SOIL FUNGAL COMMUNITIY PATTERNS IN A VIRGINIA SALT MARSH

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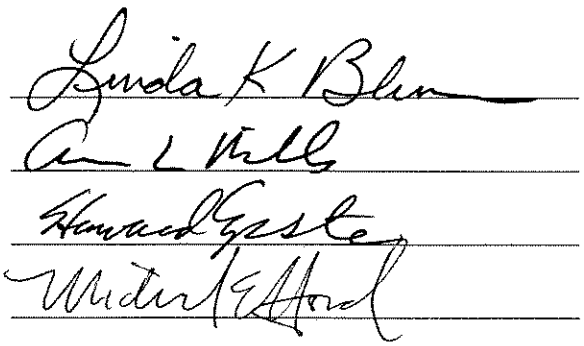

ABSTRACT

This comprehensive investigation of salt marsh soil fungal community patterns demonstrated that hyphal soil fungi were not only abundant, but also alive and active in the salt marsh environment. Work on general soil fungal community composition is sparse, and in wetlands even more rare due to the expectation that the high salinity, frequently saturated environment of the salt marsh soil is not conducive to fungal growth. However, this study found soil fungi in Upper Phillips Creek salt marsh (Virginia Coastal Bays watershed) comprise an average of 96% of the total soil microbial biomass. Total soil fungal and soil bacterial abundance was measured in patches of *Spartina patens* and *Juncus roemerianus*, and across low, mid, and high marsh regions using Acridine Orange Direct Counting (AODC). Total fungal abundance was negatively correlated with soil C/N, unassociated with soil organic matter and positively correlated with soil moisture and live standing stock. Total fungal abundance was also negatively correlated with belowground root mass and root C/N. Soil bacterial abundance and soil fungal abundance were found to be positively correlated across marsh region and vegetation zone, potentially due to the fungal role of making carbon substrate available to bacteria, as in the case of lignin.

Cytoplasm-filled fungal hyphae were enumerated using fluorescein diacetate staining (FDA) and compared to total fungal estimates. The abundance of live (cytoplasm-filled) hyphae was significantly, but weakly, correlated with soil moisture and soil organic matter, but uncorrelated with soil C/N, root biomass and root C/N, indicating that differences in plant community types are not strong factors in determining the abundance of live soil fungal hyphae. Conversely, marsh location, including distance

from the creek bank, may be influencing the abundance of live fungi through variations in flooding, salinity, and soil type. The lack of correlation between these two measurements may be due to differing rates of decay and decomposition of the evacuated hyphal lengths across marsh regions or between plant zones. Genetic community profiling via Terminal Restriction Fragment Length Polymorphisms (T-RFLP) produced strong Non-metric Multidimensional Scaling (NMDS) groupings of relative fungal community genetic similarity by both vegetative zone and marsh region. This leads to questions of fungal residency in the salt marsh soil, or, perhaps continual inoculation via standing dead fungal communities, tidal waters or airborne forest spores. These results provide strong evidence that fungi are critical components of the salt marsh soil microbial community.

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CHAPTER ONE: INTRODUCTION

1.1 Fungi and the Salt Marsh

As one of the world's most productive ecosystems and an indispensable buffer zone between land and sea, the salt marsh plays a critical role in both the estuarine environment and the overall health of the planet. Wetlands operate as giant environmental sponges by absorbing large volumes of water, and thus minimizing the impacts of flooding and erosion on recharging groundwater. Moreover, salt marsh plants and a thriving microbial community help to purify water by absorbing and metabolizing toxins and nutrient loading from the mainland. Through nutrient inputs and carbon dioxide fixation salt marshes may produce nearly 10 tons of organic matter per acre, per year (White, 1989). While much of this productivity is respired by wetland microbes and consumed by higher organisms, a significant portion is tidally exported, and serves as an importance source of carbon and energy for consumers in estuarine waters (Peterson and Howarth, 1987; Robertson *et al.*, 1988; Rublee, 1982; Saetre and Baath, 2000; Scala and Kerkhof, 2000; Scheiner and Gurevitch, 2001; Schmit, 2001) Consequently, a thriving marsh environment is key to overall coastal ecosystem sustainability. Organic carbon inputs are crucial to continued marsh sustainability, indicating the tremendous need to understand carbon sequestration and organic matter decay in these environments.

The filamentous fungi residing on plant litter are well-known key players in both carbon sequestration and organic matter decay in the marsh ecosystem (Newell, 2001), with the total annual fungal production within standing-dead marshgrass-shoot material likely to be about three times the total bacterial production (Newell and Wasowski, 1995). However, while fungi on aerial plants have received considerable study (Newell 2001 and references therein), the fungal communities of the salt marsh soil, where the

critical tasks of nutrient cycling and sediment stability take place, have historically been overlooked by investigators. In fact, almost half a century was to pass between the fungal observations of (Bayliss-Elliott, 1930) in the soils of Dover Estuary, Wales, and the next investigations of their abundance and potential importance in the salt marsh sediment (Padgett *et al.*, 1986; Padgett *et al.*, 1989; Pugh, 1962, 1979).

Almost all fungi require oxygen to grow (exceptions only for gut inhabitants) and, thus, many scientists assumed that only limited fungal activity takes place in the salt marsh soil due to the anaerobic, reducing conditions that are characteristic of this environment. However, fungi have the structural advantage of hyphae for transporting nutrients. These long, filamentous tubes, enclosed by cell walls, form the organ of vegetative growth in most fungi. This network can be used to move resources from nutrient rich areas to nutrient poor areas, allowing continued fungal growth (Jones and Lawton, 1995). Research also suggests that fungi are capable of conducting oxygen through their hyphae from the salt marsh soil surface, to the advancing mycelia front up to 11 cm below the surface (Padgett et al., 1989; Padgett and Celio, 1990). Fungi may also be able to exploit the periodic free oxygen introduced into the soil when the water table drops (Dacey and Howes, 1984), by animal burrowing activity (Montague, 1982), and by plant oxygenation of roots and rhizomes (Mendelsssohn *et al.*, 1981; Teal and Kaniwisher, 1966). Based on this evidence, and the knowledge that fungi comprise the majority of soil biomass in agricultural, grassland and forested ecosystems, contributing largely to sediment stability, plant distribution, and overall ecosystem health (Tisdall, 1991), it seemed likely that a large and influential soil fungal community might exist in the salt marsh soils.

First, this study reports the abundance of total and cytoplasm-filled hyphae in Upper Phillips Creek salt marsh soils on the Eastern Shore of Virginia. Next, a comprehensive investigation of the patterning of the soil fungal community is described to demonstrate that hyphal soil fungi were not only abundant, but also alive and active in the salt marsh environment. Biotic and abiotic factors potentially influencing the fungal community were measured, including bacterial, vegetative, and soil characteristics. These results provide strong evidence that the fungi are critical components of the salt marsh soil microbial community.

1.2 Environmental Influences on Soil Microbial Abundance

Biotic and abiotic gradients in the salt marsh environment assist researchers in the delineation of low, mid, and high marsh regions that reflect different ecological conditions (Adam, 1990). Investigating the effects of these gradients on the biotic marsh structure, recent research has begun to link trends in salinity, organic content, water content and elevation in the salt marsh to patterns of microbial community composition (Caravaca *et al.*, 2005; Franklin *et al.*, 2002; Koretsky *et al.*, 2005). Additionally, chronic and acute disturbances – natural and anthropogenic – can alter the marsh soil, changing bacterial distribution in the sediment (Langezaal *et al.*, 2003). Fungi are closely linked to bacteria in the decomposition process, and often compete for resources in the soil community, so it is not unlikely that fungi are influenced by some of these same soil parameters. My first objective in this study was to determine the affects of several biotic and abiotic factors on soil microbial abundance in Upper Phillips Creek salt marsh.

1.2.1 Tidal Inundation

In tidally flooded marshes, distance from the creek bank (ie frequency of flooding) has been indicated as strongly influential in vegetation distribution and, either directly or indirectly, in soil microbial community structure. Recent studies can be found documenting the influence of soil pH (Cookson *et al.*, 2007; Kahkonen *et al.*, 2007; Li *et al.*, 2007), salinity (Park *et al.*, 2006; Wichern *et al.*, 2006), and moisture (Chen *et al.*, 2007; Cisneros-Dozal *et al.*, 2007) on vegetative and microbial communities in virtually every environment. The impact of moisture content and redox potential on fungi is particularly relevant in salt marshes given the observations of Mansfield and Barlocher (1993) who found that concentrations of ergosterol, a sterol typical of higher fungi, to be negatively correlated with redox potential, indicating less growth in more water logged soils and at greater depths. For the current investigation, I hypothesized that tidal flooding in Upper Phillips Creek salt mash would affect the fungal community both directly with the higher salinity and oxygen stress these waters place on the ecosystem, as well as indirectly through changes in the bacterial community due to increasing redox conditions. Similarly, variations in freshwater inputs along this gradient may also be important through their effect on the availability of oxygen in the sediments.

1.2.2 Vegetation Gradients

In mid Atlantic tidal marshes such as Upper Phillips Creek, halophytic grasses such as *Spartina alterniflora, Spartina patens, Salicornia virginica, Distichilis spicata* and *Juncus roemerianus* are organized into characteristic monotypic patches and/or found occurring in distinct areas concurrent with tidal inundation variation. This conspicuous

zonation of salt marsh plant species has been described by a number of authors (ie Crain *et al.*, 2004; Levine *et al.*, 1998), and evaluations of the environmental factors effecting this distribution, such as salinity, hydroperiod, wrack disturbance and neighboring vegetation, are prevalent (ie Brinson and Christian, 1999; Ervin, 2005; Silvestri *et al.*, 2005; Traut, 2005)

In the investigation of saprophytic organisms, both quality and quantity of substrate supply has been linked to microbial enzymatic activity and microbial community composition across the well-studied forest and grassland systems (Bardgett *et al.*, 1998; Bardgett *et al.*, 1999; Gebauer and Taylor, 1999; Grayston *et al.*, 2001; Killham, 1990; Lang and Jagnow, 1986; Norton and Firestone, 1991). In particular, it is suggested that differences between aboveground species presence and species dominance likely exerts strong selective pressures on the soil microbial (bacterial) community through plant specific changes in the quantity and variety of compounds lost through rhizodeposition and litter and root senescence (Grayston *et al.*, 1998). Thus soil microbial activity and properties related to microbial activity, such as aggregate stability, are determined by the dominant vegetative species.

Abiotic factors such as soil water content and organic carbon availability which have strong effects on the soil microbial community are often tightly linked to the established plant community (Drenovsky *et al.*, 2004; Grayston et al., 2001), allowing the plant community to exert strong selective pressures on the soil microbial community. Burke *et al.* (2002) displays how marsh plant species might affect the microbial community in both size and composition, while Hines *et al.* (1999) and Marschner *et al.* (2004) are among those lending evidence to the complex interactions between plants and

the root associated bacterial and fungal communities. These studies, and many others, imply that through plant specific changes in the quantity and variety of compounds lost through above ground litter and root senescence, different soil microbial communities should be expected in areas of dissimilar plant make-up. Consequently, I expected that differences in sediment and dominant halophyte characteristics (organic matter content, above and below ground biomass, carbon/nitrogen content) would be correlated with differences in the fungal community. Specifically, I hypothesized that in low, mid and high marsh regions microbial biomass will be greater in *Juncus roemerianus* patches, where above ground biomass was greatest (greatest detrial input) and belowground biomass was lowest (decreased competition for belowground nutrients) as opposed to the surrounding *D. spicata-S. patens* dominated area.

1.2.3 Soil Nutrients and Energy

Further investigation into potential plant effects on soil microbial communities requires measurement of soil nutrients, a group of variables closely tied to microbial substrate decomposition. Carbon in the soil is a function of the historical vegetative cover and productivity, and is both a nutrient and the only source of energy for fungi. Nitrogen may be a limiting nutrient in salt marsh primary production (Howarth, 1988), with nitrogen levels and availability (due to mineralization by microorganisms) affecting plant productivity, standing biomass, and diversity and abundance of plant species (Valiela, 1983). Similarly, the ratio of carbon to nitrogen available in the soil can shift the environment to one that favors a specific microbial community structure. However, in

many salt marsh ecosystems, it is phosphorus rather than nitrogen that is generally recognized as the primary limiting nutrient (Valiela and Teal, 1974).

