across sites. The lack of a plant or site effect in winter indicates that temperature (or

temperature-dependent processes), and soil organic matter dynamics may be the strongest effects on soil bacterial community structure in UPCM.

What are some of the potential mechanisms by which inundation regime in its altering of soil redox potentials and decomposition processes affects bacterial community structure? First, differences in freshwater inundation and tidal inundation provide different proportions of terminal electron acceptors (TEAs) for bacterial respiration, shifting the dominant metabolic pathways among Fe(III) reduction, sulfate reduction, or methanogenesis, for example. This may, in turn, alter the relative abundance of the major bacterial players responsible for these reactions. Although it was not directly measured in this study, the decreasing relative abundance of sulfate reducing bacteria, or other major functional groups, such as iron reducers or methanogens, could also contribute significantly to the observed site effect. There is an absence of published data on the abundance of these different groups, as well as the process rates they are responsible for carrying out across marsh regions or inundation gradients. Devereux [1996] found sulfate reducing bacteria (SRB) comprised a large portion (up to 30%) of the total rRNA extracted from the rhizosphere of marsh plants. Neubauer [2005] showed that microbial metabolic pathways (including Fe (III) reduction, methanogenesis and sulfate reduction) were regulated by physiochemical factors in a brackish marsh, and by plant activity in a freshwater marsh. This indicates an interaction could exist between marsh inundation regime and plant effects on bacterial function across different regions of a single marsh. In future work, an analysis of SRB, methanogenic bacteria, iron reducers and other groups in concert with total bacterial community fingerprinting may demonstrate what

contribution these functional groups have on total community structure changes across marsh regions.

A second way inundation-related factors can structure bacterial communities is by affecting organic matter pools in the soil. Frequent, prolonged inundation leads to a lowering of soil redox potentials and increased anoxia, causing incomplete decomposition of plant detritus and roots and an increase in SOM and dissolved organic carbon (DOC) [Parnas, 1975; Reddy and Patrick, 1975; Brinson, et al., 1981; Rabenhorst, 2001; Bossio, et al., 2006]. SOM is lower in low marsh soils, as mineral sediment comprises a large portion of total soil volume; additionally, root production is lower in the low marsh than in the upper marsh of UPCM [Blum and Christian, 2004]. Thus, differences in SOM quality and abundance of labile C and N between tidally influenced and fresh water areas of the marsh are established. In the wider scope of microbial ecology, the concentration, source, or type of organic carbon is a widely recognized control on bacterial community structure in the environment [Crump, et al., 2003; Girvan, et al., 2003; Drenovsky, et al., 2004; Marschner, et al., 2004; Waldrop and Firestone, 2004; Docherty, et al., 2006].

It has been demonstrated that different bacterial groups will preferentially decompose different OM pools [Waldrop and Firestone, 2004; Docherty, et al., 2006; Kramer and Gleixner, 2006]. Some preferentially decompose plant-derived OM in the forms of root tissue, newly senesced root tissue or other macroorganic matter, such as stem and leaf litter. Other bacteria utilize the standing pools of SOM, fermentation products exuded from plants or produced by other bacteria, while others find carbon sources outside of SOM, such as methanogenic bacteria utilizing CO_2 [Kramer and

Gleixner, 2006]. Thus, the site effect on bacterial community structure observed in UPCM soils is likely a combination of covarying and cascading responses. The result is a complex community in which some bacteria are responding primarily to availability of alternate TEAs, or redox conditions as caused by inundation regime, rhizosphere bacteria responding to carbon exudation from roots, others to the amount or quality of SOM already present as a result of earlier microbial processing, including products of bacterial and fungal metabolic processes. In this study, I was unable to separate the individual contribution of SOM, elevation and tidal inundation on soil bacterial community structure, as these three variables were highly correlated (Fig 3.2, Table 3.2). This could be explored in field manipulations or greenhouse experiments in the future.

Will functional community differences necessarily be reflected in DGGE community DNA fingerprints? It is known that microbial respiration processes in a fresh marsh versus a tidally influenced low marsh can be very different, and/or change seasonally, [Howarth and Teal, 1979; Thomas, 2004; Neubauer, et al., 2005], and I observed in this study that community structure in summer, as revealed by 16S rDNA methods, varies by marsh site, suggesting a link between soil bacterial community structure and function in UPCM. (Figs 3.16A and 3.18A, Tables 3.6a and 3.6c). Yet, it has been demonstrated in numerous laboratory experiments that a reduction in bacterial diversity via serial dilution of mixed cultures does not affect bacterial function, such that the functional redundancy of hyper-diverse natural bacterial communities is well-accepted [Degens, 1998; Franklin and Mills, 2006].

It is possible, as a result of the functional redundancy of natural bacterial communities, that a change in bacterial community function would not necessarily be

reflected in community diversity. Thus, one could argue that the community structure differences observed in this study across an inundation gradient are not likely yielding functional differences in the community. It should be emphasized that diversity was not measured in this study, but differences in community *identity* were. Bacterial community identity can be viewed as the qualitative community composition as measured by DNA fingerprinting methods such as DGGE. This and other molecular fingerprinting methods capture a snapshot of the dominant bacterial phylotypes; thus they are not an exhaustive metric of the actual diversity. In addition, diversity is a measure of the richness and evenness of a community; two communities could have very different species composition, or have the same species present in different relative amounts, and still have equivalent diversity values [richness, evenness, Shannon-Wiener diversity (H')] [Hellmann and Fowler, 1999; Sterling and Wilsey, 2001]. The present study was intended not to describe the diversity of the soil bacteria, but to produce a qualitative fingerprint representing dominant bacterial phylotypes present in the soil. 16S-DGGE captures the most abundant members of a community, and is not a complete measure of diversity or its components, richness and evenness. Thus, there is good reason to conclude that the qualitative differences in community identity observed here reflect variation in bacterial community function and processes across the inundation gradient.

4.2 Effects of Plants on Bacterial Community Structure

It was surprising to observe no significant effect of overlying plant community on soil bacterial community structure in any of the three sampling events. There are at least three explanations for this finding; two of which are related to methodological issues.

1.) *Absence of an observed plant effect could be the result of the type of soil sample collected.* No distinction between rhizosphere and bulk soil was made during sample collection. Thus, community DNA was extracted from a homogenized sample of soil and fine root materials. The samples were taken from the upper 3 cm of marsh soil, which are densely populated with plant roots. A few experiments have compared bulk and rhizosphere soil communities separately and showed plants exerted control on the structure of rhizosphere communities, and had little to no effect on bulk soil bacterial communities [Kowalchuk, et al., 2002; Costa, et al., 2004]. Kang and Mills [2004] removed large root and plant material from soil samples, but made no distinction between bulk and rhizosphere material. These authors found the bacterial community changed both in response to plant succession and a soil moisture gradient. It is possible in the present study, as in {Burke, 2002 #164}, that marsh soil bacterial communities are inherently more responsive to abiotic factors such as salinity, redox or soil carbon content than to plants. Nevertheless, the dense root systems of these particular marsh plant species and the high belowground productivity in UPCM suggest that the bacteria in these samples were likely within the realm of root influence.

2. A suboptimal sampling design could cause failure to reveal any plant community effects. The presence of the same plant communities in all three regions of the salt marsh was exploited in this study to build a complete block experimental design with plant community and marsh site as class variables. Although very few instances of significant site-plant interactions were observed here (in belowground biomass and root OM, Table 3.1a), genetic variation, aneuploidy, or phenotypic plasticity related to environmental differences may have some impact on plant ecology [Ackerly, et al., 2000;

Seliskar, et al., 2002] and could possibly affect bacterial communities. Little to no evidence of phenotypic plasticity was observed in *J. roemerianu*s, *S. patens* or *D. spicata* in this study site. However, in the case of any plant-site interaction, a nested design, or manipulations involving marsh mesocosms or lab microcosms could be more powerful in determining the effect of plants on bacterial community structure. As with any ecological study, there is a trade off between the noisy, real-world observations in the field and the more controlled systems created in field manipulations and laboratory simulations.

3.) Soil bacterial communities are resistant to plant community differences, but depend on them for supply of carbon in the form of dead roots. Lack of an observed plant community effect in this study does not mean that the bacterial communities are uninfluenced by the surrounding plants. Although plant species can affect rhizosphere bacterial community composition, especially those bacteria which colonize root tissues and surfaces[Rovira, 1965; Wieland, et al., 2001; Marschner, et al., 2004], these differences were overshadowed by the combined effects of SOM content and inundation (including TEA supply and soil anoxia) in UPCM. On the spatial scales measured in this study, it may be that plentiful carbon resources are more important to bacterial communities than source of carbon (ie. plant species). Christian et al. [1978] found no significant effect of glucose addition on subsurface sediment bacterial activity--ATP and total adenylate concentrations, community adenylate energy charge ratio (CEC), and C^{14}-uptake--in a Sapelo Island, GA salt marsh. Furthermore, clipping all aboveground *S. alterniflora* stems to eliminate primary productivity had no effect on bacterial activities either. The authors hypothesized that the resistance of bacterial metabolism to perturbations could be due to two reasons: 1) bacteria are limited by some

"physiochemical space limitation" (limited in physical space or via accumulation of metabolites which inhibit growth) or 2) bacteria are most dependent on the large stocks of slowly decaying dead roots present in marsh soils. The authors conclude that the results of their study favor the second hypothesis. There is some recent evidence that bacterial community composition is resistant to changes in marsh plant productivity, even in the rhizosphere [Piceno and Lovell, 2000].

High densities of roots, both live and dead, were found in this study (Fig 3.10), suggesting that the strong link between bacterial community productivity and dead roots found in Sapelo Island marsh soils may also exist in UPCM soils. Significant differences in dead root OM across sites and plant communities were also observed in this study (Fig 3.4), but dead root *mass* was equivocal (data not shown). Though my results do not show a direct relationship between dead root mass and bacterial community composition, it is likely that effects of decaying roots on bacterial productivity will affect, if indirectly, bacterial community composition.

4.3 The Need for Balance: SLR and marsh surface accretion

Eustatic sea level rise (SLR) is a pressing concern for coastal habitat managers [Vellinga and Klein, 1993; Yanez-Arancibia and Day, 2004]. How will the results of this research impact predictions about response of marsh ecosystems to sea level rise and marsh transgression? If the present rate of SLR and current plant productivity continues, marsh surface accretion will be maintained at rates sufficient to allow expansion of the wetlands [Kastler, 1993; Kastler and Wiberg, 1996] and there will be a continued slow transgression of Upper Phillips Creek Marsh into the upland forest. If this change is gradual, a "transgression of bacterial communities" is expected as high marsh converts to

low marsh and high marsh encroaches upon forested areas. Bacterial community structure will reflect the slow changes in inundation, salinity, belowground productivity that will occur along the changing tidal gradient.

Alternatively, increased SLR and/or increased frequency or severity of coastal storms and associated physical disturbance could drive much more rapid changes in UPCM. Deposition of wrack in conjunction with increased flooding would likely cause loss of *J. roemerianus* stands in the low marsh [Brinson and Christian, 1999]. Ponded salt water in the high marsh will stress plants and cause mortality, collapse of the peaty marsh base, and create new depressed areas that can coalesce into new creek networks, increasing tidal flooding to the high marsh, and hastening transition from high to low marsh habitat [Brinson, et al., 1995; Christian, et al., 2000].

Could this rapid environmental change be reflected in microbial community structure? The colonization of devegetated areas by microalgae and cyanobacteria (Underwood 1997) could alter the detrital pool and lead to the development of unique bacterial and fungal decomposer communities [Hannen, et al., 1999], which may be very different from the previous high marsh microbial communities. Changes in soil or sediment properties will occur in areas undergoing state change (eg. high marsh → low marsh, forest→ high marsh) [Brinson, et al., 1995] SOM accumulation and belowground productivity will have to be maintained to prevent rapid marsh loss. Under this scenario, marsh ecosystem function could be preserved by the great functional redundancy reported in laboratory manipulations of natural bacterial communities [Degens, 1998; Franklin and Mills, 2006; Wertz, et al., 2006]. Degens [1998], and others have shown that general decomposition processes are not altered by great reductions in diversity or

changes in community structure. Even process rates have been shown to be maintained after a great reduction in diversity via serial dilution and re-growth [Franklin, et al., 2001; Wertz, et al., 2006].

The most important factor for stable marshes may be the continued presence of healthy marsh vegetation, providing abundant carbon sources to soil bacteria. Changes may arise when salt stress on plants lowers productivity and reduces carbon supply from roots endangering the constant supply of live and dead root stocks for microbial decomposition. Though soil bacterial communities may be resistant to change, once a resistant system has been perturbed from its nominal state, it can be very difficult for the system to return to that prior state [Christian, et al., 1978]. Selection will favor members of the bacterial community which survive best in the low-carbon, high salinity, low redox potential environment. Numerous ecological studies have demonstrated the structuring force of limited resource availability on biological communities, including microbes [Waldrop, et al., 2006], phytoplankton [Sanders, et al., 1987] and invertebrates [Dayton, 1971].

Chapter 5. Conclusions

Variations in bacterial community structure are linked to site differences ultimately related to inundation regime, namely SOM content and elevation. Though stark plant zonation exists within each marsh region, any plant community effects on structuring the bacterial communities sampled in this study are masked by the presence of environmental gradients that correspond to marsh location. It is likely that the plants' most important role is in providing a constant, large supply of decaying root and litter material for bacterial utilization.

Methods for sampling and analyzing bacterial communities will never be perfect, but improvements can be made that could increase success of studies of plant and site effects. Though distinction between rhizosphere and bulk soil is difficult to make in the upper 3 cm of marsh soils, homogenation of small roots and soil may have made it difficult to see any plant effects. High variation and low replication lowered the statistical power of the16SrDNA-DGGE analysis used here. In spite of this, significant differences in bacterial community composition were still observed, suggesting these trends would become more significant with increased analytical power. Though DGGE has been demonstrated to be a quick and powerful tool in microbial ecology [Kowalchuk, et al., 2002; Marschner, et al., 2004], researchers must understand the limitations of the method, including gel variability, one band-one clone assumption and all other inherent limitations of PCR-based methods [Wang and Wang, 1997; Wintzingerode, et al., 1997].

Further work growing out of this project should include manipulative experiments to assess relative contributions of environmental variables to bacterial community composition and abundance. More frequent (i.e. seasonal) monitoring of bacterial

communities may provide further evidence for the complex interaction between bacterial community composition and soil temperature in salt marshes, as it has in aquatic habitats [Pomeroy and Wiebe, 2001; Kan, et al., 2006]. Characterization of important functional groups of bacteria, along with measurement of bacterial processes could determine if bacterial community structure and function are related in UPCM.

Simultaneous assessments of microbial community composition and function (respiration, sulfate and iron reduction, methanogenesis), especially in areas of UPCM experiencing subsidence or tidal creek encroachment, are needed to show if the results of this work correspond to actual changes in bacteria function. These studies will facilitate further understanding the role of bacteria in marsh transgression. A major step towards these goals could rest in establishing a program of frequent marsh soil sampling and development of standard community analysis methods as part of routine VCR-LTER monitoring.

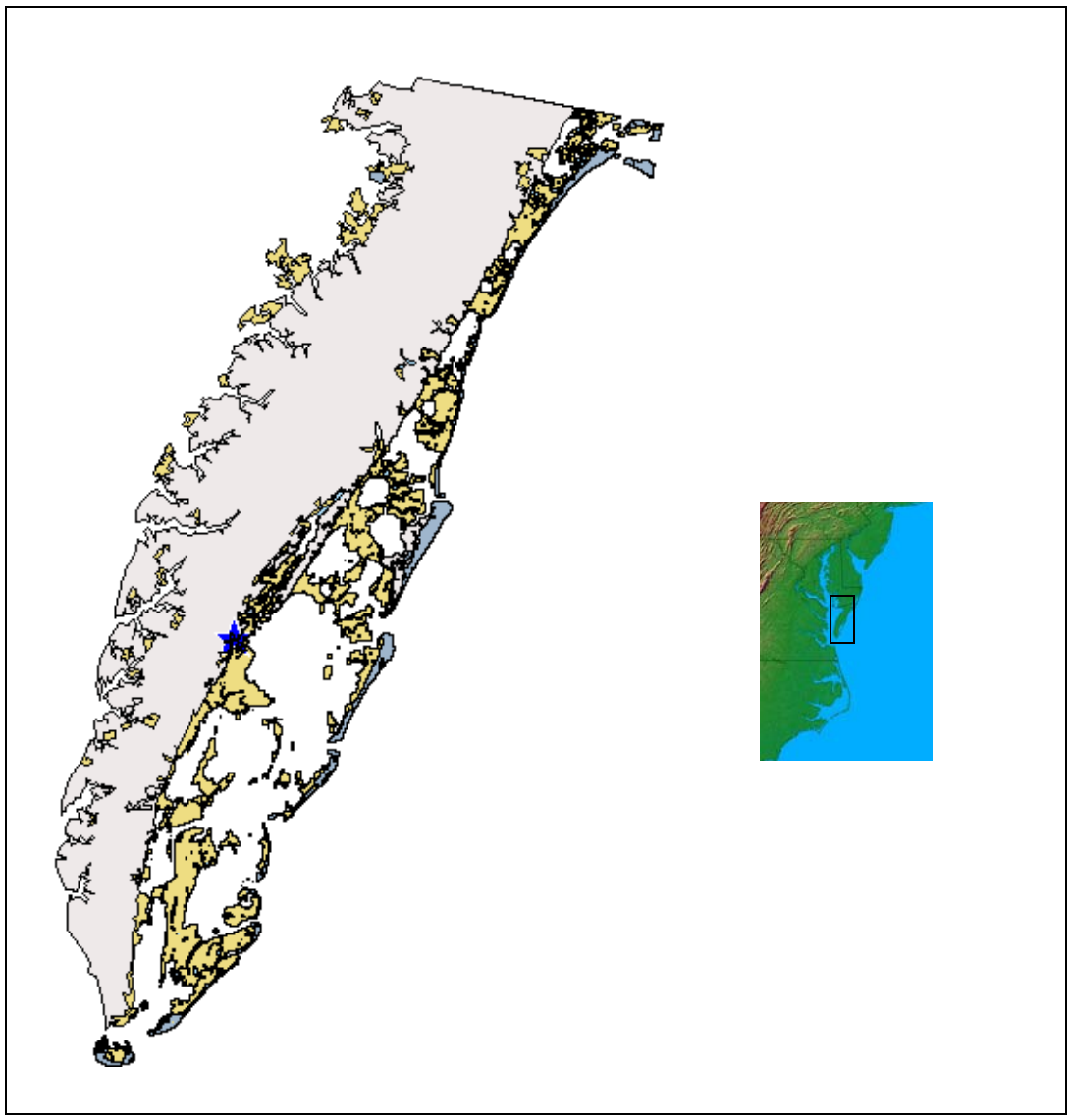


Fig 1.1 Location of study site (marked with star) within the Virginia Coast Reserve Long Term Ecological Research (VCR-LTER) mega site, on the Eastern Shore of Virginia (shown in inset).

Fig 1.2. Aerial photo of Upper Phillips Creek Marsh. General locations of transects are indicated by Low, Mid and High labels. The letter J indicates a patch of *J. roemerianus*, while S-D indicates area of *S. patens-D. spicata* mix. 'Die Off' marks large, recent die back region of *S. patens* near this study's sites. Note the boardwalk and uppermost part of creek on the left of the photo. This photo was taken on June 9, 2004 by the author.

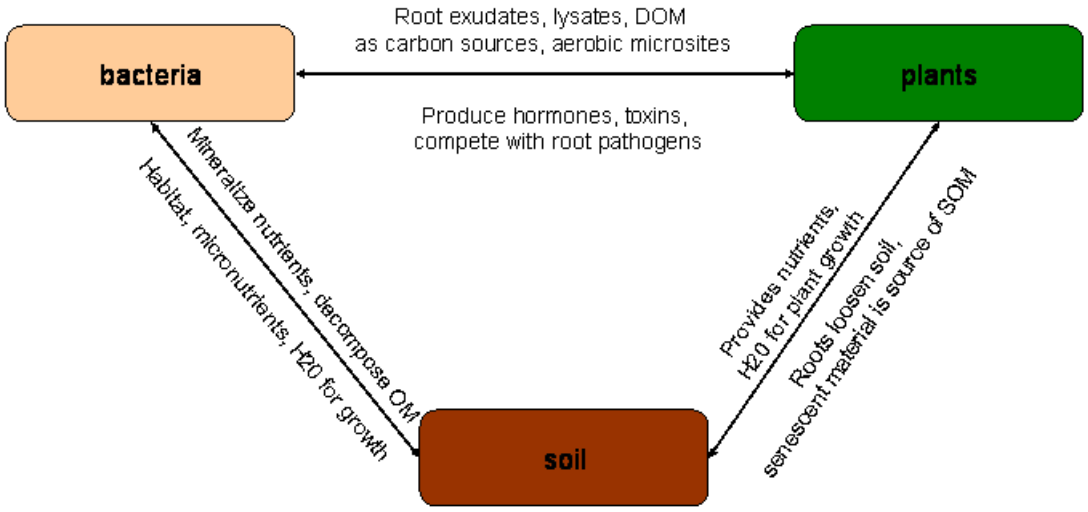


Fig 1.3. Conceptual diagram summarizing interactions among plants, soil and soil bacteria.

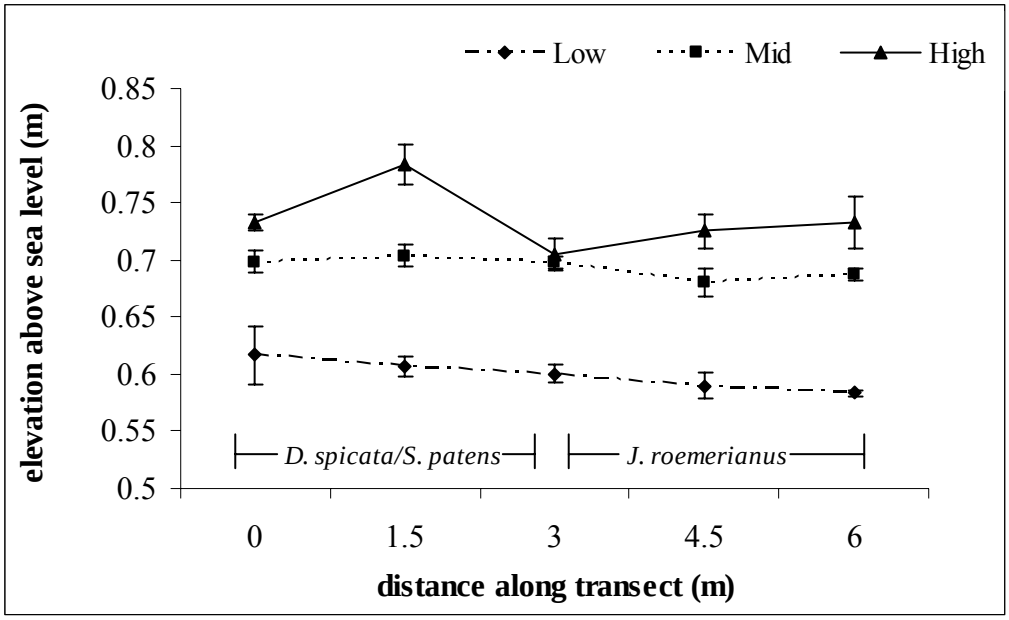


Fig 3.1. Mean marsh elevation at positions along experimental transects, corresponding to location of cores used for bacterial community analysis. Zero position on transects was arbitrarily placed in *D.spicata-S.patens* community, and the 3m position centered upon the transition between the two plant communities. Bars represent one SE of the mean from three replicate transects.

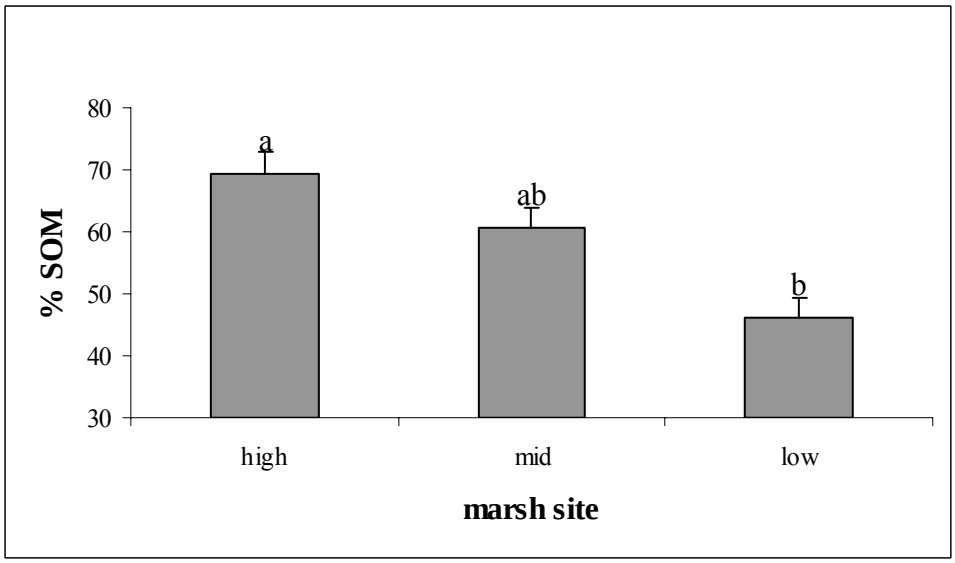


Fig 3.2 Mean soil organic matter content of salt marsh locations as sampled in July 2004 and July 2005. Data were collected from small cores used for bacterial community analysis. Error bars represent one SE of the mean of 9 replicates.

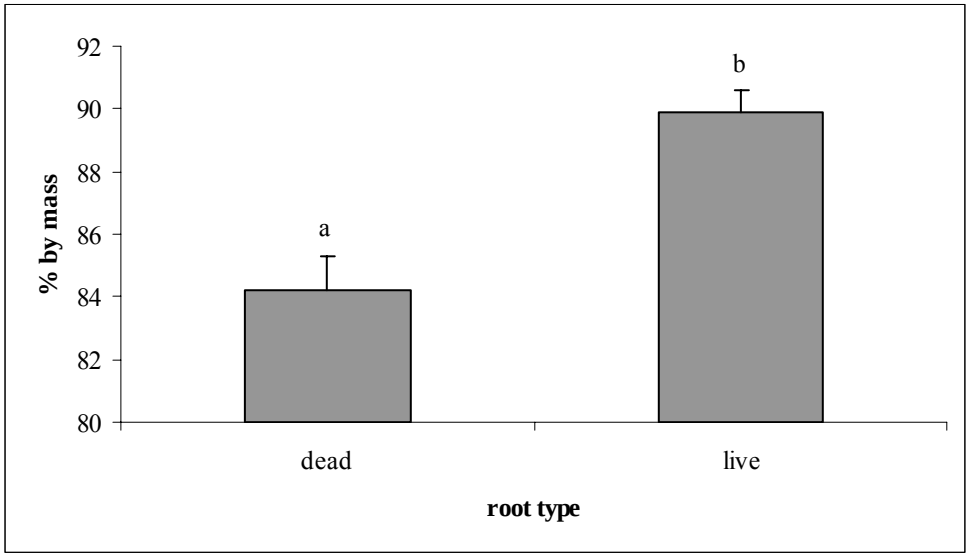


Fig 3.3 Live versus dead root organic matter content, averaged over the entire core depth (20cm). Data were collected from belowground biomass cores collected in June 2005. Error bars represent one standard error, n=27.

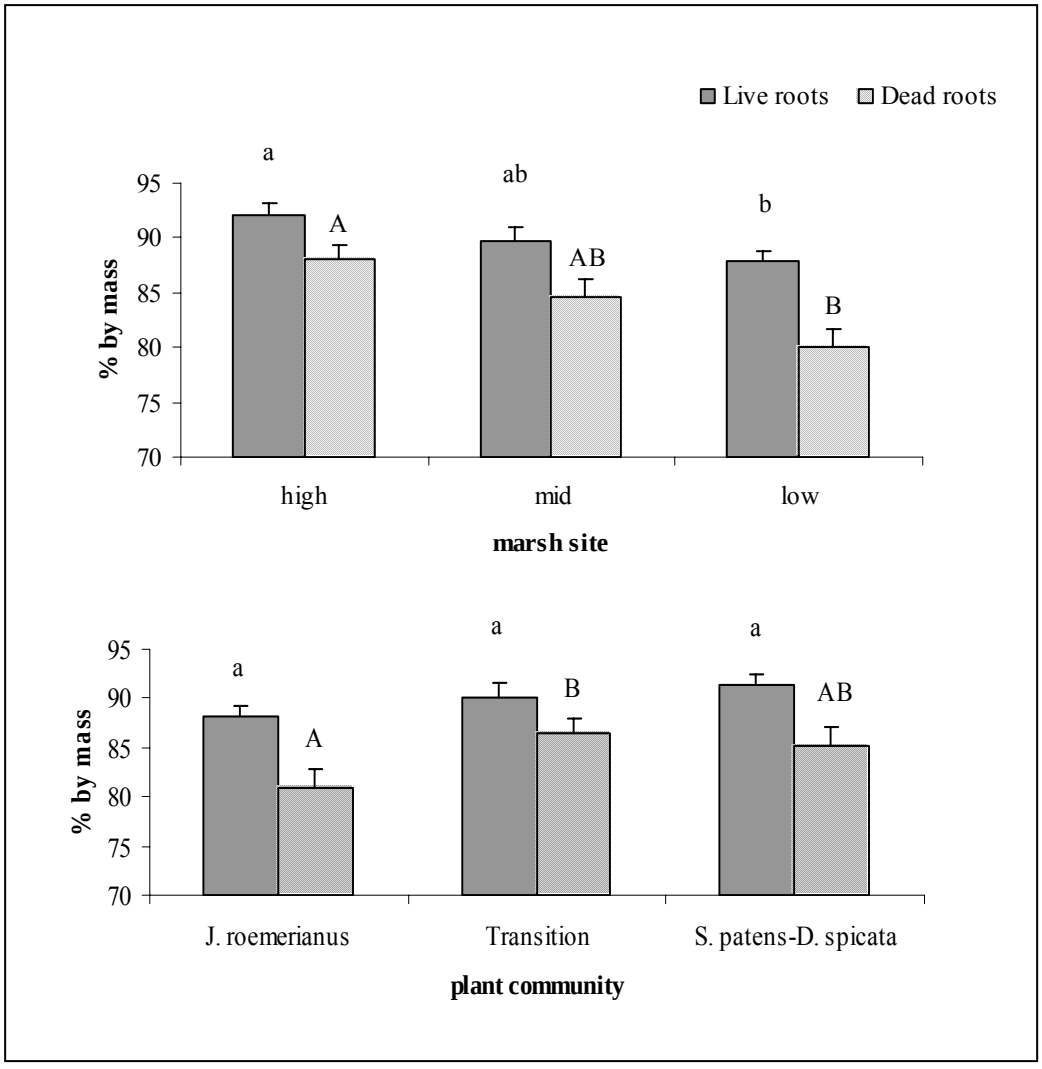


Fig. 3.4 Root organic matter content by marsh site (top) and by plant community (bottom). Error bars represent one SE of the mean of 9 replicates. Groups that share the same letter and case are not significantly different for one another. Root organic matter data was gathered from belowground biomass samples collected in June 2005. Samples were collected close to transects used for bacterial community analysis.

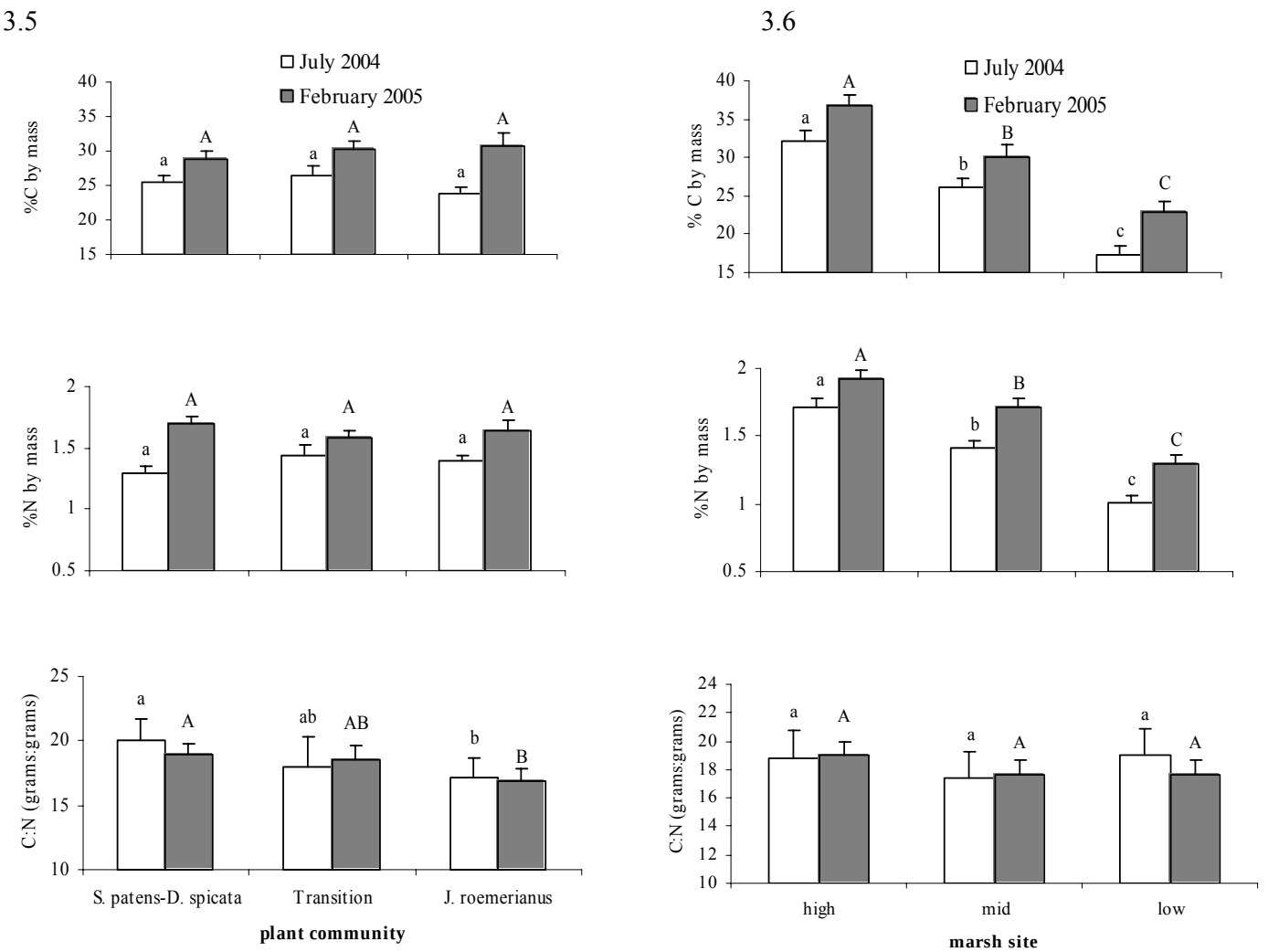


Fig 3.5 and 3.6. Soil Nutrients (%C, %N, C:N) by plant type (left) and marsh site (right) for July 2004 and February 2005. Groups sharing same letter and case are not significantly different. Error bars are one SE. n=27. Nutrient data were collected from shallow cores used for bacterial community analysis.