In a study of improved verses unimproved agricultural soils, Innes *et al.* (2004) found significantly greater total soil microbial biomass and activity in the more fertile (improved) soils, but greater fungal abundance in the unimproved soil type. Additional studies have displayed a shift in soil microbial community structure from one favoring fungi to one favoring bacteria as grassland communities improved in soil fertility and nutrient availability (Bardgett *et al.*, 1996; Bardgett et al., 1998; Bardgett and McAlister, 1999; Grayston et al., 2001). This is not surprising given the nutrient 'mining' techniques of fungi as they extend their hyphae over great distances through the soil. Consequently, I hypothesized that fungal abundance would be inversely related to soil organic matter and soil C/N in Upper Phillips Creek salt marsh.

1.3 Relationship between bacterial and fungal abundance in the salt marsh soil

A second objective of this study was to investigate the relationship between soil fungal abundance and soil bacterial abundance across tidal inundation and vegetation gradients. Recent studies have pointed to the existence of both a positive and a negative relationship between the bacteria and fungi comprising the aquatic microbial community (Buchan *et al.*, 2003; Burke *et al*., 2002, 2003; Gulis and Suberkropp, 2003; Mille-Lindblom and Tranvik, 2003; Moller *et al.*, 1998; Wohl and McArthur, 2001). The most common situation reported in the literature is the existence of an inverse relationship between bacterial and fungal abundance in sediment and litter microbial communities. Support for this relationship in aquatic environments was reported by Mille-Lindblom

and Tranvike (2003) who found that, although bacterial biomass is generally much lower than fungal biomass on aquatic plant litter, the presence of bacteria consistently obstructed the development of a large fungal biomass. In contrast, Wohl and McArthur (2001) illustrated the inhibitive properties of fungi on bacterial growth, and attributed this phenomenon to fungal actions including the elimination of suitable colonization strata for bacteria, the exploitation of essential bacterial nutrients, and the inhibitory compounds often secreted by fungi. Furthermore, Miao and Qian (2005) showed that of 46 marine fungal isolates tested, 70% inhibited the growth of at least one target bacterial species, although eleven of 19 bacteria species showed anti-fungal activity.

Work in grassland systems indicates that fungal and bacterial abundance varies quantitatively with plant productivity, or in the case of saprophytic microbes, substrate supply (Grayston et al., 2001). A few studies even indicate a positive relationship between the two groups, possibly explained by fungal activity making carbon substrate available to bacteria, as in the case of lignin (Moran and Hodson, 1989; Ruttimann *et al.*, 1991). Bardgett *et al.* (1993) showed that while bacterial abundance in soil microbial communities is favored in soils with high fertility and nutrient availability, increased fungal abundance is favored in the converse situation. As mineral miners, fungi are able to exist, thrive, and subsequently out compete bacteria in soils of low nitrogen or phosphorous content, or other nutrient poor environments (Dighton, 2003). Consequently, I hypothesized that the percentage of total microbial biomass constituted by fungi would be greatest in the high marsh region, where pore water concentrations of both ammonia and phosphate have been observed to be at their lowest (Blum and Christian, 2004; Blum *et al.*, 2004).

1.4 Live and Active Fungal communities

Studying sizes of microbial communities can be a tool for determining microbial dynamics and can lend considerable insight into the scale at which microbial interactions become important. However, it is necessary to go at least two steps further to conclude without a doubt that fungi are not only abundant, but are also alive and active in their soil environments. Distinguishing between cytoplasm-filled (live) fungal hyphae and determining genetic community fingerprints across environmental and vegetative gradients are the evidence necessary for such an assessment.

1.4.1 Percentage of Cytoplasm-Filled Hyphae Across Fungal Communities

Several studies mentioned above have noted salt marsh soil fungal communities, but none identified live fungi verses total fungal biomass. Saprophytic fungi in soils are non-discrete organisms that are well adapted to live in spatially heterogeneous environments. Hyphae are rigid filamentous structures that contain varying amounts of cytoplasm. Fungi can move the cytoplasm through this network depending on environmental conditions, especially the amounts and ratios of nutrient resources, as well as the physical distribution of nutrients (Carlisle, 1994; Davidson, 1998; Klein and Paschke, 2004). Furthermore, it is widely recognized that most fungal hyphal lengths in soil do not contain active cytoplasm (Harris, 1994; Ingham and Horton, 1987; Stahl and Parkin, 1996). In order to determine the patterning of live fungal abundance across the salt marsh, and better understand the relationship between bacterial and fungal abundance in the soil, different staining techniques were used to separately visualize total and live (cytoplasm-filled) fungal hyphae.

1.4.2 Relative Genetic Similarity Across Fungal Communities

Fungi may be selective in their source of a food base or may be restricted in terms of resource exploitation by other fungal species or other microbes (Mikola, 1998; Schmit, 2001; Wardle and Yeates, 1993). To determine if soil fungi are actively responding to environmental cues, it is important to not only collect abundance data but also information regarding the genetic structure of the fungal community. One way to do this is through the determination of relative genetic similarity between samples. Although gene sequencing and species identification has become routine, sequencing large numbers of clones is cumbersome, expensive and often very time consuming (Tiedje *et al.*, 1999). Terminal Restriction Fragment Length Polymorphism (T-RFLP) offers an alternative to gene cloning and sequencing by allowing for community composition comparison based on DNA fragment size of the target gene once it has been subject to multiple base-pair (sequence-specific) cutting by restriction enzymes. Tiedje *et al.* (1999) found five times greater success at detecting and tracking specific ribotypes using T-RFLP than Denaturing Gradient Gel Electrophoresis (DGGE), and MacNaughon *et al.* (1999) estimated that DGGE can only detect 1-2% of the microbial populations representing the dominant species present in an environmental sample. T-RFLP targeting rRNA genes has been successfully used to characterize fungal communities in soil, and can be a first step molecular fingerprinting technique for the identification of specific fungal taxa (Burke *et al*., 2005 and references therein).

Understanding how fungi are distributed in the salt marsh is a perquisite to evaluate what factors influence community structure. Thus, the spatial scale of the community must be described. Understanding the scales at which microbial processes are

carried out is motivated by a desire to gain insight into the fundamental properties of natural systems or for predicting ecosystem behavior (Boesch *et al*., 2000). Such a scale is defined by grain size (sampling unit), sampling interval, and extent (total area included in the study). Since no structure can be detected that is smaller than the grain size, or larger than the extent of the study, the sampling design defines the observational window for spatial pattern analysis (Legendre and Legendre, 1998).

While the majority of ecological studies employ a grain size far to large to permit microscale analysis, several examples can be found of microbial colonization or community structure patterning in grassland and forest soils (Both *et al.*, 1992; Kuperman *et al.*, 1998; Morris, 1999; Ritz *et al.*, 2001; Robertson et al., 1988; Saetre and Baath, 2000), in shallow coastal aquifers (Franklin *et al.*, 1999), in the open ocean (Duarte and Vaque, 1992; Mackas, 1984) and in salt marsh and marine sediments (Berardesco *et al.*, 1998; Danovaro *et al.*, 2001; Moran *et al.*, 1987; Scala and Kerkhof, 2000). While an exhaustive investigation of fungal community heterogeneity in salt marsh soils was beyond the scope of this study, the scale of fungal community structure chosen for investigation was appropriate within in the constraints of the physical and chemical environment properties measured, and followed logically with the work of previous authors (Franklin *et al.*, 2002).

1.5 Significance of This Study

Fungi are quite possibly the most critical group of organisms regulating soil processes. They play a major role in soil formation and stability through rock dissolution and particle bonding (Tisdall 1991), provide fertility for primary production through

decomposition of soil organic matter and nutrient mineralization (Ingham *et al.*, 1985; Joner and Jakobsen, 2005), and even stand as a food source for bacteria and numerous invertebrates (McGonigle, 1995; Silliman 2003). They have been well studied in grassland and forest soils, and even on standing dead marsh grasses and in tidal waters, but their presence and persistence in the salt marsh soils has not been fully addressed. This study serves, for the first time, to provide an informative picture of the structure of the soil fungal community in the salt marsh, as well as pertinent biotic and abiotic factors that may be influencing its size and activity. There have been no prior studies designed to systematically describe this community, particularly in the areas of live verses total fungal abundance and relative genetic similarity across inundation and vegetative gradients.

CHAPTER 2:

MATERIALS AND METHODS

2.1 Site Description and Transect Establishment

The site chosen for this study of soil microbial communities was Upper Phillips Creek salt marsh (Fig. 2.1). This integral piece of the Virginia Coastal Bay Watershed is located on the seaward side of the southern Delmarva Peninsula of Virginia, and is situated within the Virginia Coast Reserve/Long Term Ecological Research area (VCR/LTER). The marsh is located behind a relict sand ridge, and the surrounding uplands are either farmlands or pine-forest wood lots with very low slope (Blum *et al.*, 2004). One relatively unique feature of this marsh is the appearance of *Juncus roemerianus* (black needlerush) and *Spartina patens* (saltmeadow cordgrass) in all three major zones of tidal inundation (low, mid and high marsh). Typically, *J. roemerianus* is found above mean high tide to spring tide levels, placing it almost exclusively in high marsh zones along the Atlantic coast. At all experimental transects, the regions of *S. patens* under investigation are also accommodating to *Distichilis spicata*, and in the low marsh *Spartina alterniflora* as well. This consistent distribution of plant communities makes Upper Phillips Creek salt marsh an ideal place to investigate plant effects on microbial community structure across inundation gradients.

Three transects were constructed in each marsh region – low, mid and high – in Upper Phillips Creek salt marsh, in order to investigate the microbial community characteristics across the inundation gradient. Each transect was marked with a 6-m rope spanning the transitional zone between monotypic *J. roemerianus* and a mixed association of *S. patens* and *D. spicata* (Fig. 2.2).

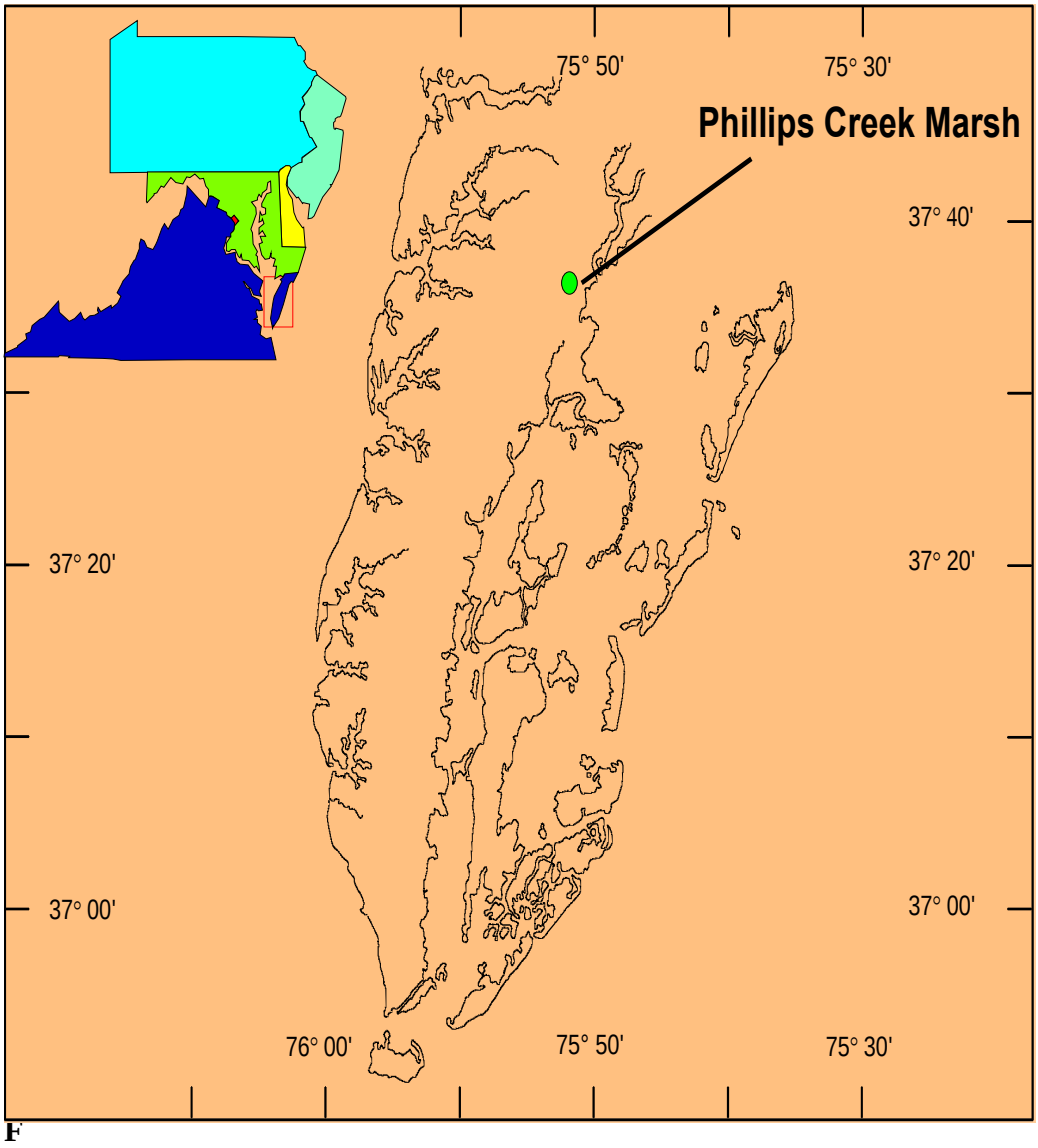


Figure 2.1. The location of the Upper Phillips Creak Salt Marsh study site on the eastern shore of Virginia (lower Delmarva Peninsula).

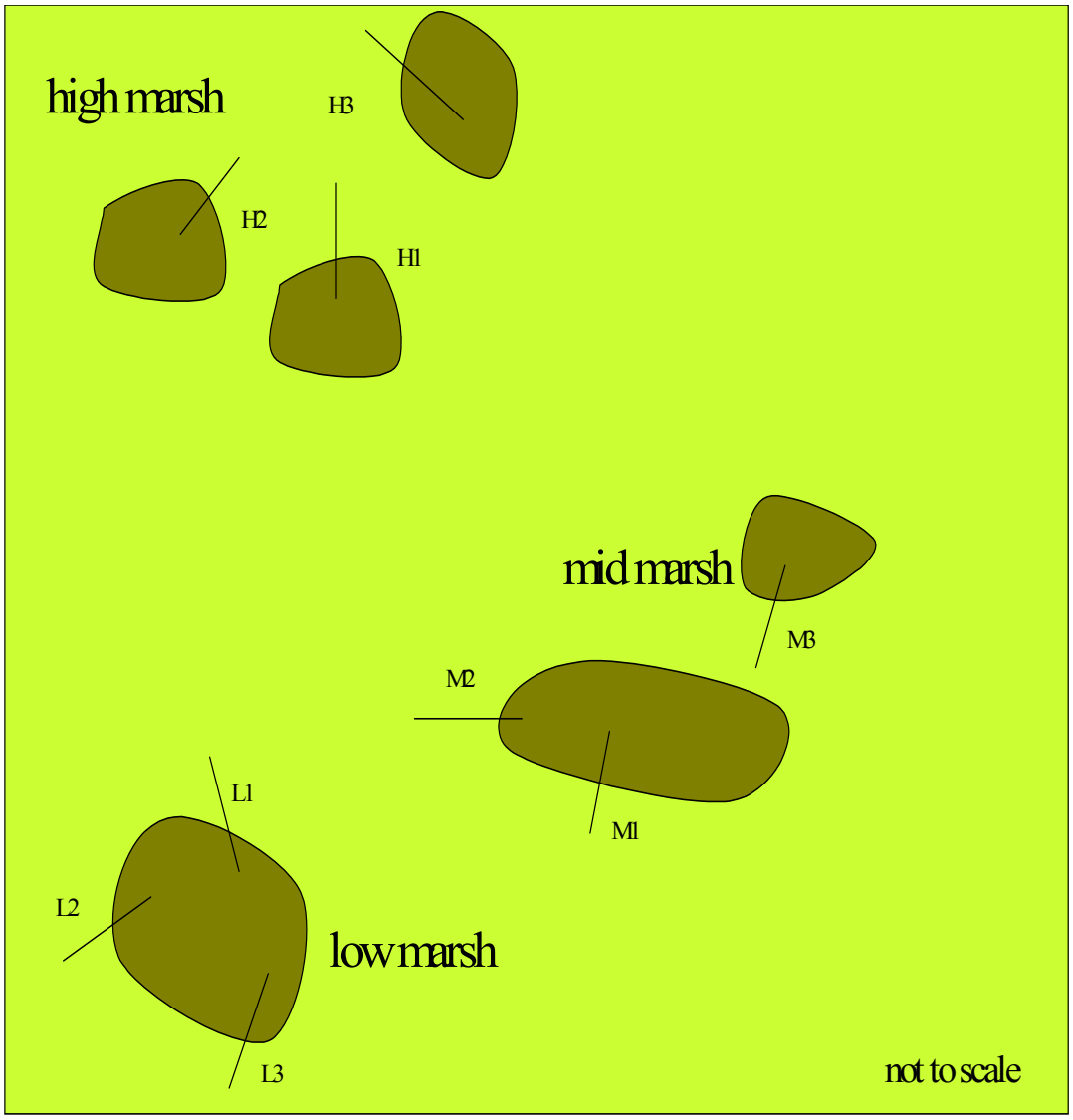


Figure 2.2. Three 6 m transects were constructed in each region of the salt marsh – low, mid and high. Each transect spanned similar vegetation zones – monotypic patches of *Juncus roemerianus*, the surrounding *Spartina patens – Distichilis spicata* dominated marsh, and the transitional zone between the two. Dark areas represent *J. roemerianus* monotypic patches, surrounded by the lighter colored *S. patens – D. spicata*.

2.2 Soil Microbial Abundance

2.2.1 Total Microbial Abundance and Biomass

Samples from the marsh surface were collected along the transects during August 2004 and July 2005 as 8 cm^3 soil cores and used to measure total and live microbial abundance just below the marsh surface. These same cores were subsequently used to determine fungal community structure and to measure several soil properties including moisture, organic matter content and soil C/N. Cores from 2004 were collected from three transects only - one each in the low, mid and high marsh. These transects were designated as L3 (low marsh #3), M2 (mid marsh #2), and H1 (high marsh #1). Each was randomly selected from two other transects in that marsh region. On these transects, samples were collected 10 cm apart for one meter in both the *J. roemerianus* and the *S. patens/D. spicata* dominant regions. The 2005 cores were collected at 10-cm sampling locations along a 4-m stretch in the same transects as the 2004 samples (H1, M2 and L3). These 40 samples span the transitional zone between the two plant regions, with the outermost samples falling in the exact locations as the July 2004 samples. Additionally, the six other established transects (H2, H2, H3, M1, M3, L1, L2) were sampled at 12 locations along the same 4-m region that the complete sampling took place in the main transects. These transects will be referred to as the replicate transects.

For core collection, a flat 2-cm x 2-cm Plexiglas template was attached to the end of a wooded rod, which served as the handle when the template was placed against the marsh surface at the point of sampling. A pocketknife was used to cut the marsh surface roughly along the edge of the template and scoop the soil core out of the ground at a depth of approximately 2-cm. As a result, soil samples were approximate cubes

measuring 8 cm^3. Soil cores were transferred to the lab in a cooler and stored at –80^oC until processed.

Bacterial and fungal abundance estimates from each sample core were determined via Acridine Orange Direct Counting (AODC), a microscopic enumeration technique (Hobbie et al., 1997). The measured sample (approximately 1 g) was ground in 100 mL of a 2% formaldehyde solution with the addition of 0.1 mL Triton. For the staining procedure, 10.0 mL filtered de-ionized water followed by 1.0 mL Acridine Orange solution was placed into the filter column, followed by an aliquot of sample varying in volume. If necessary, ten-fold sample dilutions were made to give between 20-200 bacterial cells per microscopic field. Prepared slides were viewed through a florescence microscope, oil emersion lens (100x; ocular magnification 10x), and counted in accordance with general AODC guidelines. Fungal abundance estimates are reported as hyphal length per gram of dry material by the hyphal intersection approach of Jones and Mollison (1948). Bacterial abundance is reported as the number of cells g^{-1} dry weight (DW) material.

Each soil microbial abundance measurement was then converted into microbial biovolume. For bacterial cells, the method of Krambeck *et al.* (1981) was used. First, a sampling of 20 cells was measured at 100x magnification, with one mark of the eyepiece micrometer equal to 0.5-μm. These measurements were then converted into cell volume using the formula:

$$V = (\pi/4)\, w^2\, (l\text{-}w/3)$$

where V = biovolume; w = width of the bacterial cell; and l = length of the cell.

The volumes were averaged and the resulting value for the biovolume of one bacterial cell from this population was used to convert the AODC data from units of bacterial cells g^{-1} DW sample to μm^3 bacteria g^{-1} DW sample. Fungal abundance was determined by measuring hyphal diameter at 1000x magnification (100x oil emersion lense; 10x ocular magnification) and treating hyphae as cylinders for biovolume conversions.

Bacterial and fungal biovolume measurements were converted to picograms of carbon g^{-1} DW sample using the assumptions of Newell and Statzell-Tallman (1982). They assume fungal dry mass is equal to approximately 0.55 of fungal biovolume, but that no such conversion is necessary for bacteria. They also assume a general case of dry soil + microbe as containing 35% carbon. The following equations were used accordingly, with C = picograms carbon g^{-1} sample:

Bacterial Carbon: $C = biovolume\ um^3/g\ soil \times 0.354\ pg\ C/um^3$

Fugal Carbon: $C = biovolume\ cm^3/g\ soil \times 0.55 g/cm3 \times .35\ (\%\ C) \times 10^{12} pg/1g$

2.2.2 Live Fungal Abundance

Although many stains are currently available for direct microscopic enumeration of microorganisms (Coleman, 1980; Hobbie *et al.*, 1997; Latt and Statten, 1976), fluorescein diacetate (FDA) is unique in its proven ability to distinguish live from dead fungal hyphae. As a fluorogenic substrate, FDA functions as a true vital stain by remaining non-fluorescent until enzymatically hydrolyzed. Therefore, only metabolically active organisms appear fluorescent under the microscope and those in resting stages (such as spores) lack the enzymatic activity necessary to cleave the FDA molecule, remaining unstained (Ingham and Klein, 1984; Soderstrom, 1977).

In order to obtain an estimate of both living and total fungal biomass, FDA must be used in conjunction with a stain that does not distinguish fungi based on viability. In this investigation, Acridine Orange Direct Counting (AODC) and FDA staining estimates were performed separately on each soil sample. Comparison of fungal abundance numbers obtained from these procedures produced relative live soil fungal abundance at each sampling location.

For both total and live abundance estimates, 1.0 g moist weight of soil sample was homogenized and dilutions of each suspension stained and viewed via florescence microscopy. The FDA stain concentration and incubation time were modified from Soderstrom (1977) and Green *et al.* (2006) and are described as follows. The stain was prepared by dissolving 20 mg FDA lipase substrate ($C_{24}H_{16}O_{7,}$ Sigma-Aldrich Chemical Co., Milwaukee, WI) in 10.0 ml of regent-grade acetone for a final solution concentration of 47.6 μM FDA ml^{-1}. Samples were ground with triton as described for AO staining (above) and incubated for 3 h at 37^{o}C. After incubation was complete, 2 ml of acetone was added to the suspension in order to terminate FDA hydrolysis. Fungal abundance estimates were again reported as hyphal length per gram of dry material by the hyphal intersection approach of Jones and Mollison (1948). Live soil fungal abundance was also expressed as a proportion of total hyphae as determined by dividing the fungal abundance estimate observed with the FDA staining procedure by the abundance estimate determined using the AODC staining procedure. Henceforth, live (cytoplasm-filled) fungi will be referred to as live hyphae while total fungi will continued to be referred to simply as fungal hyphae.