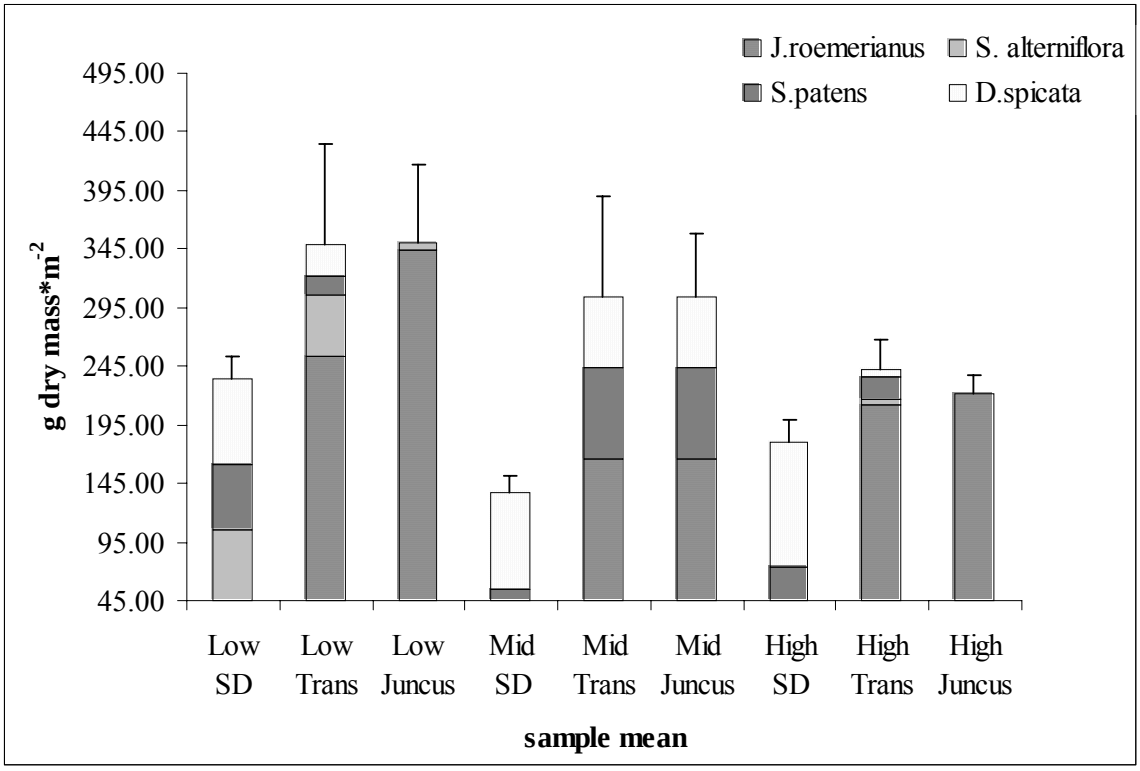


Fig 3.7 Aboveground biomass and community composition by plant community type and marsh zone. SD= *S. patens-D.spicata*, Trans = transition zone, Juncus = *J. roemerianus*. Note the presence of *S. alterniflora* in the low marsh samples.
Clip plots were taken near sites of belowground biomass sampling, parallel to transects used for community analysis and bacterial abundance. Error bars represent 1 SE of the mean of 3 replicate total biomass measurements for each group.

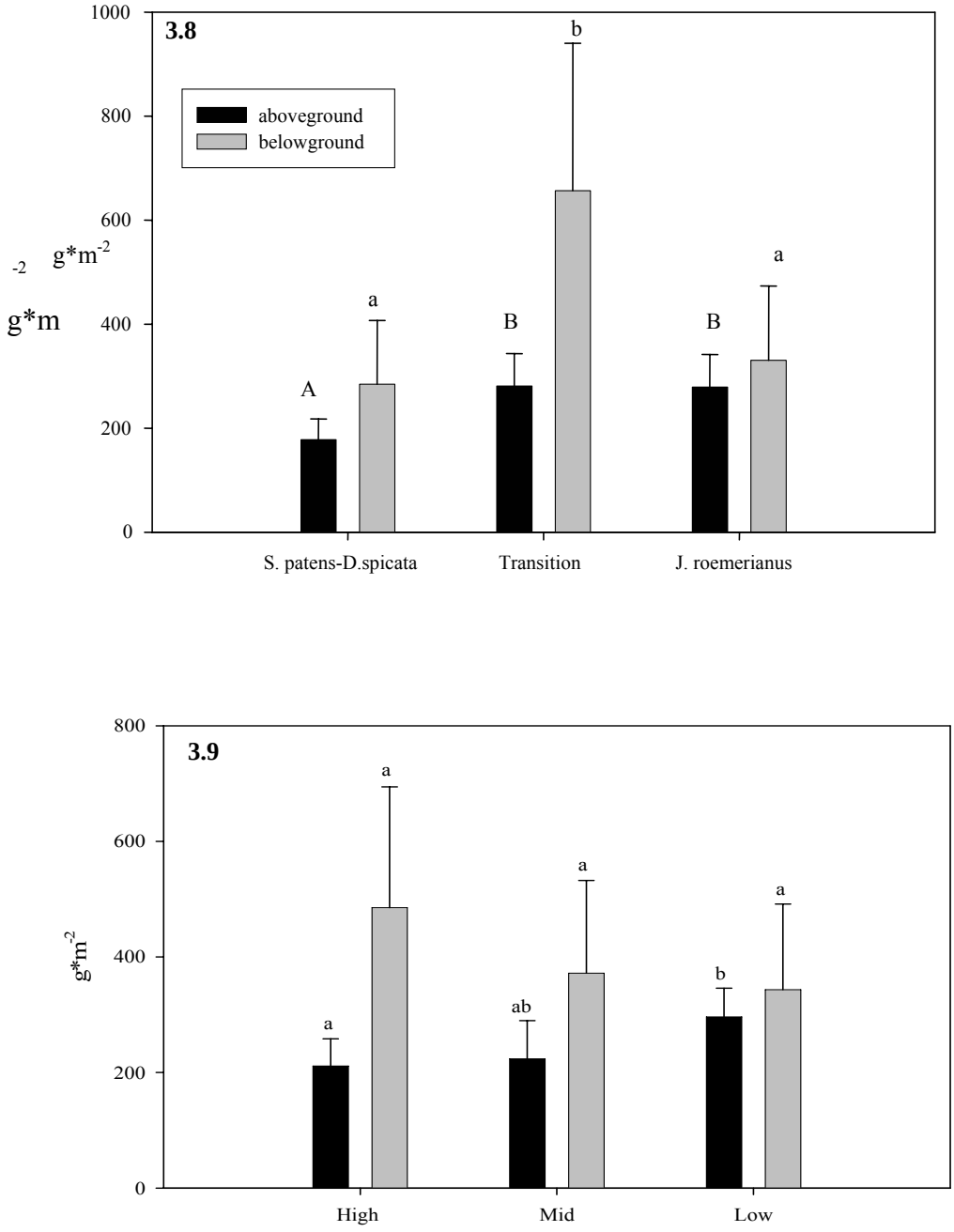


Fig. 3.8 and **3.9**. Mean above and belowground biomass for plant community (top) and marsh site (bottom). Error bars represent one standard error of mean of 9 replicates in aboveground biomass group, +/- 95% CL in belowground group.

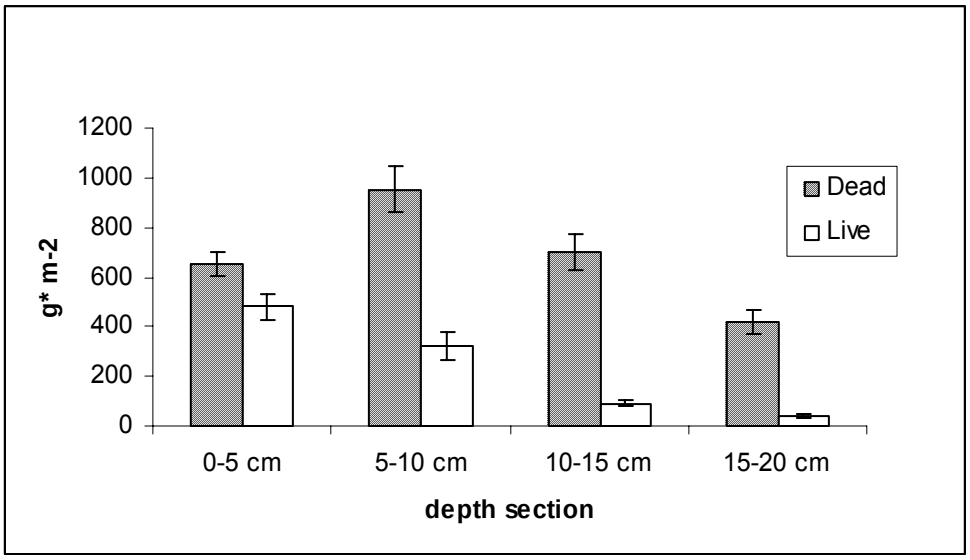


Fig 3.10. Live and Dead root mass over the depth of the belowground biomass cores. Error bars are one standard error. n=27

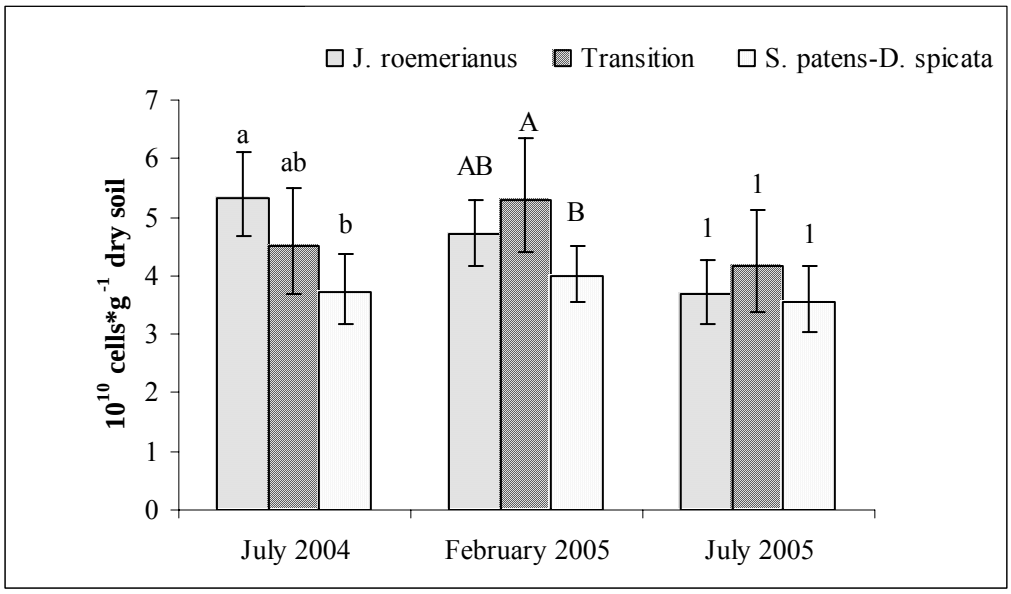


Fig 3.11

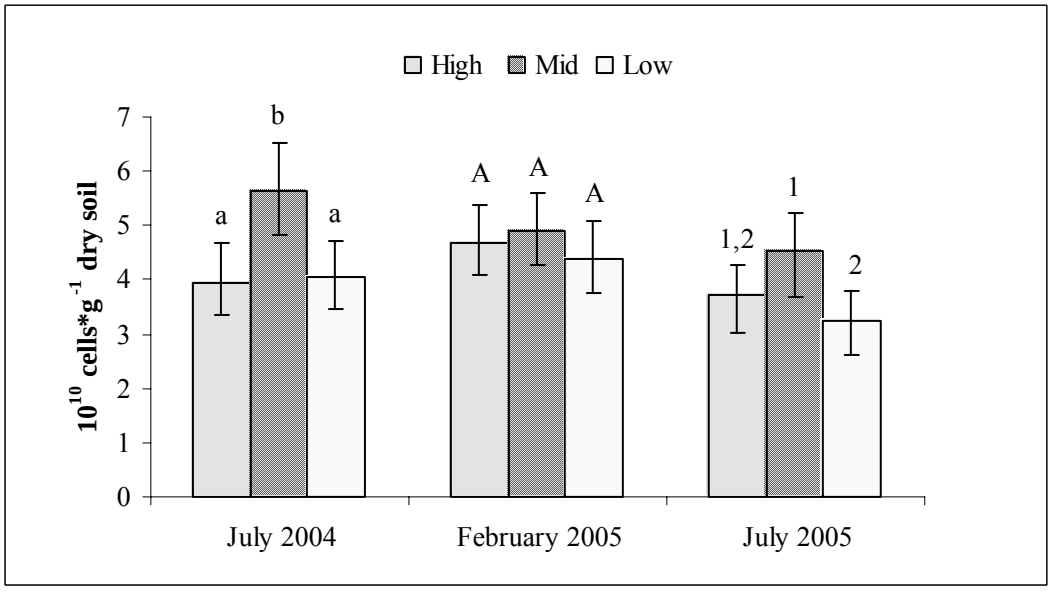


Fig 3.12

Figs 3.11 and **3.12**. Total soil bacterial abundance by plant community (top) and marsh site (bottom) over the three dates sampled. Error bars are 95% confidence intervals for mean of 9 replicates. Bars sharing the same letter and case or numeral are not significantly different.

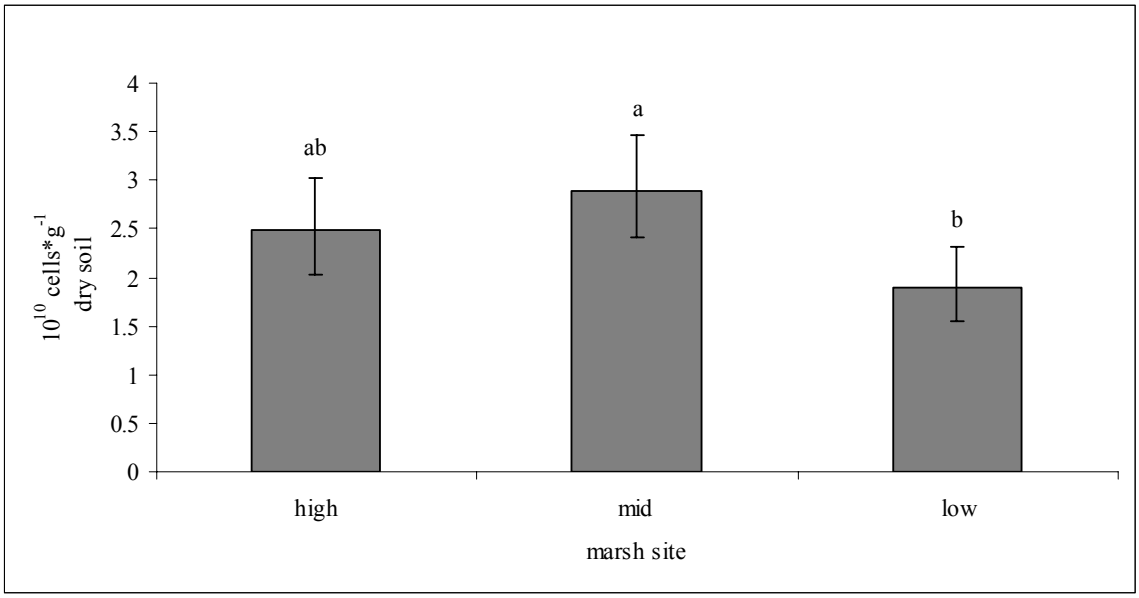


Fig 3.13

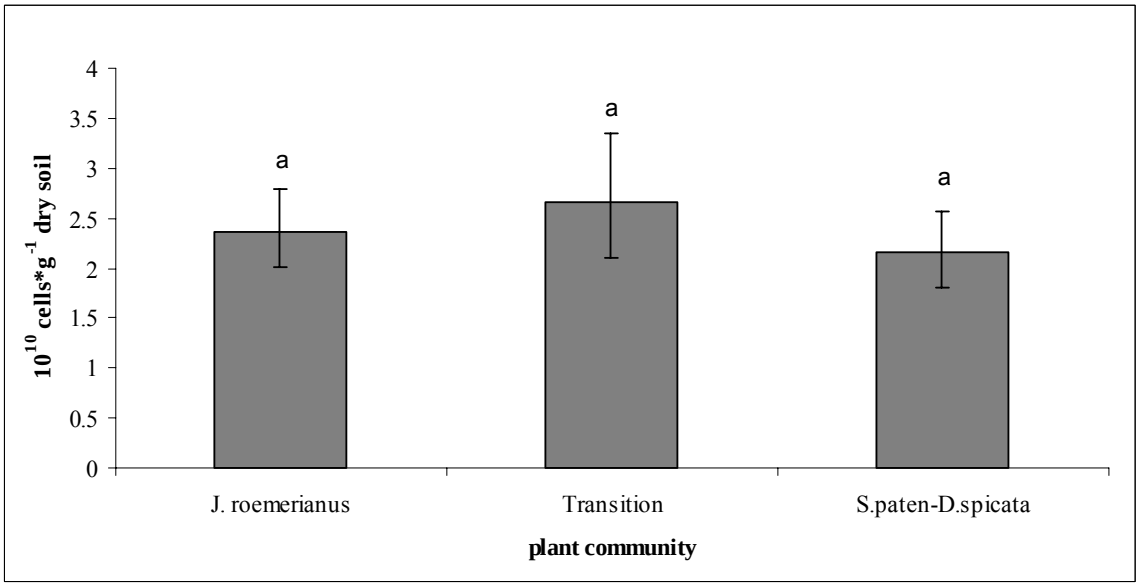


Fig 3.14

Figs 3.13 and 3.14. Live Bacterial abundance on July 25, 2005 by marsh site (top) and plant community (bottom). Error bars represent 95% confidence intervals of the mean of 9 replicates.