2.3 Environmental Measurements

2.3.1 Soil Nutrients, Moisture and Organic Matter

The soil cores collected for microbial abundance estimates were also used to determine soil moisture and soil organic matter patterns across the marsh. First, wet weights were recorded and samples dried at 70^{o}C to a constant mass. These samples were then reweighed (dry weight), ignited (450^{o}C, overnight), and weighed again (ash-free dry weight). The carbon and nitrogen content of these soil cores was determined using dry material and a CHNS/O analyzer (Carlo Erba, NA 2500). Samples were dried, ground and proportioned into tin capsules at weights between 10-15 mg. C/N was calculated as a ratio of the masses of carbon and nitrogen in the tinned sample. Data for marsh profiling including ammonia, salinity, and pH trends were obtained from previous work by Blum and Christian (2004). Elevation of the marsh surface above sea-level was recorded according to the Brownsville Farm marker BRNV (37.4606902856, -75.8347114622) at 1.225 m above mean sea level. Elevation data + or – 2.0 cm was collected at five points along each transect using a surveyor's laser level and stradia rod.

2.3.2 Aboveground Biomass and C/N

Aboveground biomass in each marsh region and plant zone was measured using 0.0625 m^2 clip plot samples taken in August 2005, the month of peak salt marsh aboveground biomass (Tolley and Christian, 1999). Three clip plots were taken along each of the nine transects (27 samples), one in each plant zone (*J. roemerianus*, Transitional, *S. patens/D. spicata*). Samples were sorted based on plant species and viability. *Juncus roemerianus* samples were only considered dead if they were more than

90% brown. Organic matter content of aboveground material was estimated via loss-on-ignition for 24 h at 450^{o}C. A separate portion of each sample was dried, ground and 2-5 mg was measured into tin capsules for C/N analysis (as described above for soil samples). Total aboveground nitrogen at each sampling locale was determined using ash-free dried weights of aboveground material and percent nitrogen values.

2.3.3 Belowground Root Mass and C/N

Three soil cores (5-cm diameter x 20-cm deep) were collected from each transect, one in each plant zone in 2005,during June, the month of peak salt marsh belowground biomass (Blum, 1993). Cores were processed within 48 h. Roots were separated into live and dead categories at 5-cm depth intervals prior to weighing. The live roots from an entire core were pooled, ground, and 2-5 mg were pressed into tin capsules. Samples were analyzed as described above for soil C/N.

2.3.4 Statistical Analysis

Analysis of Variance (ANOVA) was used to determine the effects of the independent variables marsh region and plant zone (and the interaction term) on live and total microbial abundance, addressing the hypotheses that both tidal inundation and plant type independently effect fungal community structure in the salt marsh environment. One-way ANOVA and post-hoc ANOVA using Ryan's Q test for all unplanned pair-wise comparisons were used for simple analysis across one independent variable. A post-hoc ANOVA using Tukey's test was used to make comparisons of the region x zone interaction (region*zone) (Sokal and Rohlf, 1995).

Multiple Analysis of Variance (MANOVA) was used to determine the significance of the main effects of marsh region and plant zone, to identify interactions between the response variables, and determine if there is a significant multivariate effect. MANOVA is considered to be more informative than simply completing multiple ANOVA tests on multiple variables because it captures the interrelatedness of the dependent variables and also keeps from an inflation of the α–value, an unwelcome consequence of performing multiple ANOVAs (Scheiner and Gurevitch, 2001). With MANOVA, we will protect ourselves from concluding that microbial communities differ between marsh region and plant zone if these differences are simple due to chance. Pearson's Correlation coefficients were examined in order to elucidate which transect characteristics have potentially strong or weak influence on soil microbial abundance.

All analyses were performed with a confidence level of 0.05 using the statistical package SAS v9.1 (SAS Institute, Cary, NC). Post hoc ANOVA with Ryan's Q test was performed in SAS proc glm and Tukey's test was preformed in SAS proc mixed. Scatter plots were used to view the relationship between sample fungal abundance and distance between samples, providing a quick, visual assessment of any potential gradients along the transects.

2.4 Fungal Community Composition

2.4.1 DNA Extraction and Quantitation

For nucleic acid extraction, approximately 0.25 g of each soil sample was transferred to a 2-mL screw-top tube as provided in the UltraClean Soil DNA Isolation Kit (MoBio Laboratories, Solana Beach, CA). DNA was extracted in accordance with the manufacturer's instructions, as modified by Clement and Kitts (2000). DNA was quantitated using the Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen Corporation, Carlsbad, CA, USA) and a BioLumin 960 microassay reader (Molecular Dynamics, Piscataway, NJ). Wavelength absorbance was measured at 260 nm (A_{260}), thus permitting the collection of a linear fluorescent signal in the range of 0.2-2.0 ng DNA.

The mean concentration of DNA per gram dry weight (DW) soil was determined by marsh region and by plant zone so that patterns of soil fungal abundance and soil DNA extracted could be compared. As described above for microbial abundance, one-way Analysis of Variance (ANOVA) with Ryan's Q test was used for a simple analysis across each of these independent variables. ANOVA using Tukey's test was used to make comparisons under the region x zone interaction (region*zone) (Sokal and Rohlf, 1995).

2.4.2 Amplification and Restriction Digest

Molecular analysis of the fungal community via Terminal Restriction Length Fragment Polymorphism (T-RFLP) began with the amplification of the fungal ITS gene via Polymerase Chain Reaction (PCR). The ITS region was chosen for comparison of fungal community composition because this is currently the most sequenced DNA region

of the fungal genome and the use of ITS specific PCR primers allow us to create many copies of this gene in amounts that far out weigh the rest of the extracted genome. Consistent with T-RLFP methods, the primers used for ITS amplification were terminally labeled with a florescent tag that can be read by an automated genetic analyzer. Due to sequence variations, the terminal restriction site for each species in the community should be different and thus the digital output can provide information on the size of the product in base pairs, with each product size considered a specific species. Thus, the T-RFLP banding pattern can be used to measure species richness and evenness as well as similarities between samples (Liu *et al.*, 1997).

PCR was performed in 100-uL reaction volumes with the use a 6FAM-labeled forward primer (ITS1-F) and a HEX-labeled reverse primer (ITS4) (synthesized by Operon Technologies, Alameda, CA, USA). These primers are manufactured to target the intergenic transcribed spacer (ITS) region of the fungal ribosomal DNA operon as described by Klamer *et al.* (2002). This specific primer pair has been applied successfully in similar work by Anderson *et al.* (2003) and Blum *et al.* (2004). In order to assure a consistent starting concentration of DNA, samples were grouped according to their PicoGreen® determined DNA concentrations and the amount of water in the reaction vessel was adjusted accordingly to give like concentrations of DNA, and presumably the optimal concentration for the PCR recipe employed.

In addition to the concentrated DNA, molecular grade water, and 1.0µM of each primer, PCR reaction vessels contained 2.0mM $MgCl_2$, 1x Applied Biosystems Buffer II, 200µM of each dNTP, 0.4µg/µL BSA (bovine serum albumin), and 1.25 AmpliTaq Gold DNA polymerase. Reagents were obtained from Applied Biosystems (Foster City, CA,

USA), with the exception of BSA that was obtained from Roche Diagnostics (Inndianapolis, IN, USA). Amplification reactions were performed in an MJ Research PTC-200 Thermocycler (Waltham, MA, USA) with reaction conditions set for fungal ITS amplification according to Blum *et al.* (2004). Specifically, the PCR cycle began with 5 min initial denaturation at 94^{o}C followed by 35 cycles of 0.5 min at 94^{o}C, 2 min of annealing at 50^{o}C, and 3 min extension at 72^{o}C.

PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) for the removal of primers and other fragments less than 40bp. Samples were digested with the restriction endonuclease *Hinf*I at 5U per reaction and an incubation of 60 min at 37^{o}C, in accordance with the manufacturer's instructions (New England Biolabs, Beverly, MA, USA). Following restriction, samples were purified and desalted with the QIAquick Nucleotide Removal Kit (Qiagen, Hilden, Germany).

2.4.3 Fragment Analysis and Data Collection

For analysis of the purified ITS fragments, samples were dried in a Savant Speed Vac (GMI, Inc, Ramsey, MN, USA) for 20 min and resuspended in a mixture of 11.5 µl formamide and 0.5 µl GeneScan^{TM} – 500 ROX^{TM} size standard (Applied Biosystems, Foster City, CA, USA). Samples were denatured at 95^{o}C for 5 min, cooled on ice, and separated by capillary electrophoresis in an ABI 3130x Genetic Analyzer. Data were analyzed in GeneMapper and the T-RFLP fragments, as displayed by electrophoretic peaks, were segregated into size classes of two-base pair increments. Peaks were called at a minimum cutoff of 50 relative fluorescence units to distinguish T-RFLP fragments from background noise. Each position that contained a peak in at least one sample was

recorded as ‘1’ for peak presence and ‘0’ for peak absence. Because each peak represents a specific ITS sequence, the peak profile for each sample represents the structure of the soil fungal community at that sampling location. Like all molecular microbiologic methods, it is possible that T-RFLP will produce certain biases in community composition. For instance, if different species have different gene copy numbers then molecular results will tend to overestimate diversity. However, diversity can also be underestimated if only numerically dominant species, with their relatively very large quantity of DNA, are detected post-PCR

2.4.4 Mantel Tests

Patterns in soil fungal community composition were investigated using the Mantel test, non-metric multidimensional scaling (NMDS), and semi-variogram analysis. The Mantel test is a statistical test of the correlation between two matrices of the same rank. It was used here to investigate spatial changes in the fungal community across several environmental variables including plant type, marsh region, soil organic matter, soil moisture, soil C/N, belowground root mass, aboveground biomass, elevation, and above and belowground C/N. For this application, the binary community data and each environmental variable measured were organized into separate similarity matrices using the Jaccard coefficient,

$$J = a / (a + c + d)$$

(where a is the number of positive matches, (1 and 1), and c and d represent negative matches (1 and 0 or 0 and 1) between a pair of samples) (Colwell and Austin, 1981). The

Jaccard coefficient is valuable because it prevents formation of groups based solely on negative data, relying on the presence of bands rather than absence (Lamboy, 1994). The matrices were then converted into dissimilarity matrices (1-J) and the significance of the correlation between the community matrix and each environmental matrix was tested. Environmental variables that were significantly correlated with relationship strength of +/- 0.350 or higher were merged into the same matrix in order to reduce the overall number of comparisons. This was important because the alpha-value at which Mantel test statistics were evaluated underwent a necessary Bonferroni adjustment with the addition of each environmental variable matrix. Matrices were constructed using the software package SAS v9.0 (Cary, NC, USA).

In the Mantel test, the spatial or temporal autocorrelation between two variables is taken into consideration by computing the relationship between two distance matrices. Mantel is able to account for the presence of underlying spatial autocorrelation in the data in a way that Analysis of Variance (ANOVA) cannot because ANOVA assumes independence of observations and without independence, the estimate of degrees of freedom is exaggerated (Fortin and Gurevitch, 2001). The correlation significance between the two matrices is tested by subjecting the rows and columns of one of the matrices to random permutations and recalculating the correlation coefficient between the matrices after each permutation. The significance of the observed correlation is the proportion of these permutations that lead to a higher correlation coefficient. The higher the number of permutations the more accurate the significance test, with the minimum number of permutations recommended by Manly (1997) as 1000. Mantel tests were performed in Microsoft Excel (Redmond, WA, USA) using the PopTools Add-In and

results were reported as the standardized matrix correlation coefficient, ρ, and its associated p-value.

2.4.5 Non-Metric Multidimensional Scaling

Non-metric multidimensional scaling (NMDS) analysis was performed in the statistical package SAS v9.0 (SAS Institute Inc., Cary, NC, USA). NMDS analysis required the previously constructed dissimilarity matrices created from fungal T-RFLP binary data. The general goal of NMDS is to detect meaningful underlying dimensions that allow the researcher to explain observed similarities or dissimilarities between samples (StatSoft 2003). These dissimilarities are represented visually as distances in plots constructed from n-dimensional eigenvector NMDS solutions. Factor analysis requires that the underlying data are distributed as multivariate normal, and that the relationships are linear. NMDS imposes no such restrictions.

In this study, the pattern of association in fungal communities across marsh region and plant zone was determined from plots constructed with the two-dimensional and three-dimensional eigenvector NMDS solutions. As a non-parametric technique, the NMDS model structure was not specified *a priori* but is instead determined from the data structure.

2.4.6 Geostatistics

Geostatistical analysis of environmental data allows for the determination of the spatial or temporal correlation between observations. One approach, the semivariogram (or, simply, the variogram), was used to integrate the spatial arrangement of soil samples

with the differences in the composition of the fungal community at that sampling location. The variogram is an empirical estimate of the covariance of the observation increment (distance between samples and community structure), defined as half the average squared distance between paired data values, and is formulated:

$$\gamma(h) = \frac{1}{2n} \sum_{i=1}^{n} \left(Z(X_i) - Z(X_i + h) \right)^2$$

where gamma is the semivariogram value plotted on the dependent axis, h is the separation distance between a pair of points (samples), Z(i) is the attribute value at location i, and n is the number of pairs with the specified separation distance, also referred to at the lag distance (Isaaks and Srivastava, 1989). Variograms representing fungal abundance in the low, mid and high marsh regions were constructed in the software package GEOEAS (EPA, Washington, DC, USA). Relative dissimilarity values, calculated from the Jaccard similarity matrices, were used for fungal community variogram construction rather than the GEOEAS program because multivariate binary data have been discovered to be inappropriate for creating variograms in the traditional sense (Franklin and Mills, 2003; Lilleskov *et al.*, 2004; Mackas, 1984; Mummey and Stahl, 2003). Prior to constructing the variograms, the appropriate lag distance (distance within which pairs are taken) was determined for all pairs of sampling points and binning was used to separate the sample pairs into groups depending on the appropriate lag distance. Sturge's rules was applied to determine the number of bins (Legendre and Legendre, 1998), and lag distance was calculated as the maximum pair distance divided by 2 and then subdivided by the number of bins required (Englund and Sparks, 1991). Thus the 40 samples for each transect were organized into 10 bins with a lag distance of

approximately 2-m. A covariance variogram was used rather than the standard variogram because the former is more resistant to erratic values as it accounts for the lag means and the lag values (Isaaks and Srivastava, 1989). The last third of each variogram was removed, as variograms are generally not valid beyond this estimate.

The variogram was then used to fit a model of spatial autocorrelation structure in the microbial communities of the low, mid and high marsh sampling transects. Ranges, sills, nuggets, and the appropriate geostatistical model were identified for each variogram. The range is the distance at which the variogram plateaus, indicating that the distance has been reached where an increase in the separation distance no longer causes a corresponding increase in the average squared difference between pairs of values. Fungal community patch-size is indicated by the range value on the relative genetic dissimilarity variograms, and can be equated to the scale at which significant (alpha = 0.05) fungal community composition changes no longer take place along the sampling transect (Franklin *et al.*, 2002). The sill is the plateau in variance (or relative genetic dissimilarity) that the variogram reaches at its range. The nugget effect is the discontinuity often seen at the origin of the variogram and is due to factors such as sampling error or short scale variability. A nugget effect indicates that sample values separated by extremely small distances are quite dissimilar (Isaaks and Srivastava, 1989). Common models for variogram analysis include exponential, Gaussian, linear, and pure nugget effect.

CHAPTER 3: RESULTS

3.1 **Soil Microbial Abundance**

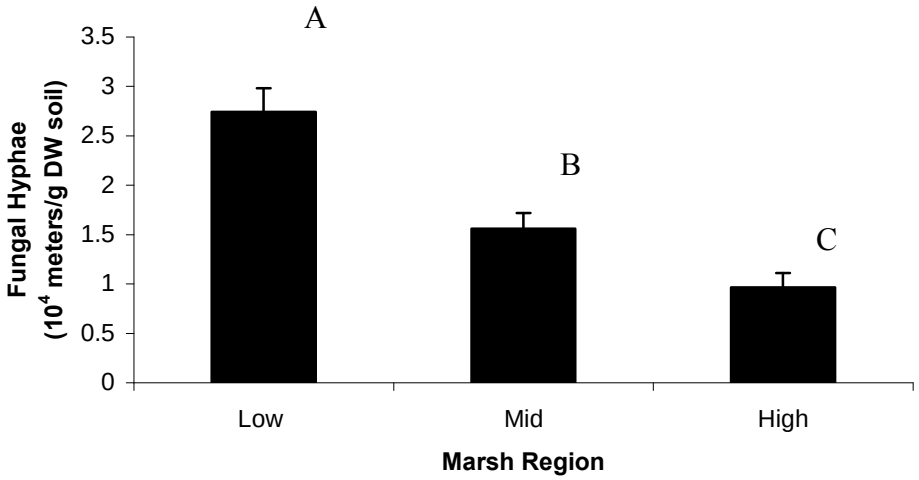
Salt marsh soil fungal abundance varied significantly across marsh region and vegetation zone during both the August 2004 and July 2005 sampling events. Recall that total microbial counts were obtained using the Acridine Orange stain and thus include both live and dead microorganisms, while live counts were performed using the enzymatically hydrolyzed FDA stain. Henceforth, total fungal hyphal lengths will simply be referred to as fungal abundance, while cytoplasm-filled hyphal lengths will be referred to as live hyphae or live fungal abundance.

3.1.1 Total Fungal Abundance

AODC results from both 2004 and 2005 displayed a greater abundance of soil fungal hyphae in the *Juncus roemerianus* zone rather than in the *S. patens/D. spicata* zone. Across zones with different tidal inundation frequency, the greatest hyphal abundance occurred in the low, or low and mid, marsh regions. The August 2004 data revealed a difference in average hyphal length of up to 1.2×10^4 m g^{-1} dry weight (DW) of soil between vegetation types (zones), and up to 1.8×10^4 m g^{-1} DW soil across marsh regions (Fig. 3.1). There was a significant main effect for both marsh region, $F(2, 57) = 23.54$, $p < 0.05$ and plant zone, $F(1, 58) = 5.04$, $p < 0.05$. Post ANOVA comparisons displayed a significant difference between all marsh regions and between all plant zones when main effects were observed separately. However, analyzing data for comparisons between each region x zone combination deemed the main effects inconclusive by presenting a significant interaction term $F(3, 57) = 3.49$, $p < 0.05$ (Table 3.1). Fungal abundance in the low marsh *J. roemerianus* zone was significantly different from all

Figure 3.1. Soil fungal abundance data from August 2004 was grouped according to marsh region (a) and vegetation zone (b). Pair-wise comparisons were performed and bars with letters in common are not significantly different. Error bars represent +1 standard error.

(a)



(b)

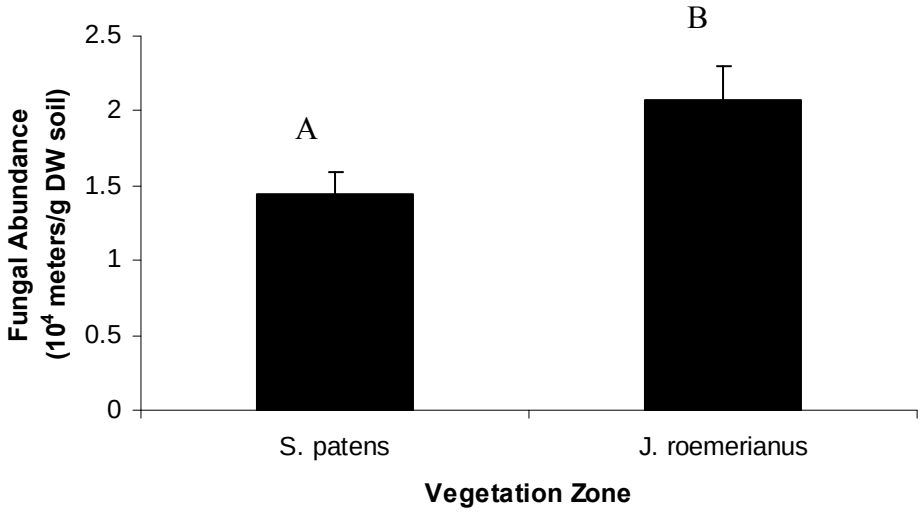


Figure 3.2. Soil fungal abundance from August 2004 cores was compared between each marsh region-plant zone combination. Bars with letters in common are not significantly different. Error bars represent +1 standard error.

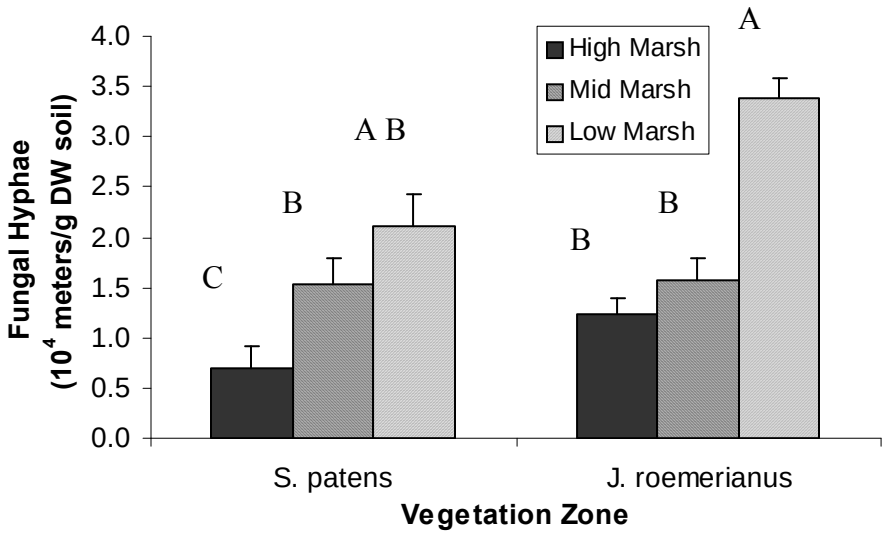


Table 3.1. Fungal abundance ANOVA results for August 2004 sampling. Main effects of marsh region and plant zone were tested in SAS proc GLM, and the interaction effect of region x zone was tested in proc MIXED. Significance (*) is indicated at the 95% confidence level.

Source	DF	Type III SS	Mean Squared Error	F Value	Pr>F
region	2	3.27E+09	1.63E+09	23.54	<.0001*
zone	1	5.77E+08	5.77E+08	5.04	0.0287*
region*zone	2	-	-	3.49	0.0375*

other sampling locations excluding the low marsh *S. patens*. No other sites differed significantly from one another (Fig. 3.2).

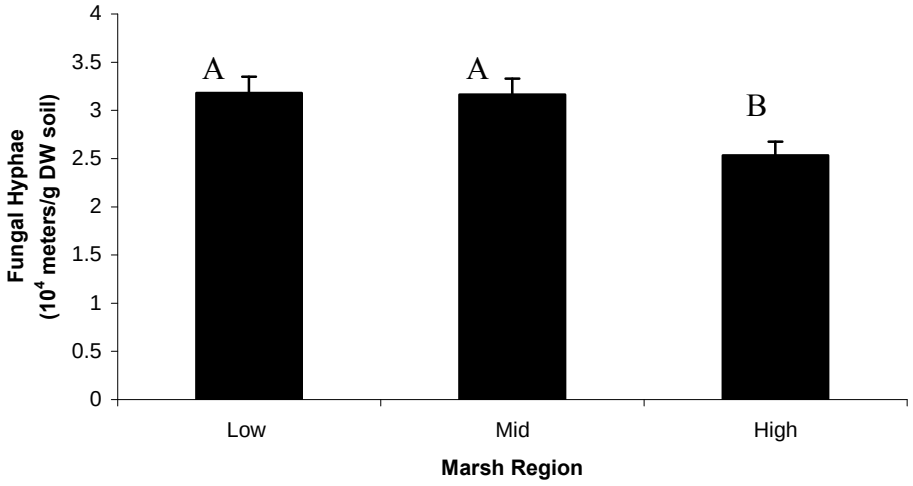
Further sampling in July 2005 supported the August 2004 results and revealed the abundance patterns in the transitional area (between the two distinct vegetative zones) to be intermediate to the values seen in the monotypic patches (Fig. 3.3). In 2005, there was a significant main effect for both marsh region, $F(2, 183) = 5.49$, $p < 0.05$, and plant zone $F(2, 183) = 16.62$, $p < 0.05$. Abundance varied significantly between the *J. roemerianus*, the transitional, and the *S. patens/D. spicata* soils, with a trend of decreasing abundance. A significant difference was seen between fungal abundance in the low and high marsh regions, but the mid marsh did not differ significantly from the low marsh (Fig. 3.3). Again, Tukey's comparison (a procedure that is particularly conservative for unequal samples sizes, as is the 2005 data) revealed the presence of an interaction between the independent variables region and zone, $F(4, 181) = 2.41$, $p < 0.05$. Significant differences in soil fungal abundance were seen only between the low marsh *J. roemerianus* samples and all *S. patens* samples (low, mid and high marsh regions) and between the low marsh *J. roemerianus* and the high marsh *J. roemerianus* samples (Fig. 3.4).