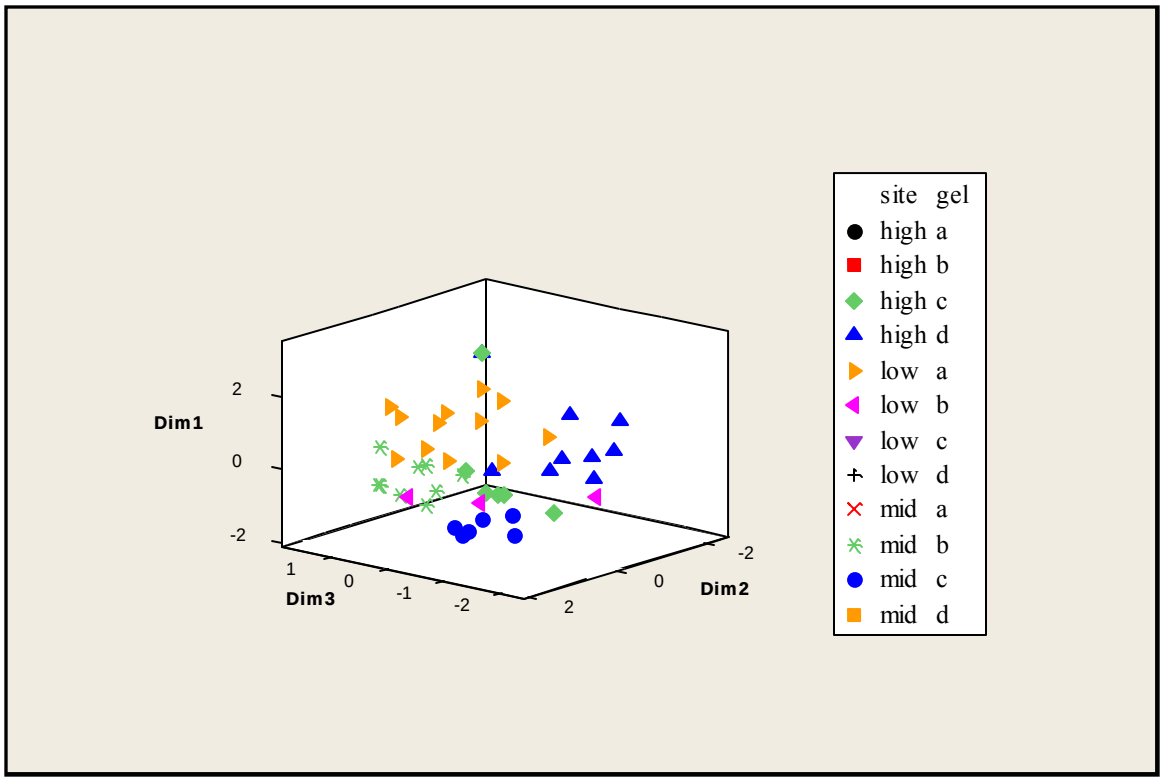
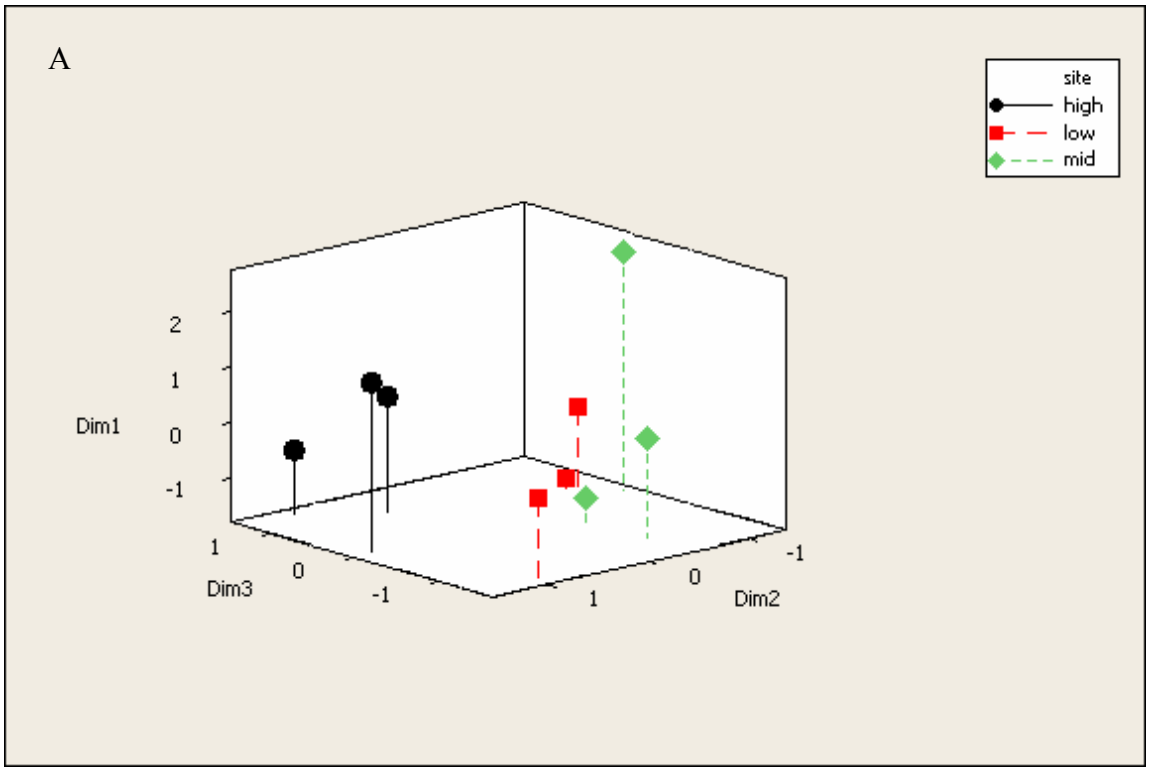
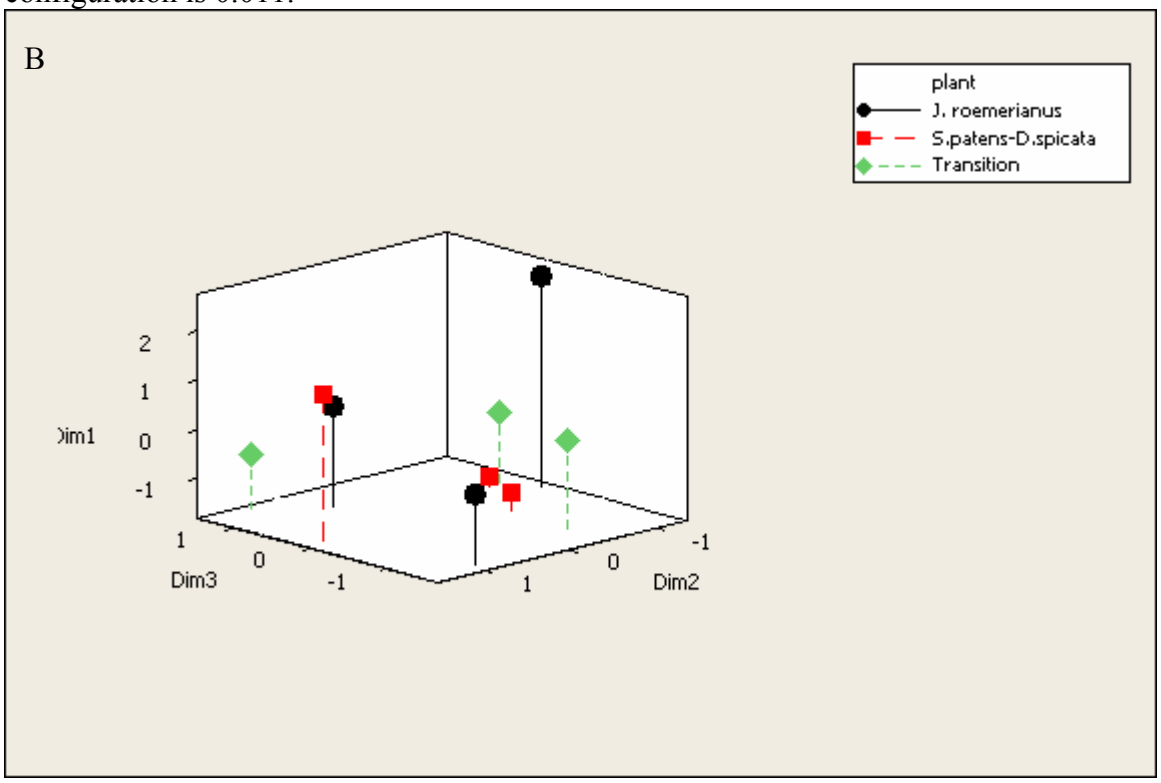
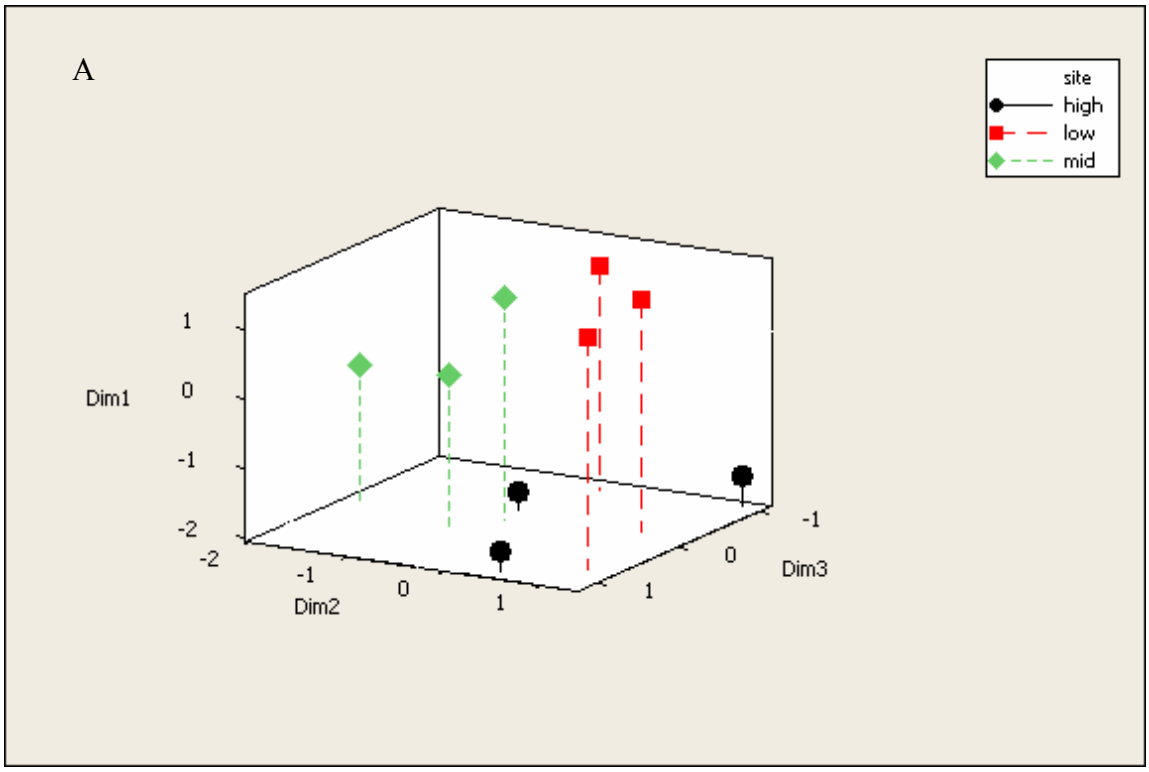


Fig 3.15 Example of an NMDS configuration from the July 2005 full dataset (n=45). Graph shows samples by their gel assignment and marsh location. Not all groups in legend actually exist. Groups of interest are *low a*, and *low b*, *mid b* and *mid c*, *high c* and *high d*. Note that samples from a particular gel are frequently more alike than samples from the same marsh location.

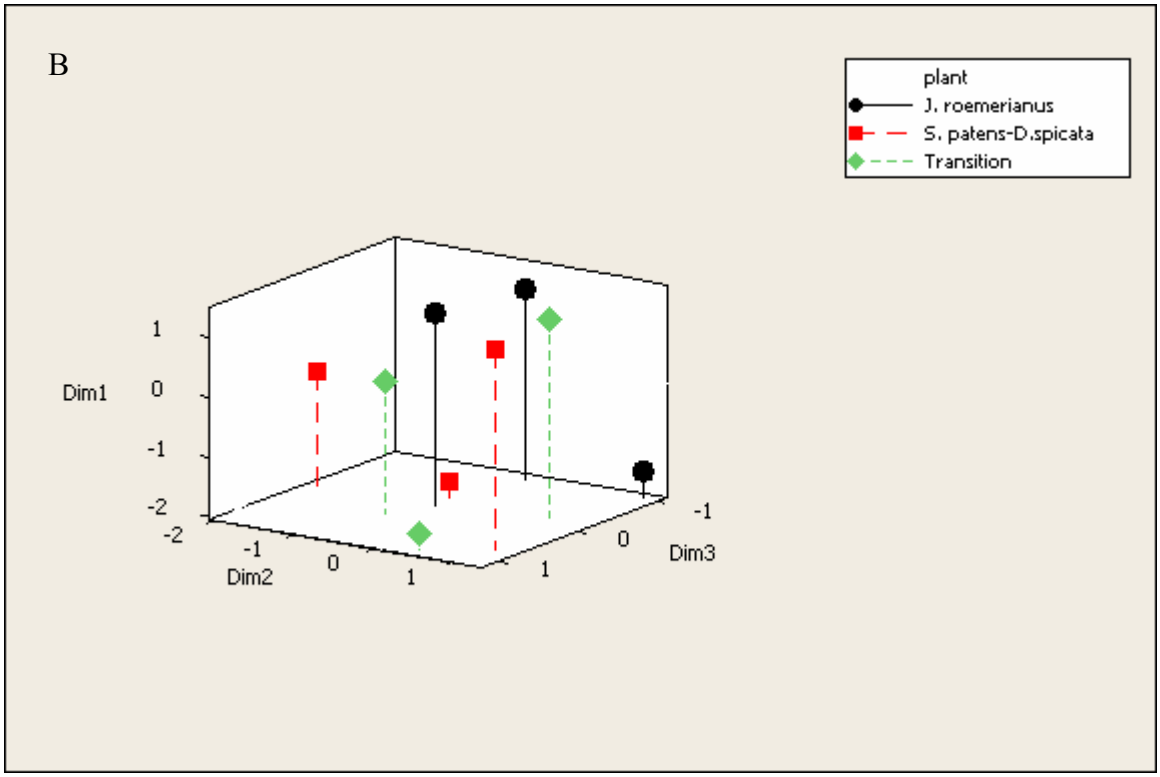


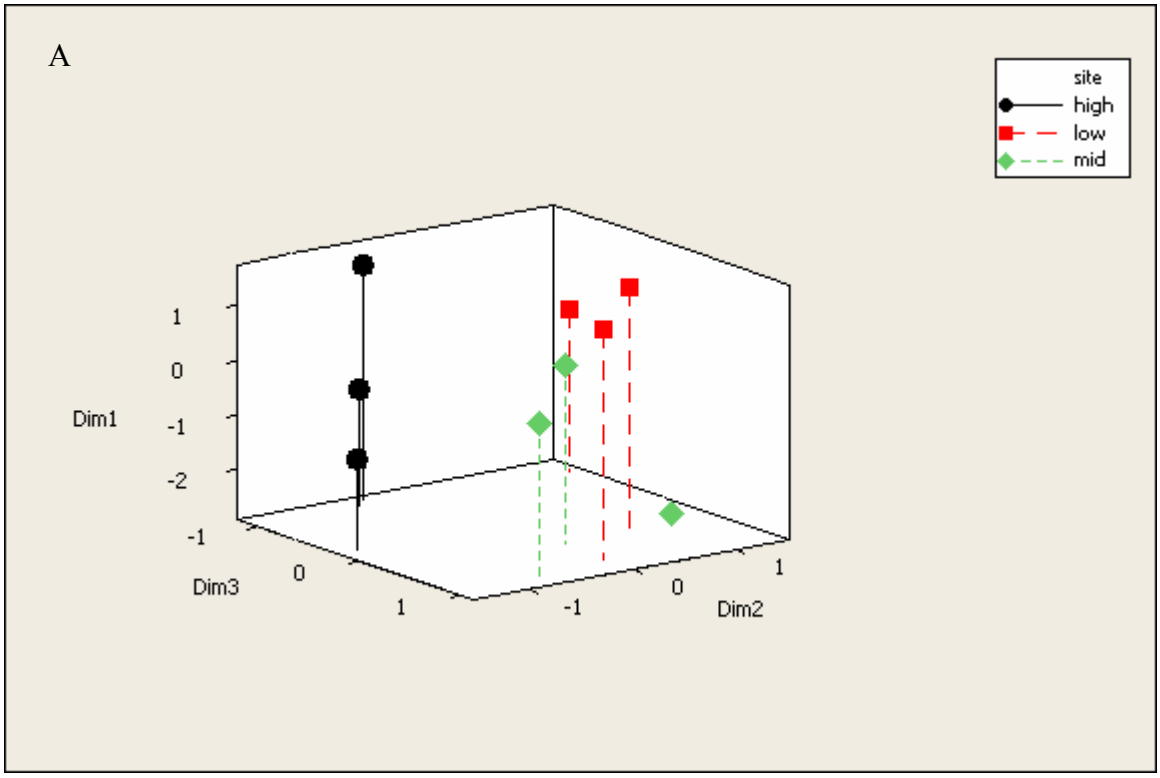
Figs 3.16A and **B**. NMDS plots of July 2004 subset "A" community dissimilarity, showing groupings by site (A) and plant community types (B). Stress for this configuration is 0.011.



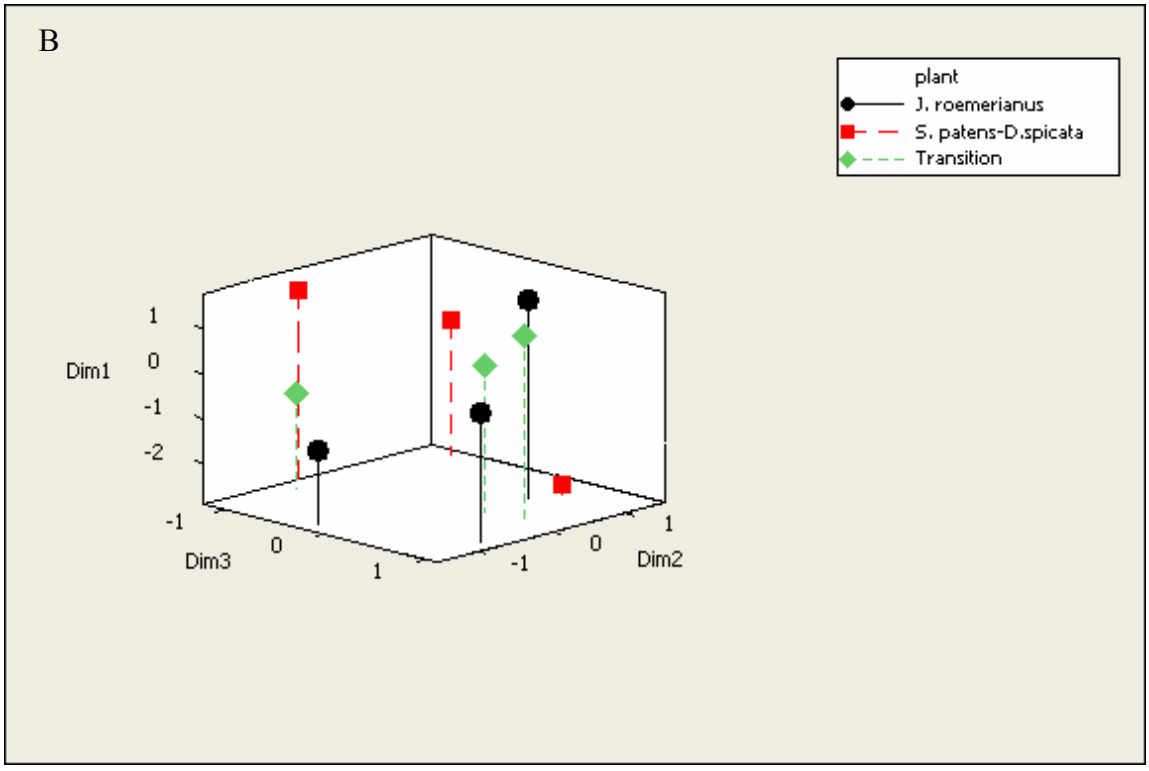


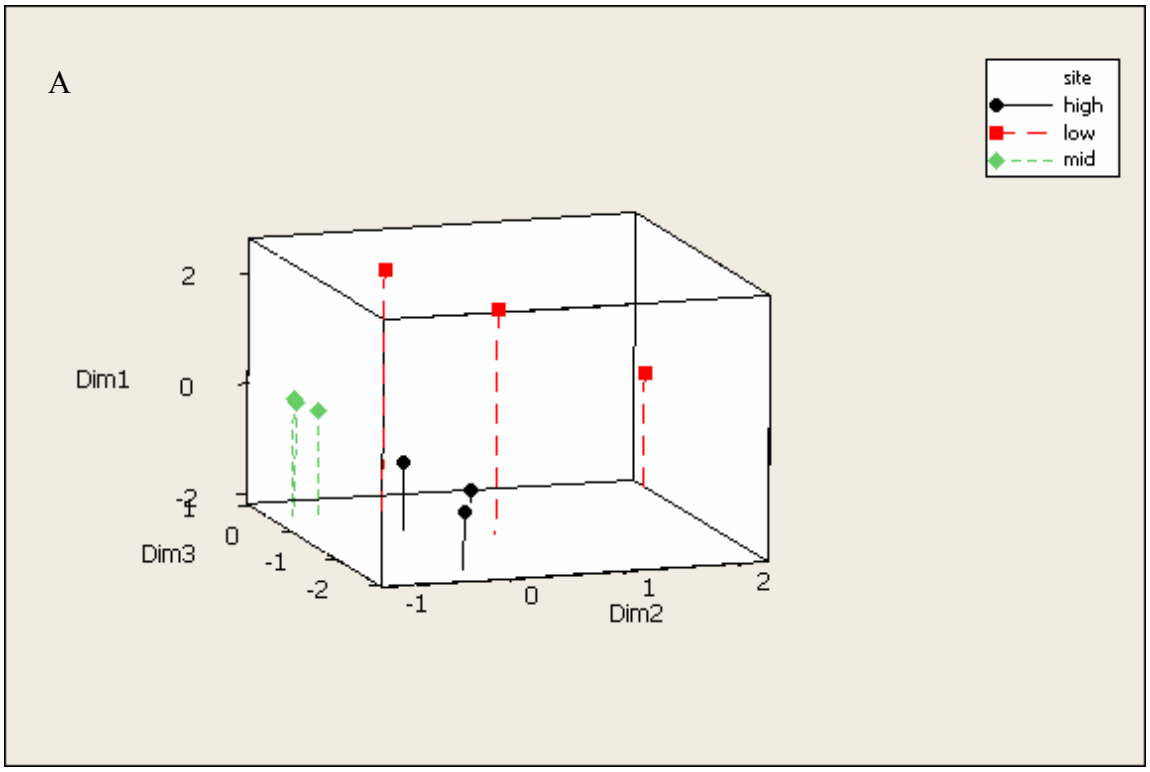
Figs 3.17A and **B**. NMDS plot of July 2004 subset "B" community showing groupings by site (A) and plant community (B). Stress for this configuration is 0.076.





Figs 3.18A and **B**. NMDS plots of February 2005 (subset) bacterial community dissimilarity showing groupings by site (A), and by plant community (B). Stress = 0.055.





Figs 3.19A and **B**. NMDS plots of July 2005 (subset) bacterial community dissimilarity showing marsh site (A) and plant community groupings (B). Stress = 0.017

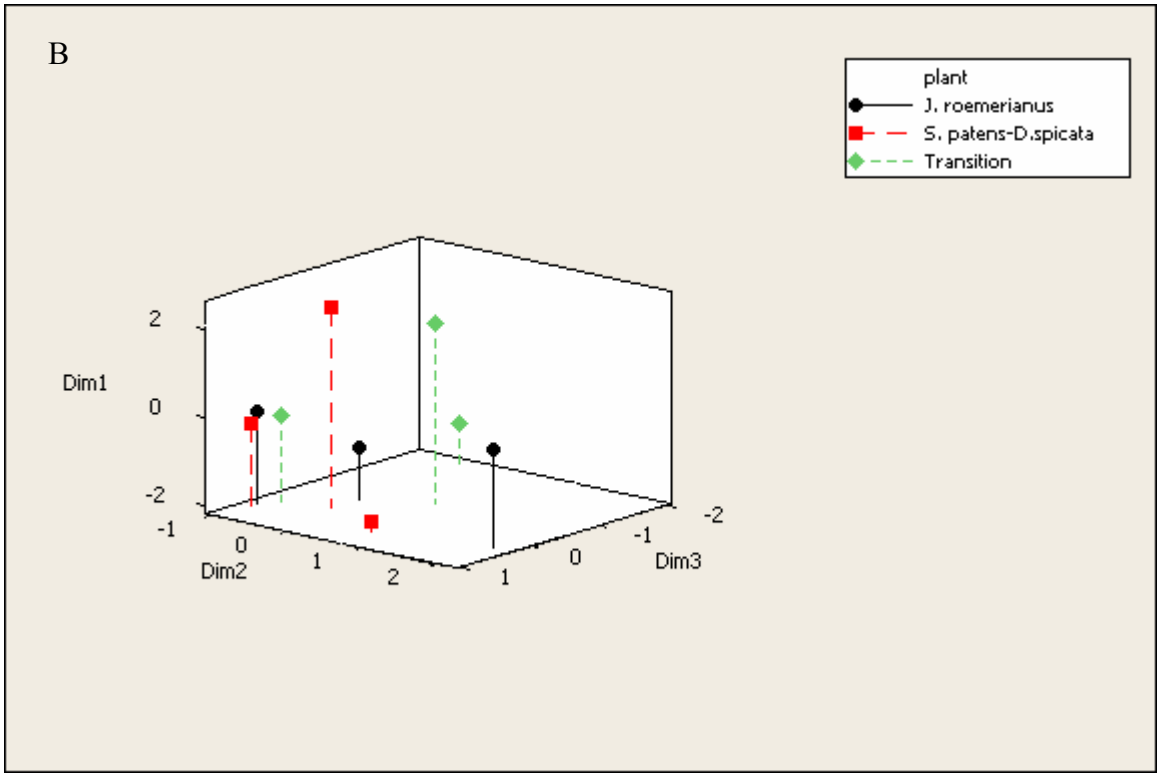


Table 2.1 Variables Measured in this Study and Dates Sampled

Variable	**Date Measured**
Elevation	1-June-04
SOM	14-July-04, 11-Feb-05, 25-July-05
AODC	14-July-04, 11-Feb-05, 25-July-05
Live Bacteria	25-July-05
Soil C:N, C, N	14-July-04, 11-Feb-05
Live Root C:N, C, N	late June ‘05
Aboveground biomass	mid-August ‘05
Belowground biomass	late June ‘05
Bacterial Community DNA	14-July-04, 11-Feb-05, 25-July-05

Table 2.2 Description of Common 16S rDNA Primers for Bacterial Fingerprinting

Citation	Primers	Sample Type	16s DGGE purpose
Kang and Mills 2004	63f/518r	silt loam soil	universal, band pattern
Kowalchuk et al 2002	968f/1378r	rhizosphere/bulk soil	V6/V8, gene library
Wantanabe et al 2001	314f/926r 968f/1401r	oil contaminated ground H20	universal
Duineveld et al 2000	968f/1401r	loamy soil	universal, RT-PCR
Nakatsu et al 2000 (after Ovreas et al 1997)	338f/518r 968f/1406r 46f/1100r 340f/358r	silty clay/ loam soil	bac V3 bac V6/V9 Archaea Archaea V3
Marchesi et al 1998	63f/1387r	"difficult" samples ie, Oral, deepsea, epilithon	develop new primers for "difficult" samples
Hansen et al. 1998	515f/1408r GM5f/907r 9f/1512r	pure, mixed cultures from waste gas biofilter	16S PCR, RFLP
El Fantroussi et al 1998	63f/518r 338f/518r	soil, treated with urea herbicides	universal, band pattern, sequencing
Muyzer et al 1993	341f/534r	sediment microbial mat, biofilms, pure culture	DGGE development

Table 2.3 Sequence Information for Common 16S rDNA Primers

Primer ID	Sequence	Target Region
"Universal"		
63F	5'-CAG GCC TAA CAC ATG CAA GTC-3'	
518R (534R)	5'-ATT ACC GCG GCT GCT GG-3'	V3
338F	5'-ACT CCT ACG GGA GGC AGC AG-3'	V3
1387R	5'-GGG CGG WGT GTA CAA GGC-3'	V9
968F	5'-AAC GCG AAG AAC CTT AC-3'	V6
515F	5'-GTG CCA GCA GCC GCG GTA A-3'	V3
1408R	5'-TGA CGG GCG GTG TGT ACA AGG C-3'	V9
GM5F (341F)	5'-CCT ACG GGA GGC AGC AG-3'	V3
DS907R	5'-CCC CGT CAA TTC CTT TGA GTT T-3'	V6
9F	5'-GAG TTT GAT CCT GGC TCA G-3'	
1512R	5'-ACG GCT ACC TTG TTA CGA CTT-3'	
Archaea		
46F	5'-C/TTA AGC CAT GCG/A AGT-3'	
1100R	5'-T/CGG GTC TCG CTC GTT G/ACC-3'	
340F	5'-CC TAC GGG GC/TG CAG/C CAG-3'	Arc V3
519R	5'-TTA CCG CGG CG/TG CTG-3'	Arc V3

GC clamp:
5'-cgc ccg ccg cgc gcg gcg ggc ggg gcg ggg gca cgg ggg g-3' (Muyzer et al, 1996, Nakatsu et al. 2000, Watanabe et al. 2001)

Table 3.1a: MANOVA P-values (Probability that r > F) n=9

Effect	Aboveground biomass	AODC	Ash free dry weight	Below ground biomass	Belowground C:N	Elevation	Live cells	Sediment C:N	Sediment N	Sediment C	Root OM
Overall	**0.002**	0.16	**0.002**	**<.001**	0.18	**<.001**	**0.05**	0.11	**<.001**	**<.001**	**<.001**
Site	**0.02**	**0.03**	**<.001**	0.12	0.94	**<.001**	**0.01**	0.45	**<.001**	**<.001**	**<.001**
Plant	**0.002**	0.39	0.39	**0.001**	0.06	0.15	0.34	0.07	0.32	0.34	**<.001**
Site*Plant	0.42	0.71	0.83	**0.001**	0.28	0.68	0.34	0.14	0.89	0.21	**0.03**

Table 3.1b: MANOVA Test criteria and F approximations for H_0 of no overall Site effect

Statistic	Value	F Value	Num DF	Den DF	Pr > F
Wilks' Lambda	0.01424297	12.75	22	38	<.0001
Pillai's Trace	1.53959703	6.08	22	40	<.0001
Hotelling-Lawley Trace	30.3249287	25.19	22	28.733	<.0001

Table 3.1c MANOVA Test criteria and F approximations for H_0 of no overall Plant effect

Statistic	Value	F Value	Num DF	Den DF	Pr > F
Wilks' Lambda	0.111635131	3.34	22	38	<.001
Pillai's Trace	1.30998193	3.45	22	40	<.001
Hotelling-Lawley Trace	3.93047084	3.26	22	28.73	<.001
Roy's Greatest Root	2.41016338	4.38	11	20	<.001

Table 3.1d: MANOVA Test criteria and F approximations for H_0 of no overall Site*Plant Effect

Statistic	Value	F Value	Num DF	Den DF	Pr > F
Wilks' Lambda	0.06906162	1.72	44	74.644	0.0199
Pillai's Trace	1.77690862	1.60	44	88	0.0316
Hotelling-Lawley Trace	4.48736082	1.82	44	43.057	0.0260
Roy's Greatest Root	2.39984469	4.80	11	22	0.0009

Table 3.2 Pearson Correlation Coefficients, p-value and n for Continuous Variables. Significant p-values are highlighted with an *.

	Aboveground biomass	AODC	SOM	Belowground Biomass	Live root C:N	Root OM	Elevation	Live Bacteria	Soil C:N	Soil Moisture	Soil N	Soil C
Aboveground Biomass	1.00	-0.03	-0.36	0.01	0.23	-0.20	**-0.45**	-0.34	**-0.44**	0.14	-0.31	**-0.49**
		0.90	0.07	0.95	0.24	0.31	0.02*	0.09	0.02*	0.49	0.12	0.01*
	27	26	26	27	27	27	27	26	26	27	27	27
AODC		1.00	**0.49**	0.24	0.24	0.30	0.15	0.29	0.01	**0.42**	0.31	0.30
			0.01*	0.24	0.25	0.14	0.45	0.14	0.97	0.03*	0.12	0.14
		26	26	26	26	26	26	26	25	26	26	26
SOM			1.00	**0.40**	-0.09	**0.58**	**0.75**	**0.49**	0.37	-0.11	**0.80**	**0.90**
				0.04*	0.67	<.01*	<.01*	0.01*	0.07	0.61	<.01*	<.01*
			26	26	26	26	26	26	25	26	26	26
Belowground Biomass				1.00	-0.02	**0.38**	0.19	0.28	0.25	0.05	0.25	0.31
					0.91	0.05*	0.35	0.17	0.23	0.80	0.21	0.11
				27	27	27	27	26	26	27	27	27
Live Root C:N					1.00	0.15	0.09	-0.18	-0.19	-0.15	0.03	-0.05
						0.47	0.67	0.38	0.35	0.45	0.87	0.82
					27	27	27	26	26	27	27	27
Live Root OM						1.00	**0.45**	0.13	**0.40**	-0.26	**0.44**	**0.58**
							0.02*	0.52	0.04*	0.19	0.02*	<.01*
						27	27	26	26	27	27	2 7
Elevation							1.00	0.32	0.28	-0.31	**0.73**	**0.74**
								0.11	0.17	0.12	<.01*	<.01*
							27	26	26	27	27	27
Live Bacteria Cells								1.00	0.17	0.20	0.37	**0.46**
									0.41	0.33	0.06	0.02*
								26	25	26	26	26
Soil C:N									1.00	0.10	-0.01	0.37
										0.63	0.97	0.06
									26	26	26	26
Soil Moisture										1.00	**-0.43**	**-0.40**
											0.02*	0.04*
										27	27	27
Soil N											1.00	**0.90**
												<.01*
											27	27
Soil C												1.00
												27

Table 3.3 Regression of DGGE Band Richness with Bacterial Abundance and %SOM

Date	r^2	
	Bacterial Abundance	%SOM
July 2004 A	0.05	0.36
July 2004 B	0.16	0.28
February 2005	0.17	--
July 2005	0.17	0.65

Table 3.4 Specified Dimensions and Corresponding Stress Values of NMDS Model Fits of 4 Community Fingerprint Subsets. Stress values in the range of 0.01-0.05 are generally considered acceptable. Note that stress values decrease as dimensionality increases.