To allow fungal abundance patterns along each transect to be compared, abundance was plotted against the sample's position along the transect in each marsh region (Fig. 3.5). There is a general decrease in abundance along each transect: higher numbers were associated with *J. roemerianus,* and decreased along the length of the transect to its end in 3m into the *S. patens* community. This trend is strongest in the low marsh ($R^2 = 0.614$), intermediate in the mid marsh ($R^2 = 0.315$), and weakest in the high

marsh (R^2 = 0.002) (Fig. 3.5). These values are derived from the heavily sampled transects of 2005 – H1, M2 and L3. The six remaining transects, which were sampled less intensely, exhibit the same pattern as the more intensely sampled transects; higher soil fungal abundance is generally observed in the *J. roemerianus* vegetation zone (Fig. 3.6).

Figure 3.3. Soil fungal abundance data from July 2005 was grouped according to marsh region (a) and vegetation zone (b). Pair-wise comparisons were performed and bars with letters in common are not significantly different. Error bars represent +1 standard error.

(a)



(b)

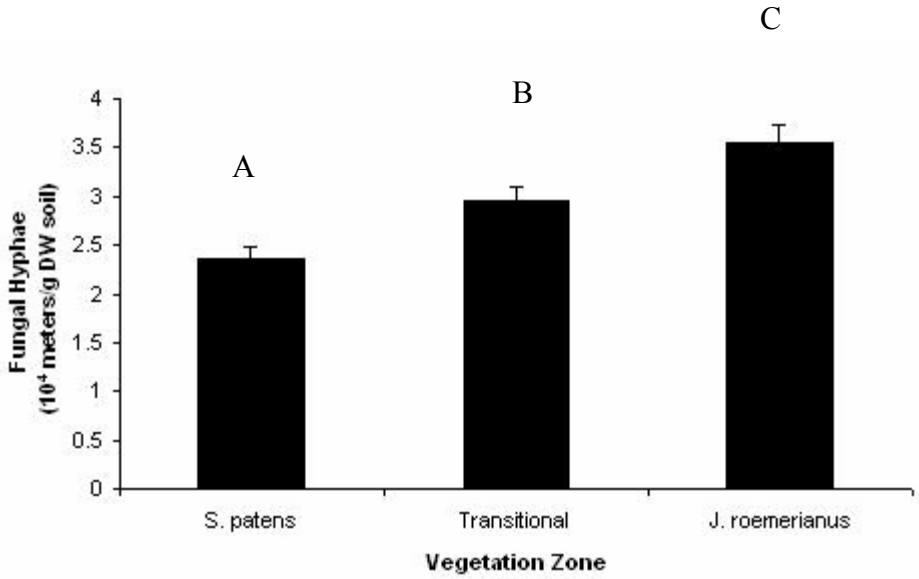


Figure 3.4. Soil fungal abundance from July 2005 cores was compared between each marsh region-plant zone combination. Bars with letters in common are not significantly different. Error bars represent +1 standard error.

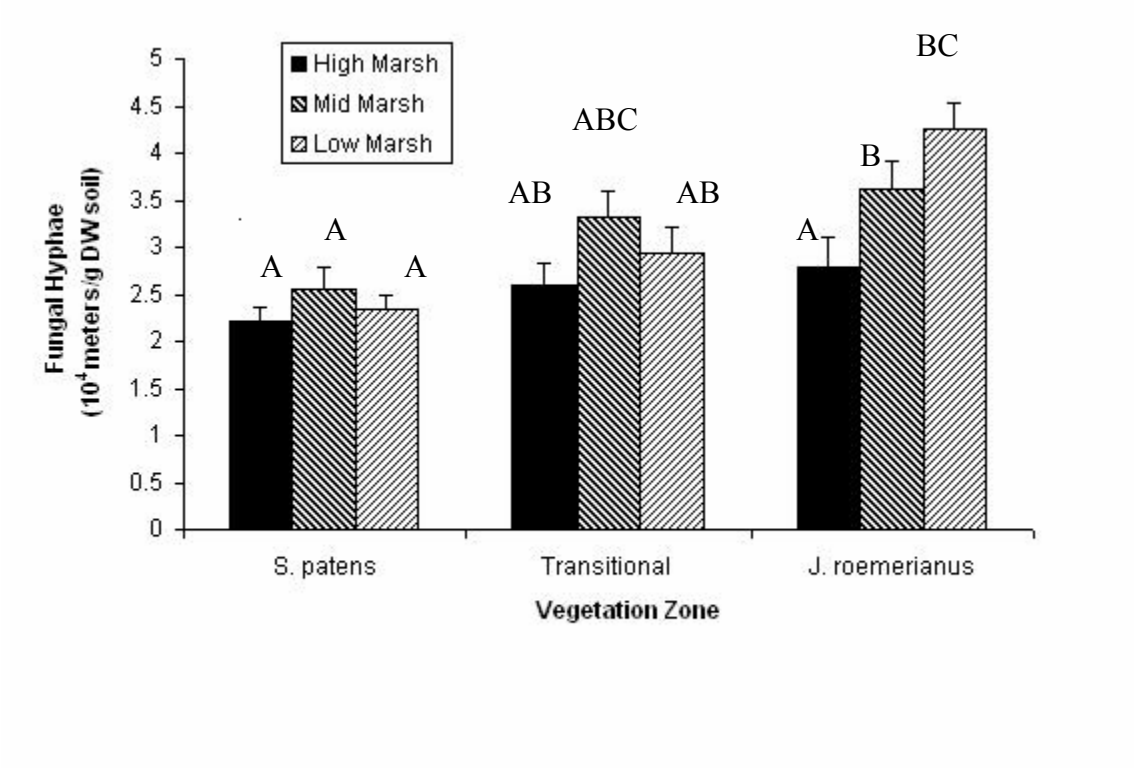
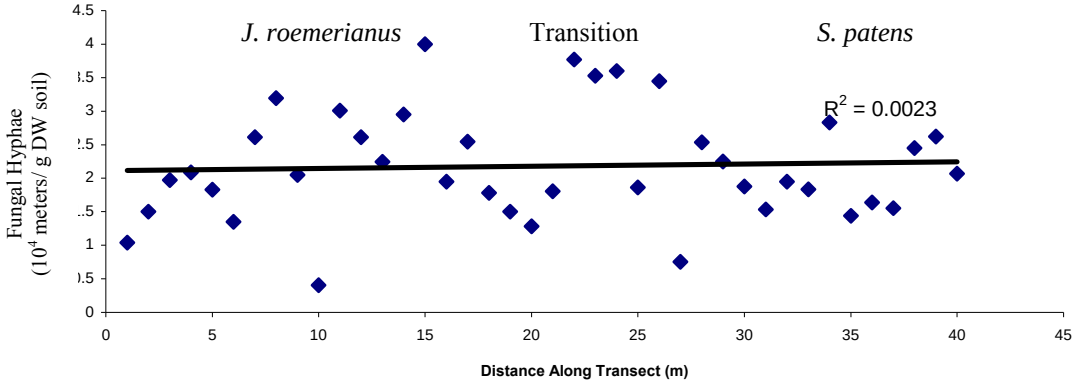


Table 3.2. Fungal abundance ANOVA results for July 2005 sampling. Main effects of marsh region and plant zone were tested in SAS proc GLM, and the interaction effect of region x zone was tested in proc MIXED. Significance (*) is indicated at the 95% confidence level.

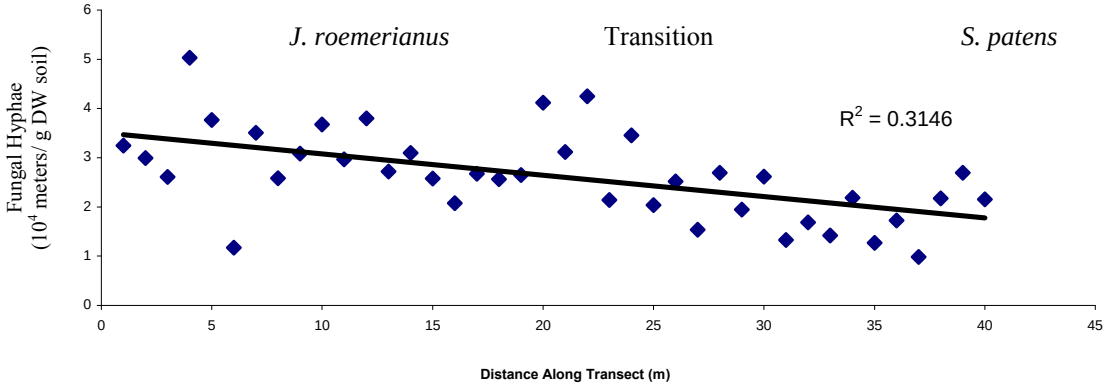
Source	DF	Type III SS	Mean Squared Error	F Value	Pr>F
region	2	1.79E+09	8.96E+08	5.49	0.0048*
zone	2	4.89E+09	2.44E+09	16.62	<.0001*
region*zone	4	-	-	2.41	0.0508

Figure 3.5. Scatter plots with trend lines were used to examine the strength of the relationship between plant zone and soil fungal abundance in each marsh region, with distance between samples as the continuous variable. Samples were collected in July 2005, and each point represents one soil core.

(a) High Marsh



(b) Mid Marsh



(c) Low Marsh

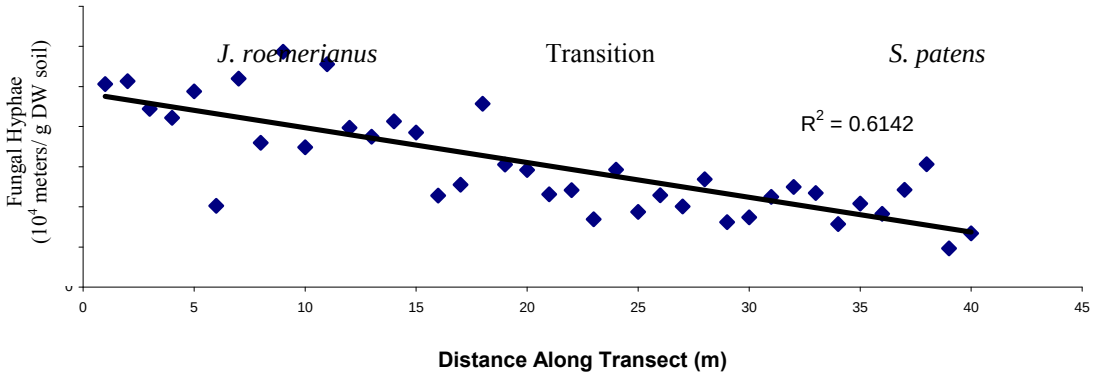
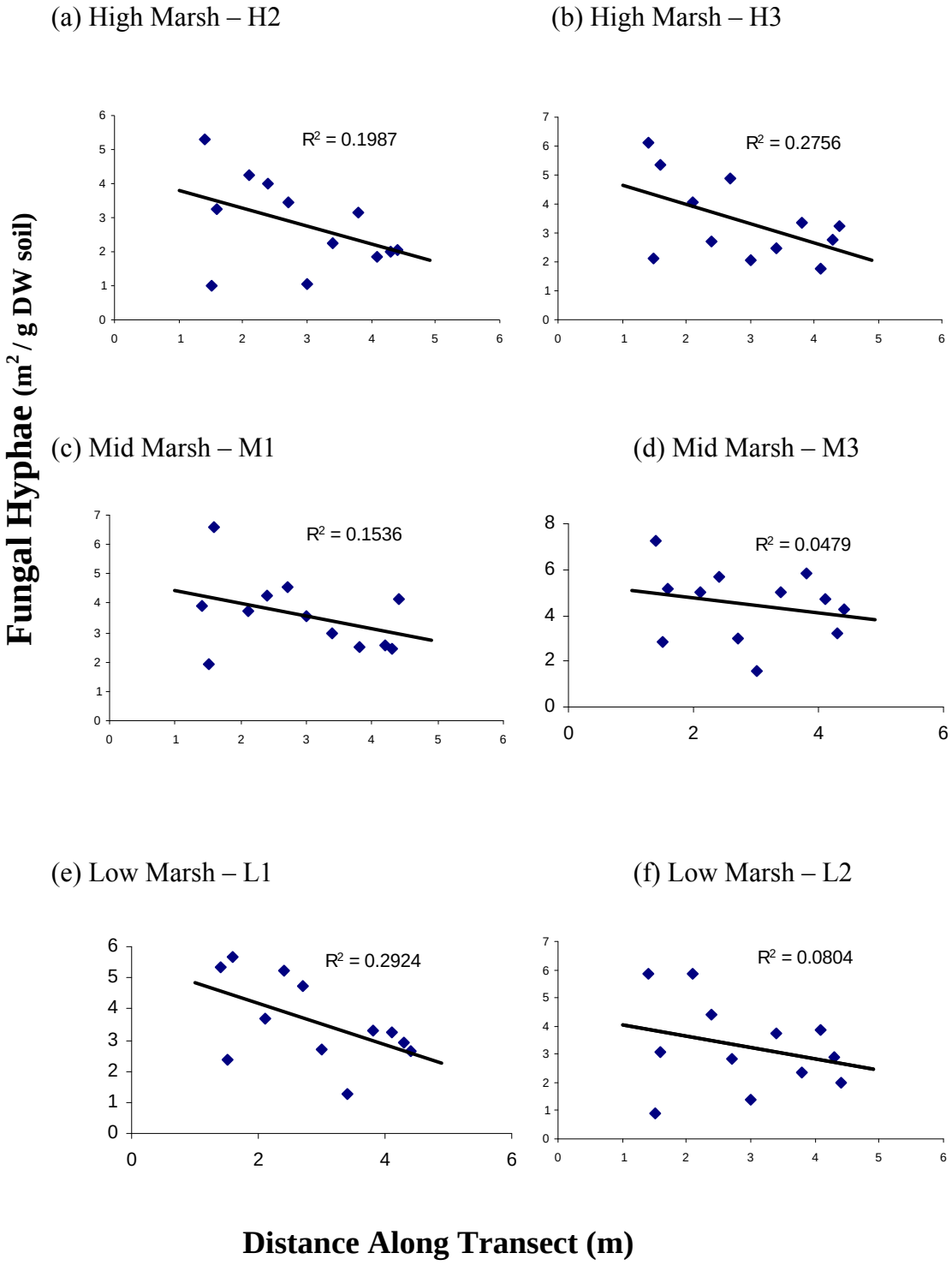


Figure 3.6. – Scatter plots with trend lines were also used to examine the strength of the relationship between plant zone and soil fungal abundance along replicate transects. Samples were collected in July 2005, and each point represents one soil core.



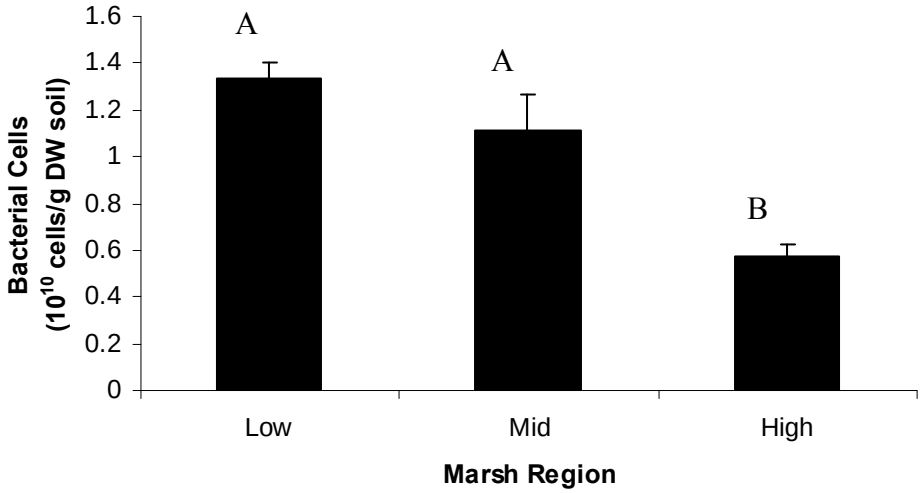
3.1.2 Total Bacterial Abundance

Bacterial abundance patterns were strikingly similar to fungal abundance across both marsh region and vegetation zone. In 2004, bacterial abundance was greatest in the low marsh (x = 1.33 x 10^{10} cells g^{-1} DW soil) and lowest in the high marsh (x = 5.71 x 10^{9} cells g^{-1} DW soil). Abundance in the low and mid marsh regions was significantly different from the high marsh (Fig. 3.7a). Abundance was significantly different across plant zones and averaged 8.44 x 10^{9} cells g^{-1} DW soil in the *S. patens*, and 1.16 x 10^{10} cells g^{-1} DW in the *J. roemerianus* (Fig. 3.7b). There was both a significant region effect $F(2, 57) = 34.96$, $p < 0.05$, and a significant plant effect $F(1, 58) = 4.86$, $p < 0.05$, but no clear region x zone interaction $F(2, 58) = 0.82$, $p < 0.05$ (Table 3.3). The high marsh *S. patens* was significantly different from all other sites (Fig. 3.8).

In 2005, soil bacterial abundance also was lowest in the high marsh at 1.10 x10^{10} cells g^{-1} DW soil and differed significantly across all marsh regions (Fig. 3.9). The transitional zone showed intermediate abundance between the two distinct plant zones (Fig. 3.9). There were very strong region and zone effects at $F(2, 183) = 23.89$, $p < 0.05$ and $F(2, 183) = 30.43$, $p < 0.05$, respectively. The interaction term was significant at $F(4, 181) = 3.25$, $p < 0.05$ (Fig. 3.10, Table 3.4).

Figure 3.7. Soil bacterial abundance data from August 2004 was grouped according to marsh region (a) and vegetation zone (b). Pair-wise comparisons were performed and bars with letters in common are not significantly different. Error bars represent +1 standard error.

(a)



(b)

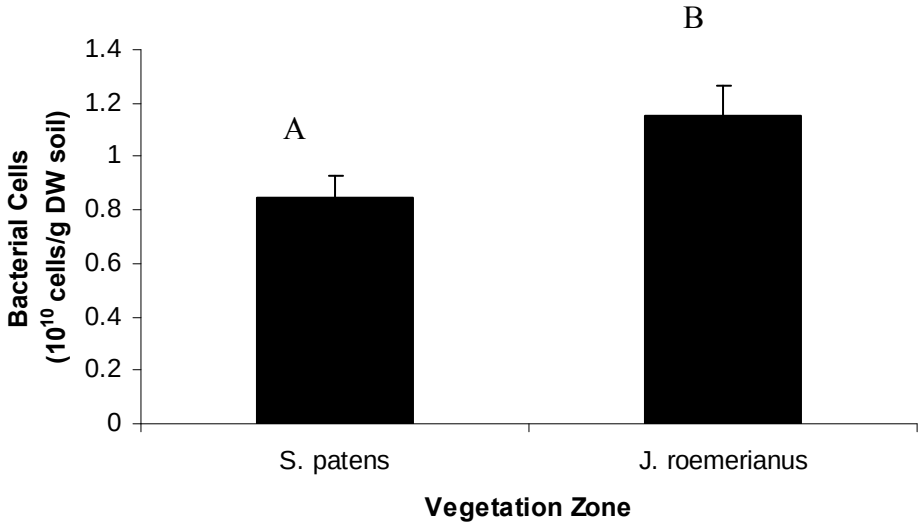


Figure 3.8. Soil bacterial abundance data from August 2004 was compared between each marsh region-plant zone combination. Bars with letters in common are not significantly different. Error bars represent +1 standard error.

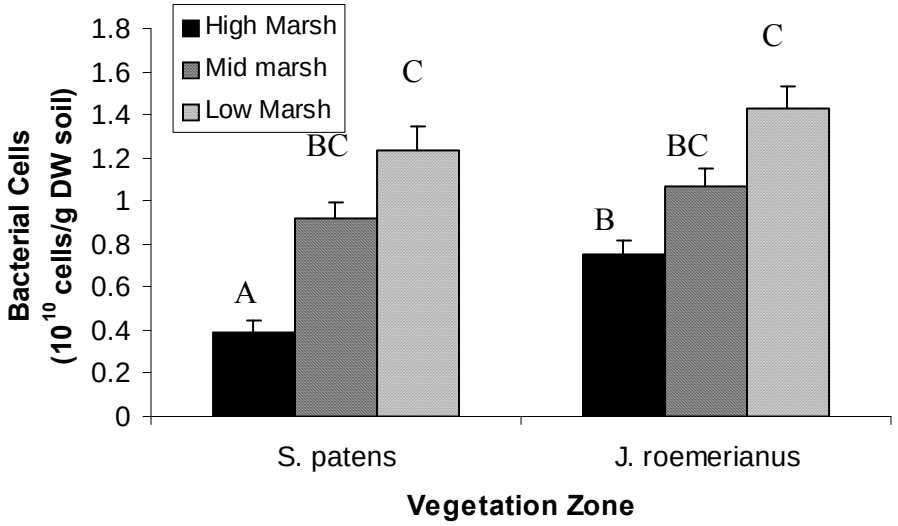
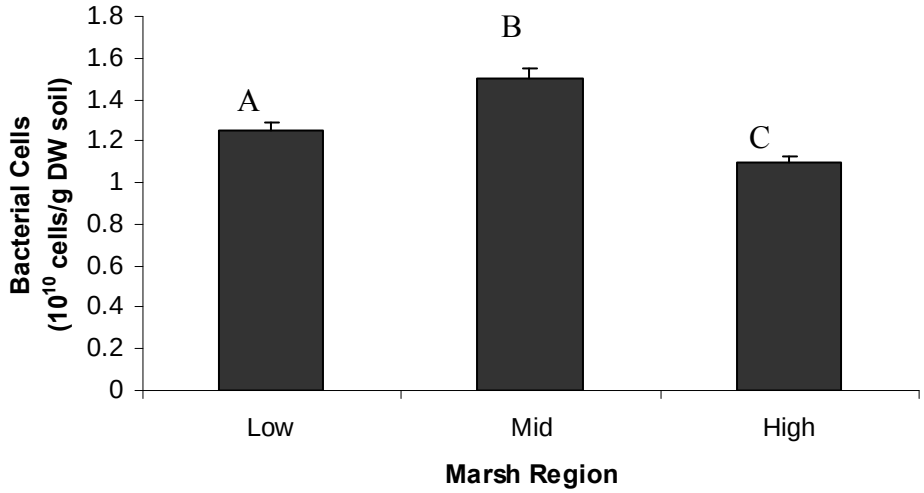


Table 3.3. Bacterial abundance ANOVA results for August 2004 sampling. Main effects of marsh region and plant zone were tested in SAS proc GLM, and the interaction effect of region x zone was tested in proc MIXED. Significance (*) is indicated at the 95% confidence level.

Source	DF	Type III SS	Mean Squared Error	F Value	Pr>F
region	2	5.81E+20	2.91E+20	34.96	<.0001*
zone	1	8.16E+19	8.16E+19	4.86	0.0314*
region*zone	2	-	-	0.82	0.4479

Figure 3.9. Soil bacterial abundance data from July 2005 was grouped according to marsh region (a) and vegetation zone (b). Pair-wise comparisons were performed and bars with letters in common are not significantly different. Error bars represent +1 standard error.