Subset	Dimensions	Stress value (S)
July 2004 A	2	0.029
	3	0.011
July 2004 B	2	0.076
	3	0.029
February 2005	2	0.098
	3	0.055
July 2005	2	0.056
	3	0.017

Tables 3.5a-3.5c. Mantel Test results of Complete Community DGGE Data. ρ = standardized matrix correlation coefficient. Alpha level has been adjusted with Bonferroni correction for multiple comparisons. Significant comparisons are marked with an *. Note the high correlation between "site" and "gel" on all three sampling dates as well as the corresponding high correlation between community dissimilarity and gel assignment.

Table 3.5a

Date	Variable A	Variable B	P	p-value	adj α level	# iterations
Jul-04	Community	SOM & Elev	**0.1554**	<0.0001*	0.0071	999
	Community	Sediment C:N	0.03326	0.3186	0.0071	999
	Community	"site"	**0.178**	<0.0001*	0.0071	999
	Community	"gel"	**0.25**	<0.0001*	0.0071	999
	Community	"plant"	0.069	0.032	0.0071	999
	"site"	"gel"	**0.575**	<0.0001*	0.0500	999

Table 3.5b

Date	Variable A	Variable B	ρ	p-value	adj α level	# iterations
Feb-05	Community	SOM & Elev	-0.0029	0.513	0.0071	999
	Community	Sediment C:N	0.022	0.359	0.0071	999
	Community	"site"	**0.222**	<0.0001*	0.0071	999
	Community	"gel"	**0.288**	<0.0001*	0.0071	999
	Community	"plant"	0.025	0.234	0.0071	999
	"site"	"gel"	**0.476**	<0.0001*	0.0500	999

Table 3.5c

Date	Variable A	Variable B	P	p-value	adj α level	# iterations
Jul-05	Community	SOM & Elev	**0.19**	<0.0001*	0.0071	999
	Community	Sediment C:N	-0.050	0.2725	0.0071	999
	Community	Aboveground	0.030	0.3918	0.0071	999
	Community	Belowground	-0.14	0.9319	0.0071	999
	Community	"site"	**0.30**	<0.0001*	0.0071	999
	Community	"gel"	**0.47**	<0.0001*	0.0071	999
	Community	"plant"	-0.021	0.30	0.0071	999
	"site"	"gel"	**0.50**	<0.0001*	0.0500	999

Tables 3.6a-3.6c: Mantel Test Results on Bacterial Community DGGE Subsets. (9 samples/date run on a single DGGE gel). ρ: correlation coefficient of Mantel test. Alpha value has been adjusted by Bonferroni correction for multiple comparisons. Significant comparisons indicated with an *.

Table 3.6a

Date	Variable A	Variable B	P	p-value	α-value	# iterations
July 2004	OM & Elevation	Community A	0.27	0.08	0.0125	999
	OM & Elevation	Community B	**0.75**	0.01*	0.0125	999
	Sediment C:N	Community A	0.42	0.05	0.0125	999
	Sediment C:N	Community B	-0.11	0.31	0.0125	999
	"site"	Community A	0.21	0.06	0.0125	999
	"site"	Community B	**0.56**	<0.0001*	0.0125	999
	"plant"	Community A	-0.13	0.23	0.0125	999
	"plant"	Community B	0.08	0.31	0.0125	999

Table 3.6b

Date	Variable A	Variable B	P	p-value	α-value	# iterations
February 2005	OM & Elevation	Community	0.16	0.18	0.0125	999
	Sediment C:N	Community	-0.12	0.28	0.0125	999
	"site"	Community	0.20	0.12	0.0125	999
	"plant"	Community	0.18	0.15	0.0125	999

Table 3.6c

Date	Variable A	Variable B	ρ	p-value	α -value	# iterations
July 2005	"site" model matrix	Bacterial Community	**0.48**	0.001*	0.007	999
	"plant" model matrix	Community	0.11	0.26	0.007	999
	SOM, Root OM Elevation	Bacterial Community	**0.46**	0.006*	0.007	999
	Sediment C:N	Bacterial Community	-0.25	0.06	0.007	999
	Aboveground Biomass	Bacterial Community	**-0.42**	0.003*	0.007	5000
	Belowground biomass	Bacterial Community	-0.35	0.07	0.007	5000
	Root C:N	Bacterial Community	0.093	0.33	0.007	999

APPENDIX D

PCR Conditions for 16S-DGGE

An extensive review of the literature was performed to find possible 16S rDNA primers appropriate for DGGE on sediment bacteria, e.g., [Muyzer, 1999; Crump, et al., 2003; Kang and Mills, 2004].

The following constraints for primer design in DGGE were considered:

1. Are forward and reverse primers free of complimentary regions (to prevent primer-dimer formation)?
2. Are primers free of hairpin loops or palindromic sequences (regions where primer could fold and anneal to itself)?
3. Does primer set target a highly-variable, or phylogenetically informative region of 16S rDNA?
4. Does primer set target a fragment under 500 base pairs long? DGGE for community fingerprinting functions best on fragments between 200-500 base pairs in length.
5. Is primer set generally accepted in literature as appropriate for PCR-DGGE on sediment soil or aquatic bacteria?

The following primers were selected initially for PCR-DGGE:

63F	5’-CAG GCC TAA CAC ATG CAA GTC-3’
357F	5’-CCT ACG GGA GGC AGC AG-3’
968F	5’-AAC GCG AAG AAC CTT AC-3’
518R	5’-ATT ACC GCG GCT GCT GG-3’

1401R 5’-CGG TGT GTA CCA GAC CC-3’

The following 40 bp GC clamp was attached to each forward primer:

5’-cgc ccg ccg cgc gcg gcg ggc ggg gcg ggg gca cgg ggg g-3’

(Muyzer et al, 1996, Nakatsu et al. 2000, Watanabe et al. 2001)

As the purity of oligonucleotide primers is paramount to the success of DGGE, primers were desalted, purified by polyacrylamide gel electrophoresis (PAGE), and shipped from manufacturer lyophilized. Upon receipt, primers were resuspended with sterile (filtered, autoclaved) TE buffer, pH 8.0 to 100 µM and thoroughly vortexed to mix. Both the stock solutions and aliquots of 10µM were stored at -20 °C and protected from repeated freeze-thaw cycles.

After initial PCR optimization and DGGE, primers 63F, 968F and 1401R were not selected for further use in this study, as they produce fragments of around 500 bp, and did not produce well defined bands for the sample types in this study. Primer set 357F and 518R was found to easily amplify bacterial DNA; PCR reactions were optimized to reduce non-specific priming, by lowering Taq polymerase and $MgCl_2$ concentrations, shortening anneal time, and using a 35 minute final extension at 72°C with a ramp to the hold temperature of 4 °C.

APPENDIX M
TABLES OF MEANS FOR CONTINUOUS VARIABLES

M1 Marsh Elevation
M2 Soil Organic matter
M3 Live Root Organic Matter
M4 Soil C, N, C:N
M5 Live Root C, N, C:N
M6 AODC for All sampling events
M7 Live Bacterial abundance for July 2005

Table M1. Means and standard errors (SE's) of Marsh Elevation data collected across 3 transects at each site. n=3

Dependent Variable	**Elevation**					
Site	High		Mid		Low	
	mean	SE	mean	SE	mean	SE
Position on transect (m)						
0 (*S.patens-D.spicata*)	0.733	0.007	0.698	0.009	0.617	0.025
1.5 (*S.patens-D.spicata*)	0.783	0.017	0.703	0.009	0.607	0.009
3 (Transition)	0.705	0.014	0.698	0.005	0.600	0.008
4.5 (*J. roemerianus*)	0.725	0.015	0.680	0.012	0.590	0.012
6 (*J. roemerianus*)	0.733	0.023	0.687	0.005	0.583	0.003

Table M2 . Soil and live root organic matter means and standard errors.

Dependent Variable:	Soil Organic Matter			Live Root Organic Matter		
Class Variable						
Plant	n	mean	SE	n	mean	SE
J. roemerianus	90	57.99	2.85	9	88.26	0.88
Transition	90	63.32	4.22	9	90.17	1.33
S. patens-D. spicata	90	54.84	2.76	9	91.28	1.15
Site						
High	90	69.33	3.57	9	92.02	1.11
Mid	90	60.60	3.19	9	89.77	1.18
Low	90	46.22	3.27	9	88.26	0.88

Table M3. Belowground and Aboveground biomass means and 95% confidence limits.

Dependent Variable:	Belowground Biomass				Aboveground biomass			
Class Variable								
Plant	n	mean	CL -	CL +	n	mean	CL-	CL+
J. roemerianus	9	284.80	85.84	122.88	9	279.25	50.96	62.33
Transition	9	656.81	197.97	283.39	9	280.93	51.26	62.71
S. patens-D. spicata	9	330.83	99.72	142.74	9	178.03	32.49	39.74
Site								
High	9	485.07	146.21	209.22	9	211.06	38.55	47.11
Mid	9	371.82	112.07	160.43	9	296.01	40.80	49.90
Low	9	343.16	103.43	148.06	9	223.56	54.01	66.07

95% CL used due to log-transformation

Tables M4 (top) and M5 (bottom): Means and standard errors of Sediment and Live Root Nutrient Content data.

Dependent Variable:	Sediment C:N				Sediment N				Sediment C			
Class variable	Date	n	mean	SE	Date	n	mean	SE	date	N	mean	SE
Plant												
J. roemerianus	July 2004	18	17.13	1.58	July 2004	18	1.39	0.05	July 2004	18	23.83	0.96
Transition		9	18.00	2.34		9	1.44	0.08		9	26.43	1.42
S. patens-D. spicata		18	20.11	1.63		18	1.39	0.05		18	25.44	0.99
Site												
High		15	18.82	1.97		15	1.72	0.07		15	32.21	1.20
Mid		15	19.04	1.81		15	1.41	0.06		15	26.19	1.10
Low		15	17.37	1.85		15	1.01	0.06		15	17.29	1.13
Plant												
J. roemerianus	Feb 2005	18	16.93	0.85	Feb 2005	18	1.65	0.08	Feb 2005	18	26.26	0.79
Transition		9	18.49	1.20		9	1.59	0.05		9	28.42	1.13
S. patens-D. spicata		18	18.92	0.85		18	1.70	0.05		18	27.63	0.80
Site												
High		15	19.01	0.98		15	1.92	0.06		15	36.73	1.46
Mid		15	17.65	0.98		15	1.71	0.06		15	30.14	1.46
Low		15	17.67	0.98		15	1.30	0.06		15	22.86	1.46

Dependent Variable:	Live root C:N			Live root N			Live Root C		
Class Variable	N	Mean	SE	N	mean	SE	N	Mean	SE
Plant									
J. roemerianus	9	38.56	2.11	9	1.05	0.05	9	39.85	0.73
Transition	9	37.32	1.19	9	1.11	0.03	9	41.30	0.84
S. patens-D. spicata	9	36.63	1.29	9	1.15	0.04	9	41.89	0.62
Site									
High	9	39.41	1.18	9	1.10	0.03	9	43.12	0.48
Mid	9	36.15	1.59	9	1.14	0.05	9	40.60	0.58
Low	9	36.95	1.77	9	1.08	0.05	9	39.32	0.63

Tables M6 and M7. AODC Results Grouped by Sampling Date and Live Bacterial Counts from July 2005 95% CL are reported due to log transformation prior to ANOVA

M6

Dependent Variable: AODC					
Class Variable	Date	n	Mean ($x10^{10}$)	CL- ($x10^{9}$)	CL+ ($x10^{9}$)
Plant					
J. roemerianus	July 2004	18	5.34	7.64	6.69
Transition		9	4.51	9.87	8.10
S. patens-D. spicata		18	3.73	6.38	5.45
Site					
High		15	3.96	7.20	6.09
Mid		15	5.62	9.07	7.81
Low		15	4.04	6.70	5.75
Plant					
J. roemerianus	February 2005	18	4.71	5.97	5.30
Transition		9	5.29	10.6	8.82
S. patens-D. spicata		18	4.01	5.09	4.52
Site					
High		15	4.68	6.92	6.03
Mid		15	4.89	7.22	6.29
Low		15	4.37	7.29	6.24
Plant					
J. roemerianus	July 2005	18	3.69	5.85	5.05
Transition		9	4.15	9.61	7.81
S. patens-D. spicata		18	3.57	6.14	5.25
Site					
High		15	3.71	5.80	6.88
Mid		15	4.53	7.08	8.41
Low		15	3.25	5.36	6.42

M7.

Dependent Variable:	Live Bacteria			
Class variable				
Plant	N	Mean ($x10^{10}$)	CL- ($x10^{9}$)	CL+($x10^{9}$)
J. roemerianus	18	2.37	3.54	4.16
Transition	9	2.15	3.53	4.23
S. patens-D. spicata	18	2.66	5.51	6.95
Site				
High	15	2.42	3.86	4.59
Mid	15	2.88	4.59	5.47
Low	15	2.02	3.41	4.09

APPENDIX A
TABLES OF ANOVA RESULTS FOR CONTINUOUS VARIABLES

Table A1. Marsh Elevation ANOVA Results.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	0.1557161	0.0194645	23.6	<.0001
Error	36	0.0296917	0.0008248		
Corrected Total	44	0.1854078			

Source	DF	Type III SS	Mean Square	F Value	Pr > F
site	2	0.124109	0.0620545	75.24	<.0001
plant	2	0.0059383	0.0029692	3.6	0.0376
site*plant	4	0.0031067	0.0007767	0.94	0.4511

Table A2. Soil Organic Matter Content ANOVA Table

Overall ANOVA: SOM = Plant|Site

	df	*Ss*	*Mean square*	*f-value*	*P value*
Model	8	4247.02	530.88	3.86	0.003
Error	34	4674.35	137.48		
Corrected Total	42	8921.36			
Type III Model					
Effect	*df*	*Type III ss*	*Mean square*	*f-value*	*P value*
Site	2	3261.77	1630.89	11.86	0.0001
Plant	2	392.60	196.30	1.43	0.25

Table A3. Live Root Organic Matter Content ANOVA Table

Live Roots only
3x3 ANOVA: Root OM=site|plant

Source	*DF*	*Sum of Squares*	*Mean Square*	*F Value*	*Pr > F*
Model	8	462.59	57.82	3.05	0.0235
Error	18	341.30	18.96		
Corrected Total	26	803.89			
Source	*DF*	*Type III SS*	*Mean Square*	*F Value*	*Pr > F*
Site	2	286.26	143.13	7.55	0.004
Plant	2	154.29	77.14	4.07	0.03
*site*plant*	4	22.05	5.51	0.29	0.88

Table A4. Live and Dead Root Organic Matter ANOVA Table.

Live and Dead roots: 3x3x2 ANOVA: Root OM=site|plant|status (live vs. dead)					
Source	*DF*	*Sum of Squares*	*Mean Square*	*F Value*	*p value*
Model	17	1076.38	63.32	4.73	<.0001
Error	36	481.90	13.39		
Corrected Total	53	1558.28			
Source	*DF*	*Type III SS*	*Mean Square*	*F Value*	*Pr > F*
Site	2	327.44	163.72	12.23	<.0001
Plant	2	164.99	82.50	6.16	0.005
Status	1	435.88	435.88	32.56	<.0001
*Site*Plant*	4	41.54	10.39	0.78	0.55
*Site*Status*	2	34.84	17.42	1.3	0.28
*Plant*Status*	2	31.36	15.68	1.17	0.32
*Site*Plant*Status*	4	40.32	10.08	0.75	0.56

Table A5. July 2004 Sediment Nutrient Content and C:N ANOVA Tables.

TC						TN					C:N				
Source	*DF*	*Sum of Squares*	*Mean Square*	*F Value*	*Pr > F*	*DF*	*Sum of Squares*	*Mean Square*	*F Value*	*Pr > F*	*DF*	*Sum of Squares*	*Mean Square*	*F Value*	*Pr > F*
Model	8	1452.51	181.56	11.67	<.0001	8	3.58	0.45	9.66	<.0001	8	158.14	19.77	1.95	0.086
Error	32	497.76	15.55			32	1.48	0.05			32	323.74	10.12		
Corrected Total	40	1950.27				40	5.07				40	481.88			
Source	*DF*	*Type III SS*	*Mean Square*	*F Value*	*Pr > F*	*DF*	*Type III SS*	*Mean Square*	*F Value*	*Pr > F*	*DF*	*Type III SS*	*Mean Square*	*F Value*	*Pr > F*
Site	2	1308.41	654.20	42.06	<.0001	2	2.95	1.47	31.8	<.0001	2	20.30	10.15	1	0.378
Plant	2	42.00	21.00	1.35	0.274	2	0.13	0.07	1.43	0.255	2	74.56	37.28	3.68	0.036
*site*plant*	4	102.62	25.65	1.65	0.186	4	0.04	0.01	0.22	0.923	4	75.20	18.80	1.86	0.142

Table A6. February 2005 Sediment Nutrients and C:N ANOVA Tables.

TN						TC					C:N				
Source	*DF*	*Sum of Squares*	*Mean Square*	*F Value*	*Pr > F*	*DF*	*Sum of Squares*	*Mean Square*	*F Value*	*Pr > F*	*DF*	*Sum of Squares*	*Mean Square*	*F Value*	*Pr > F*
Model	8	3.37	0.4207	7.95	<.0001	8	1431.01	178.88	6.21	<.0001	8	75.62	9.45	3	0.011
Error	36	1.90	0.0529			36	1037.69	28.83			36	113.43	3.15		
Corrected Total	44	5.27				44	2468.7				44	189.04			
Source	*DF*	*Type III SS*	*Mean Square*	*F Value*	*Pr > F*	*DF*	*Type III SS*	*Mean Square*	*F Value*	*Pr > F*	*DF*	*Type III SS*	*Mean Square*	*F Value*	*Pr > F*
Site	2	2.75	1.37	25.99	<.0001	2	1298.3	649.15	22.52	<.0001	2	16.43	8.21	2.61	0.0876
Plant	2	0.12	0.06	1.12	0.34	2	30.53	15.26	0.53	0.59	2	37.83	18.92	6.0	0.0056
*site*plant*	4	0.03	0.007	0.13	0.97	4	85.29	21.32	0.74	0.57	4	30.55	7.64	2.42	0.0659

Table A7. C:N ANOVA Table for Pooled July 2004 and February 2005 Data.

3x3x2, 3 way ANOVA of both sampling dates combined C:N					
Source	*DF*	*Sum of Squares*	*Mean Square*	*F Value*	*Pr > F*
Model	9	195.28	21.70	3.53	0.0011
Error	76	466.82	6.14		
Corrected Total	85	662.10			
Source	*DF*	*Type III SS*	*Mean Square*	*F Value*	*Pr > F*
Site	2	30.71	15.36	2.5	0.09
Plant	2	91.76	45.88	7.47	0.001
Year	1	2.70	2.70	0.44	0.51
*site*plant*	4	91.38	22.85	3.72	0.008

Table A8. Live Root C, N, C:N ANOVA Tables. Roots were pooled from 4 depth sections for nutrient analysis.

	TC						**TN**					**C:N**			
Source	*DF*	*Sum of Squares*	*Mean Square*	*F Value*	*Pr > F*	*DF*	*Sum of Squares*	*Mean Square*	*F Value*	*Pr > F*	*DF*	*Sum of Squares*	*Mean Square*	*F Value*	*Pr > F*
Model	8	95.04	11.88	5.14	0.002	8	0.12	0.02	0.94	0.512	8	129.92	16.24	0.68	0.705
Error	18	41.58	2.31			18	0.30	0.02			18	430.73	23.93		
Corrected Total	26	136.62				26	0.42				26	560.65			
Source	*DF*	*Type III SS*	*Mean Square*	*F Value*	*Pr > F*	*DF*	*Type III SS*	*Mean Square*	*F Value*	*Pr > F*	*DF*	*Type III SS*	*Mean Square*	*F Value*	*Pr > F*
Site	2	67.46	33.73	14.6	0.001	2	0.02	0.01	0.5	0.616	2	51.75	25.87	1.08	0.360
Plant	2	19.80	9.90	4.28	0.030	2	0.05	0.02	1.38	0.278	2	17.32	8.66	0.36	0.701
site*plant	4	7.78	1.94	0.84	0.517	4	0.06	0.02	0.93	0.467	4	60.86	15.21	0.64	0.644

Table A9. ANOVA Table of Aboveground Biomass Data Collected July 2005

Source	*DF*	*Sum of Squares*	*Mean Square*	*F Value*	*Pr > F*
Model	8	0.39	0.04	3.16	0.0202
Error	18	0.28	0.02		
Corrected Total	26	0.68			
Type 3 Tests of Fixed Effects					
Effect	*Num DF*	*Den DF*	*F Value*	*Pr > F*	
Site	2	18	3.56	0.05	
Plant	2	18	7.44	0.004	
*Pite*Plant*	4	18	0.82	0.53	

Table A10. ANOVA Table of Belowground Biomass Data Collected July 2005

Source	*DF*	*Sum of Squares*	*Mean Square*	*F Value*	*Pr > F*
Model	8	8.68	1.08	4.13	0.006
Error	18	4.73	0.26		
Corrected Total	26	13.40			
Source	*DF*	*Type III SS*	*Mean Square*	*F Value*	*Pr > F*
Plant	2	3.57	1.79	6.81	0.006
Site	2	0.59	0.30	1.13	0.346
*Site*Plant*	4	4.51	1.13	4.3	0.013

Table A11. July 2004 AODC ANOVA Table

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	0.49	0.06	4.75	0.0006
Error	34	0.44	0.013		
Corrected Total	42	0.93			

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Site	2	0.20	0.10	7.65	0.002
Plant	2	0.24	0.12	9.19	0.001
Site*Plant	4	0.058	0.014	1.12	0.36

Table A12. February 2005 AODC ANOVA table

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	0.17	0.02	1.79	0.112
Error	35	0.41	0.01		
Corrected Total	43	0.57			

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Site	2	0.01	0.01	0.61	0.55
Plant	2	0.09	0.04	3.86	0.03
Site*Plant	4	0.04	0.01	0.76	0.56

Table A13. July 2005 AODC ANOVA table.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	0.23	0.03	1.62	0.1546
Error	34	0.61	0.02		
Corrected Total	42	0.84			

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Site	2	0.14	0.07	3.80	0.03
Plant	2	0.03	0.02	0.72	0.49
Site*Plant	4	0.03	0.01	0.49	0.75

Table A14. Percent Live Bacteria in July 2005, ANOVA Table

Dependent Variable: Percent Live Cells					
Source	*DF*	*Sum of Squares*	*Mean Square*	*F Value*	*Pr > F*
Model	8	305.64	38.21	1.37	0.24
Error	36	1005.03	27.92		
Corrected Total	44	1310.67			
Source	*DF*	*Type III SS*	*Mean Square*	*F Value*	*Pr > F*
Site	2	30.72	15.36	0.55	0.58
Plant	2	62.30	31.15	1.12	0.34
Site*Plant	4	192.83	48.21	1.73	0.17

LITERATURE CITED

Ackerly, D., Dudley, S., Sultan, S., Schmitt, J., Coleman, J., Linder, C., Sandquist, D., Geber, M., Evans, A., Dawson, T. and Lachowicz, M. (2000) The evolution of plant ecophysiological traits: Recent advances and future directions. *BioScience* 50, 979-995.

Adams, D.A. (1963) Factors influencing vascular plant zonation in North Carolina salt marshes. *Ecology* 44, 445-456.

Apple, J.K., del Giorgio, P.A. and Kemp, W.M. (2006) Temperature regulation of bacterial production, respiration, and growth efficiency in a temperate salt-marsh estuary. *Aquatic Microbial Ecology* 43, 243-254.

Baker, G.C., Smith, J.J. and Cowan, D.A. (2003) Review and re-analysis of domain-specific 16S primers. *Journal of Microbiological Methods* 55, 541-555.

Bertness, M.D. and Ellison, A.M. (1987) Determinants of pattern in a New England salt marsh plant community. *Ecological Monographs* 57, 129-147.

Blum, L.K. (1993) Spartina alterniflora root dynamics in a Virginia marsh. *Marine Ecology Progress Series* 102, 169-178.

Blum, L.K. and Christian, R.R. (2004) Belowground Production and Decomposition Along a Tidal Gradient in a Virginia Salt Marsh. In: *The Ecogeomorphology of Tidal Marshes*, Vol. 59, pp. 47-73. American Geophysical Union.

Blum, L.K., Roberts, M.S., Garland, J.L. and Mills, A.L. (2004) Distribution of microbial communities associated with the dominant high marsh plants and sediments of the United States East Coast. *Microbial Ecology* 48, 375-388.

Bossio, D.A., Fleck, J.A., Scow, K.M. and Fujii, R. (2006) Alteration of soil microbial communities and water quality in restored wetlands. *Soil Biology & Biochemistry* 38, 1223-1233.

Brinson, M.M. and Christian, R.R. (1999) Stability of *Juncus roemerianus* patches in a salt marsh. *Wetlands* 19, 65-70.

Brinson, M.M., Christian, R.R. and Blum, L.K. (1995) Multiple states in the sea-level induced transition from terrestrial forest to estuary. *Estuaries* 18, 648-659.

Brinson, M.M., Lugo, A.E. and Brown, S.K. (1981) Primary productivity, decomposition and consumer activity in freshwater wetlands. *Annual Review of Ecology and Systematics* 12, 123-161.

Brodie, E., Edwards, S. and Clipson, N. (2002) Bacterial community dynamics across a floristic gradient in a temperate upland grassland ecosystem. *Microbial Ecology* 44, 260-270.

Buchan, A., Newell, S.Y., Butler, M., Biers, E.J., Hollibaugh, J.T. and Moran, M.A. (2003) Dynamics of bacterial and fungal communities on decaying salt marsh grass. *Applied and Environmental Microbiology* 69, 6676-6687.

Buck, T.L. (2001) High marsh plant community response to sea-level induced high marsh subsidence and ecosystem state change. Department of Biology. Greenville, Advisor, Christian, R.R., East Carolina University 154 pp.

Burke, D.J., Hamerlynck, E.P. and Hahn, D. (2002a) Effect of arbuscular mycorrhizae on soil microbial populations and associated plant performance of the salt marsh grass *Spartina patens*. *Plant and Soil* 239, 141-154.

Burke, D.J., Hamerlynck, E.P. and Hahn, D. (2002b) Interactions among plant species and microorganisms in salt marsh sediments. *Applied and Environmental Microbiology* 68, 1157-1164.

Cardon, Z.G. and Cage, D.J. (2006) Resource Exchange in the Rhizosphere: molecular tools and the microbial perspective. *Annual review of Ecology, Evolution and Systematics* 37, 459-488.

Christian, R.R., Bancroft, K. and Wiebe, W.J. (1978) Resistance of the microbial community within salt marsh soils to selected perturbations. *Ecology* 59, 1200-1210.

Christian, R.R., Stasavich, L.E., Thomas, C.R. and Brinson, M.M. (2000) Reference is a moving target in sea-level controlled wetlands. In: *Concepts and Controversies in Tidal Marsh Ecology* (Weinstein, M.P. and Kreeger, D.A., Eds.), pp. 805-925. Kluwer Academic Publishers, Dordrecht, Netherlands.

Christiansen, T., Wiberg, P.L. and Milligan, T.G. (2000) Flow and sediment transport on a tidal salt marsh surface. *Estuarine, Coastal and Shelf Science* 50, 315-331.

Costa, R., Gotz, M., Mrotzek, N., Lottmann, J., Berg, G. and Smalla, K. (2004) Effects of site and plant species on rhizosphere community structure as revealed by molecular analysis of microbial guilds. *FEMS Microbiology Ecology* 56, 236-249.

Crain, C.M., Silliman, B.R., Bertness, S.L. and Bertness, M.D. (2004) Physical and biotic drivers of plant distribution across estuarine salinity gradients. *Ecology* 85, 2539-2549.

Crump, B.C., Kling, G.W., Bahr, M. and Hobbie, J.E. (2003) Bacterioplankton community shifts in an arctic lake correlate with seasonal changes in organic matter source. *Applied and Environmental Microbiology* 69, 2253-2268.

Dar, S.A., Kuenen, J.G. and Muyzer, G. (2005) Nested PCR-denaturing gradient gel electrophoresis approach to determine the diversity of sulfate-reducing bacteria in complex microbial communities. *Applied Environmental Microbiology* 71, 2325-2330.

Dayton, P.K. (1971) Competition, disturbance, and community organization: the provision and subsequent utilization of space in a rocky intertidal community. *Ecological Monographs* 41, 351-389.

Degens, B.P. (1998) Decreases in microbial functional diversity do not result in corresponding changes in decomposition under different moisture conditions. *Soil Biology & Biochemistry* 30, 1989-2000.

Devereux, R., Hines, M.E. and Stahl, D.A. (1996) S Cycling: Characterization of natural communities of sulfate reducing bacteria by 16S rRNA sequence comparisons. *Microbial Ecology* 32, 283-292.

Docherty, K.M., Young, K.C., Maurice, P.A. and Bridgham, S.D. (2006) Dissolved organic matter concentration and quality influences upon structure of freshwater microbial communities. *Microbial Ecology* 52, 378-388.

Drenovsky, R.E., Vo, D., Graham, K.J. and Scow, K.M. (2004) Soil water content and organic carbon availability are major determinants of soil microbial community composition. *Microbial Ecology* 48, 424-430.

Duineveld, B.M., Kowalchuk, G.A., Keijzer, A., Elsas, J.D.v. and Veen, J.A.v. (2001) Analysis of bacterial Communities in the rhizosphere of chrysanthemum via denaturing gradient gel electrophoresis of PCR-amplified 16S rRNA as well as DNA fragments coding for 16S rRNA. *Applied and Environmental Microbiology* 67, 172-178.

Edmonds, W.J., Silverhorn, G.M., Cobb, P.R., C.D. Peacock, J., McLoda, N.A. and Smith, D.W. (1985) Soil Classifications and Floral Relationships of Seaside Salt Marsh Soils in Accomack and Northampton Counties, Virginia. Virginia Polytechnic Institute and State University; Virginia Agricultural Experiment Station.

Eleuterius, L.N. and Eleuterius, C.K. (1979) Tidel levels and salt marsh zonation. *Bulletin of Marine Science* 29, 394-400.

Franklin, R.B. (2004) Spatial patterns in microbial communities. Dissertation, Department of Environmental Sciences. Charlottesville, University of Virginia. Advisor, Mills, A., 342 pp.

Franklin, R.B., Blum, L.K., McComb, A.C. and Mills, A.L. (2002) A geostatistical analysis of small-scale spatial variability in bacterial abundance and community structure in salt-marsh creek. *FEMS Microbiology Ecology.* 42, 71-80.

Franklin, R.B., Garland, J.L. and Mills, A.L. (2001) The impact of dilution on microbial community structure and functional potential: a comparison of numerical simulations and batch culture experiments. *Applied and Environmental Microbiology* 67, 702-712.

Franklin, R.B. and Mills, A.L. (2003) Multi-scale variation in spatial heterogeneity for microbial community structure in an eastern Virginia agricultural field. *FEMS Microbiology Ecology* 44, 335-346.

Franklin, R.B. and Mills, A.L. (2006) Structural and functional responses of a sewage microbial community to dilution-induced reductions in diversity. *Microbial Ecology* 52, 280-288.

Fromin, N., Hamelin, J., Tarnawski, S., Roesti, D., Jourdain-Miserez, K., Forestier, N., Teyssier-Cuvelle, S., Gillet, F., Aragno, M. and Rossi, P. (2002) Statistical analysis of denaturing gel electrophoresis (DGE) fingerprinting patterns. *Environmental Microbiology* 4, 634-643.

Garbeva, P., van Veen, J.A. and van Elsas, J.D. (2004) Microbial diversity in soil: Selection of microbial populations by plant and soil type and implications for disease suppressiveness. *Annual Review of Phytopathology* 42, 243-270.

Girvan, M.S., Bullimore, J., Pretty, J.N., Osborn, A.M. and Ball, A.S. (2003) Soil type is the primary determinant of the composition of the tota and active bacterial communities in arable soils. *Applied and Environmental Microbiology* 69, 1800-1809.

Grayston, S.J., Griffith, G.S., Mawdsley, J.L., Campbell, C.D. and Bardgett, R.D. (2001) Accounting for variability in soil microbial communities of temperate upland grassland ecosystems. *Soil Biology and Biochemistry* 33, 533-551.

Green, S.J. and Minz, D. (2005). Suicide polymerase endonuclease restriction, a novel technique for enhancing PCR amplification of minor DNA templates. *Applied and Environmental Microbiology* 71, 4721-4727.

Hannen, E.J.v., Mooij, W., Agterveld, M.P.v., Gons, H.J. and Laanbroek, H.J. (1999). Detritus-dependent development of the microbial community in an experimental system: qualitative analysis by denaturing gradient gel electrophoresis. *Applied and Environmental Microbiology* 65, 2478-2484.

Hellmann, J.J. and Fowler, G.W. (1999). Bias, precision, and accuracy of four measures of species richness. *Ecological Applications* 9, 824-834.

Heuer, H., Hartung, K., Wieland, G., Kramer, I. and Smalla, K. (1999). Polynucleotide probes that target a hypervariable region of 16S rRNA genes to identify bacterial isolates corresponding to bands of community fingerprints. *Applied and Environmental Microbiology* 65, 1045-1049.

Hill, G.T., Mitkowski, N.A., Aldrich-Wolfe, L., Emele, L.R., Jurkonie, D.D., Ficke, A., Maldonado-Ramirez, S., Lynch, S.T. and Nelson, E.B. (2000). Methods for assessing the composition and diversity of soil microbial communities. *Applied Soil Ecology* 15, 25-36.

Hines, M.E., Evans, R.S., Genthner, B.R.S., Willis, S.G., Friedman, S., Rooney-Varga, J.N. and Devereux, R. (1999). Moleular phylogenetic and biogeochemical studies of sulfate reducing bacteria in the rhizosphere of *Spartina alterniflora*. *Applied and Environmental Microbiology* 65, 2209-2216.

Howarth, R.W. and Giblin, A. (1983). Sulfate reduction in the salt marshes at Sapelo Island, Georgia. *Limnology and Oceanography* 28, 70-82.

Howarth, R.W. and Teal, J.M. (1979). Sulfate reduction in a New England salt marsh. *Limnology and Oceanography* 24, 999-1013.

Huges, J.B., Hellmann, J.J., Ricketts, T.H. and Bohannan, B.J.M. (2001) Counting the uncountable: statistical approaches to estimating microbial diversity. *Applied and Environmental Microbiology* 67, 4399-4406.

Hussein, A.H., Rabenhorst, M.C. and Tucker, M.L. (2004) Modeling of carbon sequestration in coastal marsh soils. *Soil Science Society of America Journal* 68, 1786-1795.

Ibekwe, A.M., Kennedy, A.C., Frohne, P.S., Papiernik, S.K., Yang, C.-H. and Crowley, D.E. (2002) Microbial diversity along a transect of agronomic zones. *FEMS Microbiology Ecology* 39, 183-191.

Johnson, R.A. and Wichern, D.W. (1992) *Applied Multivariate Statistical Analysis*, 767 pp. Chapman Hall, London.

Johnston, J.B., Watzin, M.C., Barras, J.A. and Handley, L.R. (1995) Gulf of Mexico Coastal wetlands: Case studies of loss trends. In: *Our living resources: a report to the nation on the distribution, abundance, and health of U.S. plants, animals and ecosystems* (LaRoe, E.T., Farris, G.S., Puckett, C.E., Doran, P.D. and Mac, M.J., Eds.), 530 pp. US Dept of the Interior, National Biological Service, Washington, D.C.

Kan, A., Crump, B.C., Wang, K., Chen, F. and Tietjen, T.E. (2006) Bacterioplankton community in Chesapeake Bay: predictable or random assemblages. *Limnology and Oceanography* 51, 2157-2169.

Kang, S.H. and Mills, A.L. (2004) Soil bacterial community structure changes following disturbance of the overlying plant community. *Soil Science* 169, 55-65.

Kastler, J.A. (1993) Sedimentation and landscape evolution of Virginia salt marshes. Thesis, Department of Environmental Sciences. Charlottesville, University of Virginia. Advisor, Wiberg, P., 119 pp.

Kastler, J.A. and Wiberg, P.L. (1996) Sedimentation and boundary changes of Virginia salt marshes. *Estuarine, Coastal and Shelf Science* 42, 683-700.

Kearney, M.S., Grace, R.E. and Stevenson, J.C. (1988) Marsh loss in Naticoke Estuary, Chesapeake Bay. *Geographical Review* 78, 205-222.

Kirk, J.L., Beaudette, L.A., Hart, M., Moutoglis, P., Klironomos, J.N., Lee, H. and Trevors, J.T. (2004) Methods of studying soil microbial diversity. *Journal of Microbiological Methods* 58, 169-188.

Knowlton, S.M. Geomorphological history of tidal marshes, Eastern Shore, Virginia, from 1852-1966. Masters thesis, Department of Environmental Sciences. University of Virginia, Charlottesville.

Kowalchuk, G.A., Buma, D.S., Boer, W.d., Klinkhamer, P.G.L. and Veen, J.A.v. (2002) Effects of above-ground plant species composition and diversity on the diversity of soil-borne microorganisms. *Antonie van Leewenhoek* 81, 509-520.

Kramer, C. and Gleixner, G. (2006) Variable use of plant-and soil-derived carbon by microorganisms in agricultural soils. *Soil Biology & Biochemistry* 38, 3267-3278.

Kropf, S., Heuer, H., Gruning, M. and Smalla, K. (2004) Significance test for comparing complex microbial community fingerprints using pairwise similarity measures. *Journal of Microbiological Methods* 57, 187-195.

Kruskal, J.B. (1964a) Multidimensional scaling by optimizing goodness of fit to a nonmetric hypothesis. *Psychometrika* 29, 115-129.

Kruskal, J.B. (1964b) Nonmetric multidimensional scaling: a numerical method. *Psychometrika* 29, 115-129.

Kuehn, K.A., Lemke, M.J., Suberkropp, K. and Wetzel, R.G. (2000) Microbial biomass and production associated with decaying leaf litter of the emergent macrophyte *Juncus effusus*. *Limnology and Oceanography* 45, 862-870.

Legendre, P. (1993) Spatial autocorrelation: trouble or new paradigm? *Ecology* 74, 1659-1673.

Legendre, P. and Legendre, L. (1998) *Numerical Ecology*, 853 pp. Elsevier, Amsterdam.

Lejuene, O., Tlidi, M. and Couteron, P. (2002) Localized vegetation patches: A self-organized response to resource scarcity. *Physical Reviews* 66, pp.1-4.

Lentzsch, P., Wieland, R. and Wirth, S. (2005) Application of multiple regression and neural network approaches for landscape-scale assessment of soil microbial biomass. *Soil Biology & Biochemistr*y 37, 1577-1580.

Levine, J.M., Brewer, J.S. and Bertness, M.D. (1998) Nutrients, competition and plant zonation in a New England salt marsh. *The Journal of Ecology* 86, 285-292.

Liu, Y.M., Zhang, Q.Y., Yuan, X.P., Li, Z.Q. and Gui, J.F. (2006) Seasonal variation of virioplankton in a eutrophic shallow lake. *Hydrobiologia* 560, 323-334.

Luna, G.M., Manini, E. and Danovaro, R. (2002) Large fraction of dead and inactive bacteria in coastal marine sediments: comparison of protocols for determination

of ecological significance. *Applied and Environmental Microbiology* 68, 3509-3513.

Marchesi, J.R., Sato, T., Weightman, A.J., Martin, T.A., Fry, J.C., Hiom, S.J. and Wade, W.G. (1998) Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA. *Applied and Environmental Microbiology* 64, 795-799.

Marschner, P., Crowley, D. and Yang, C.H. (2004) Development of specific rhizosphere bacterial communities in relation to plant species, nutrition and soil type. *Plant and Soil* 261, 199-208.

Meyers, R.M., Maniatis, T. and Lerman, L.S. (1987) *Methods in Enzymology* 155, pp. 501-527.

Muyzer, G. (1999) DGGE/TGGE a method for identifying genes from natural ecosystems. *Current Opinion in Microbiology* 2, 317-322.

Muyzer, G., Waal, E.C.D. and Uitterlinden, A.G. (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied and Environmental Microbiology* 59, 695-700.

Nakatsu, Torsvic, V. and Ovreas, L. (2000) Soil community analysis using DGGE of 16S rDNA polymerase chain reaction products. *Soil Science Society of America* 64, 1382-1388.

Neubauer, S.C., Givler, K., Valentine, S.K. and Megonigal, J.P. (2005) Seasonal patterns and plant mediated controls of subsurface wetland biogeochemistry. *Ecology* 86, 3334-3344.

Nyman, J.A., Walters, R.J., Delaune, R.D. and Jr., W.H.P. (2006) Marsh vertical accretion via vegetative growth. *Estuarine Coastal and Shelf Science* 69, 370-380.

Oertel, G.F., Wong, G.T.F. and Conway, J.D. (1989) Sediment accumulation at a fringe marsh during transgression, Oyster, Virginia. Estuaries 12, 18-26.

Parnas, H. (1975) Model for decomposition of organic material by microorganisms. *Soil Biology & Biochemistry* 7, 161-169.

Piceno, Y.M. and Lovell, C.R. (2000) Stability in natural bacterial communities: II. Plant resource allocation effects on rhizosphere diazotroph assemblage composition. *Microbial Ecology* 39, 41-48.

Pinhassi, J. and Hagstrom, A. (2000) Seasonal succession in marine bacterioplankton. *Aquatic Microbial Ecology* 21, 245-256.

Pomeroy, L. and Wiebe, W. (2001) Temperature and substrates as interactive limiting factors for marine heterotrophic bacteria. *Aquatic Microbial Ecology* 23, 187-204.

Poremba, K., Durselen, C. and Stoeck, T. (1999) Succession of bacterial abundance, activity and temperature adaptation during winter 1996 in parts of the German Wadden Sea and adjacent coastal waters. *Journal of Sea Research* 42, 1-10.

Prosser, J.I. (2002) Molecular and functional diversity in soil microorganisms. *Plant and Soil* 244, 9-17.

Rabenhorst, M.C. (2001) Soils of Tidal and Fringing Wetlands. In: *Wetland soils: genesis, hydrology, landscapes and classification* (Richardson, J.L. and Vepraskas, M.J., Eds.), pp. 417. CRC Press, Boca Raton.

Ram, A.S.P., Boucher, D., Sime-Ngando, T., Debroas, D. and Romagoux, J.C.C. (2005) Phage bacteriolysis, protistan bacterivory potential, and bacterial production in a freshwater reservoir: Coupling with temperature. *Microbial Ecology* 50, 64-72.

Reddy, K.R. and Patrick, W.H.J. (1975) Alternate Aerobic and Anaerobic Conditions on Redox Potentials. *Soil Biology & Biochemistry* 7, 87-94.

Rodrigues, F.M., Diniz-Filho, J.A.F., Bataus, L.A.M. and Bastos, R.P. (2002) Hypothesis testing of genetic similarity based on RAPD data using mantel tests and model matrices. *Genetics and Molecular Biology* 25, 435-439.

Rooney-Varga, J.N., Devereux, R., Evans, R.S. and Hines, M.E. (1997) Seasonal changes in the relative abundance of uncultivated sulfate-reducing bacteria in a salt marsh sediment and in the rhizosphere of *Spartina alterniflora*. *Applied and Environmental Microbiology* 63, 3895-3901.

Rovira, A.D. (1965) Interactions between plant roots and soil microorganisms. *Annual Review of Microbiology* 19, 241-268.

Rublee, P.A. (1982) Seasonal distribution of bacteria in salt marsh sediments in North Carolina. *Estuarine, Coastal and Shelf Science* 15, 67-74.

Rublee, P.A. and Dornseif, B.E. (1978) Direct counts of bacteria in the sediments of a North Carolina salt marsh. *Estuaries* 1, 188-191.

Sakai, M., Matsuka, A., Komura, T. and Kanazawa, S. (2004) Application of a new PCR primer for terminal restriction fragment length polymorphism analysis of the bacterial communities in plant roots. *Journal of Microbiological Methods* 59, 81-89.

Sanders, J.G., Cibik, S.J., Delia, C.F. and Boynton, W.R. (1987) Nutrient Enrichment Studies in a Coastal-Plain Estuary - Changes in Phytoplankton Species Composition. *Canadian Journal of Fisheries and Aquatic Sciences* 44, 83-90.

Schauer, M., Balague, V., Pedros-Alio, C. and Massana, R. (2003) Seasonal changes in the taxonomic composition of bacterioplankton in a coastal oligotrophic system. *Aquatic Microbial Ecology* 31, 163-174.

Schwimmer, R.A. and Pizzuto, J.E. (2000) A model for the evolution of marsh shorelines. *Journal of Sedimentary Research* 70, 1026-1035.

Seliskar, D.M., Gallagher, J.L., Burdick, D.M. and Mutz, L.A. (2002) The regulation of ecosystem functions by ecotypic variation in the dominant plant: a *Spartina alterniflora* salt-marsh case study. *Journal of Ecology* 90, 1-11.

Shia, F.-K. and Ducklow, H. (1994) Temperature regulation of heterotrophic bacterioplankton abundance, production, and specific growth rate in Chesapeake Bay. *Limnology and Oceanography* 39, 1243-1258.

Silvestri, S., Defina, A. and Marani, M. (2005) Tidal regime, salinity and salt marsh plant zonation. *Estuarine, Coastal and Shelf Science* 62, 119-130.

Smalla, K., Wieland, G., Buchner, A., Zock, A., Parzy, J., Kaiser, S., Roskot, N., Heuer, H. and Berg, G. (2001) Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis: plant-dependent enrichment and seasonal shifts revealed. *Applied and Environmental Microbiology* 67, 4742-4751.

Stach, J.E.M., Bathe, S., Clapp, J.P. and Burns, R.G. (2001) PCR-SSCP comparison of 16S rDNA sequence diversity in soil DNA obtained using different isolation and purification methods. *FEMS Microbiology Ecology* 36, 139-151.

Stasavich, L. (1999) Hydrodynamics of a Coastal Wetland Ecosystem. Thesis, Department of Biology. Advisor, Brinson, M., Greenville, NC, East Carolina University. 76 pp.

Sterling, G. and Wilsey, B. (2001) Empirical relationships between species richness, evenness, and proportional diversity. *The American Naturalist* 158, 286-299.

Taylor, J.H. (1995) The effects of altered inundation and wrack deposition on nitrification, denitrification and the standing stocks of NO_3^- and NO_2^-. Thesis, Department of Biology. Advisor, Christian, R.R., Greenville, East Carolina University., 115 pp.

Thomas, C.R. (2004) Salt marsh biogeochemistry and sediment organic matter accumulation. Department of Environmental Sciences. Charlottesville, Advisor, Blum, L., University of Virginia., 165 pp.

Tietjen, T.E. and Wetzel, R.G. (2003) Seasonal and spatial distribution of bacterial biomass and the percentage of viable cells in a reservoir of Alabama. *Journal of Plankton Research* 25, 1521-1534.

Torsvik, V., Goskoyr, J. and Daae, F.L. (1990) High diversity in DNA of soil bacteria. *Applied and Environmental Microbiology* 56, 782-787.

Torsvik, V. and Ovreas, L. (2002) Microbial diversity and function in soil: from genes to ecosystems. *Current Opinion in Microbiology* 5, 240-245.

Vellinga and Klein, D.A. (1993) Climate change, sea level rise and integrated coastal zone management: an IPCC approach. *Ocean and Coastal Management* 21, 245-268.

Waldrop, M.P. and Firestone, M.K. (2004) Microbial community utilization of recalcitrant and simple carbon compounds: impact of oak-woodland plant communities. *Oecologia* 138, 275-284.

Waldrop, M.P., Zak, D.R., Blackwood, C.B., Curtis, C.D. and Tillman, D. (2006) Resource availability controls fungal diversity across a plant diversity gradient. *Ecology Letters* 9, 1127-1135.

Wang, G.C.-Y. and Wang, Y. (1997) Frequency of formation of chimeric molecules as a consequence of PCR coamplification of 16S rRNA genes from mixed bacterial genomes. *Applied and Environmental Microbiology* 63, 4645-4650.

Ward, D.M., Weller, R. and Bateson, M.M. (1990) 16S rRNA sequences reveal numerous uncultured microorganisms in a natural community. *Nature* 345, 63-65.

Watanabe, Kodama, Y. and Harayama, S. (2001) Design and evaluation of PCR primers to amplify bacterial 16S ribosomal DNA fragments used for community fingerprinting. *Journal of Microbiological Methods* 44, 253-262.

Wertz, S., Degrange, V., Prosser, J.I., Poly, F., Commeaux, C., Freitag, T., Guillaumaud, N. and Roux, X.L. (2006) Maintenance of soil functioning following erosion of microbial diversity. *Environmental Microbiology* 8, 2162-2169.

Whipps, J.M. and Lynch, J.M. (1983) Substrate flow and utilization in the rhizosphere of cereals. *New Phytologist* 95, 605-623.

Wieland, G., Neumann, R. and Backhaus, H. (2001) Variation of microbial communities in soil, rhizosphere and rhizoplane in response to crop species, soil type and crop development. *Applied and Environmental Microbiology* 67, 5849-5854.

Wintzingerode, F.v., Gobel, U.B. and Stackebrandt, E. (1997) Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis. *FEMS Microbiology Ecology* 21, 213-229.

Wu, Y., Hayes, V.M., Osinga, J., Mulder, I.M., Looman, M.W.G., Buys, C.H.C.M. and Hofstra, R.M.W. (1998) Improvement of fragment and primer selection for mutation detection by denaturing gradient gel electrophoresis. *Nucleic Acids Research* 26, 5423-5440.

Yanez-Arancibia, A. and Day, J.W. (2004) The Gulf of Mexico: towards an integration of coastal management with large marine ecosystem management. *Ocean and Coastal Management* 47, 537-563.

Yang, C.H. and Crowley, D.E. (2000) Rhizosphere microbial community structure in relation to root location and plant iron nutritional status. *Applied and Environmental Microbiology* 66, 345-351.

Yao, S., Merwin, I.A., Bird, G.W., Abawi, G.S. and Thies, J.E. (2005) Orchard floor management practices that maintain vegetative or biomass groundcover stimulate soil microbial activity and alter soil microbial community composition. *Plant and Soil* 271, 377-389.

Yoshie, S., Noda, N., Miyano, T., Tsuneda, S., Hirata, A. and Inamori, Y. (2001) Microbial community analysis in the denitrification process of saline-wastewater

by denaturing gradient gel electrophoresis of PCR-amplified 16S rDNA and the cultivation method. *Journal of Bioscience and Bioengineering* 92, 346-353.