(a)



(b)

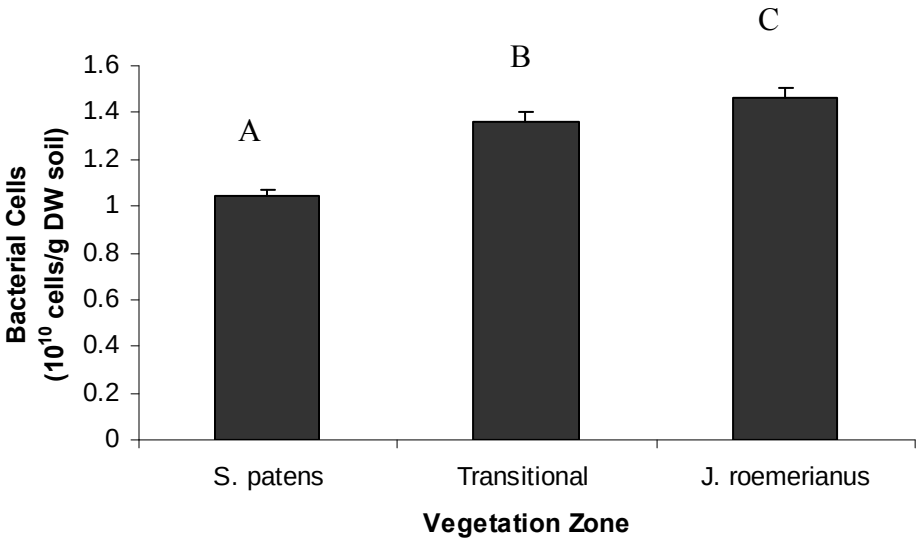


Figure 3.10. Soil bacterial abundance data from July 2005 was compared between each marsh region-plant zone combination. Bars with letters in common are not significantly different. Error bars represent +1 standard error.

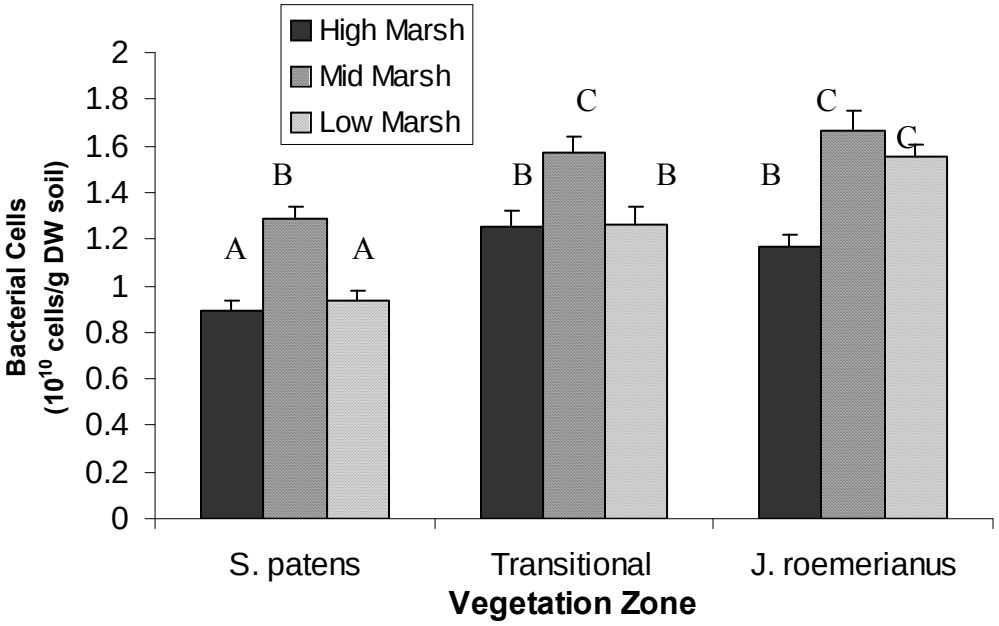


Table 3.4. Bacterial abundance ANOVA results for July 2005 sampling. Main effects of marsh region and plant zone were tested in SAS proc GLM, and the interaction effect of region x zone was tested in proc MIXED. Significance (*) is indicated at the 95% confidence level.

Source	DF	Type III SS	Mean Squared Error	F Value	Pr>F
region	2	5.47E+20	2.74E+20	23.89	<.0001*
zone	2	6.61E+20	3.30E+20	30.43	<.0001*
region*zone	4	-	-	3.25	0.0132*

3.1.3 Total Microbial Biovolume and Total Microbial Carbon Estimates

To allow direct comparison of fungal abundance with bacterial abundance, total soil microbial biovolume estimates were derived from abundance data (Fig. 3.11). Both the 2004 and the 2005 data sets revealed strong positive correlations between fungal and bacterial biovolume at $r(58) = 0.601$, $p > 0.05$, and $r(190) = 0.425$, $p < 0.05$, respectively. Conversion from microbial biovolume to microbial carbon showed a fungal contribution to total soil microbial carbon between 91.7 and 98.4 percent. (Fig. 3.12, Table 3.5). Comparison of total bacterial biovolume with live fungal biovolume revealed no relationship: $r(45) = 0.002$ (data not shown).

3.1.4 Live Fungal Abundance

Figure 3.13 displays average live fungal abundance across the *S. patens/ J. roemerianus* vegetation gradient, and between the three marsh regions. Along the transects, live fungal abundance showed a very different pattern than total fungal abundance, with less live fungi in the transition than in either the *S. patens* or *J. roemerianus* zones. However, when results were compared between each marsh region – plant zone combination, live fungal abundance was greatest in the mid marsh *S. patens* ($x = 3.6 \times 10^3$ m^{-1} g DW soil) and lowest in the low marsh *J. roemerianus* ($x = 1.38 \times 10^3$ m^{-1} g DW soil).

One-way ANOVA's for the main effects of marsh region and vegetation zone revealed a significant region effect, $F(2, 24) = 0.33$, $p < 0.05$, but no significant effect of plant zone, $F(2, 24) = 16.18$, $p < 0.05$. The interaction of region x zone was not significant (Table 3.6). Separate post-ANOVA comparisons of main effects indicated

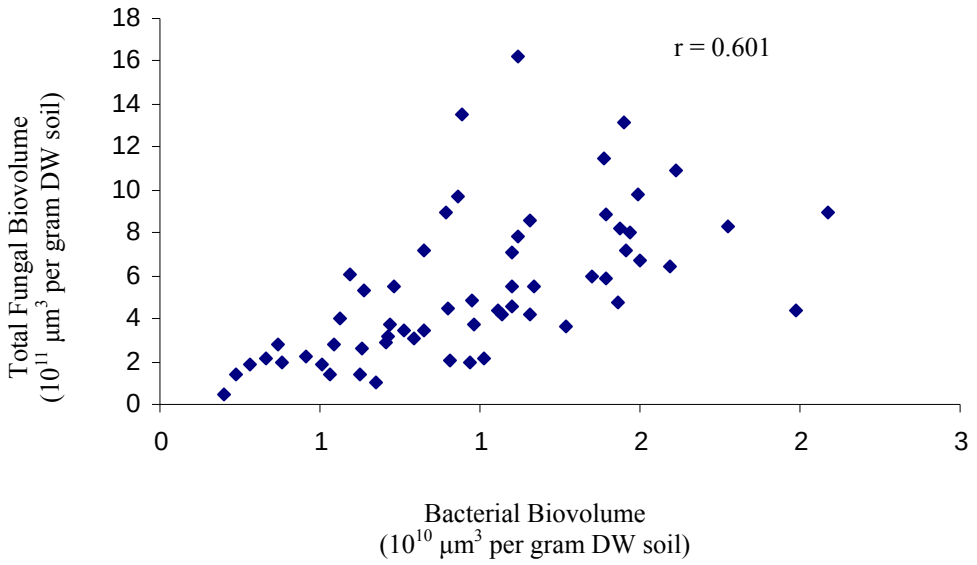
that the mid marsh had significantly more live fungi than the low or high marsh regions, and that there was no significant difference in live fungal abundance between plant zones (Fig. 3.14). Post ANOVA comparisons of live fungal abundance between each marsh region-plant zone combination confirmed that the mid marsh *S. patens* samples were significantly different from five other sample categories, including high marsh *S. patens* and transition, and low marsh *S. patens*, transition, and *J. roemerianus* (Fig. 3.3). Additionally, abundance data was more variable for live fungal abundance than for total fungal abundance, with standard error estimates ranging between 0.0337 and 0.598 with an average of 0.309 for live fungi, and a range of 0.143 - 0.309 and average 0.245 for total fungi.

3.1.5 Proportion Live Fungi

Live fungi as a proportion of total soil fungal abundance ranged between 0.064 and 0.261, and showed a pattern of general decrease from the higher to the lower marsh. The one obvious exception is the mid marsh *S. patens*, which demonstrated an average proportion of 0.261 total fungi as live. The proportion was always lower in the transition zone and there was no consistent pattern of higher proportion in *S. patens* than *J. roemerianus*. Additionally, there were no significantly different sampling locations at the 95% confidence level (Fig. 3.16).

Figure 3.11. Bacterial and fungal biovolume per was estimated from direct abundance counts and samples were plotted to determine correlation coefficients for August 2004 (a) and July 2005 (b).

(a) August 2004



b) July 2005

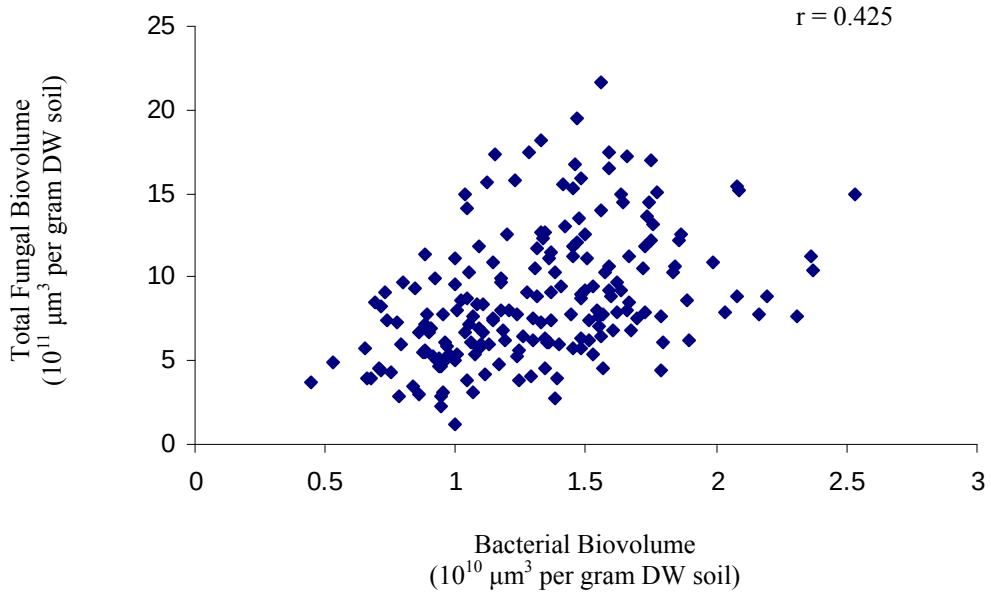
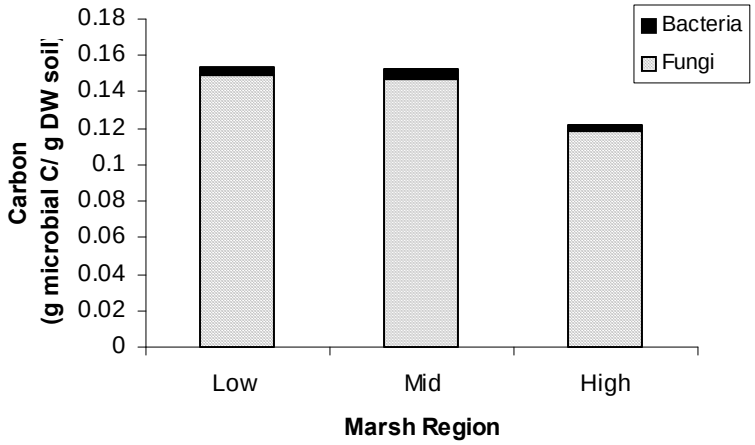


Figure 3.12. Contribution of bacterial and fungal carbon to the total microbial carbon of each soil sample was empirically determined and grouped according to marsh region (a) and vegetation zone (b).

(a)



(b)

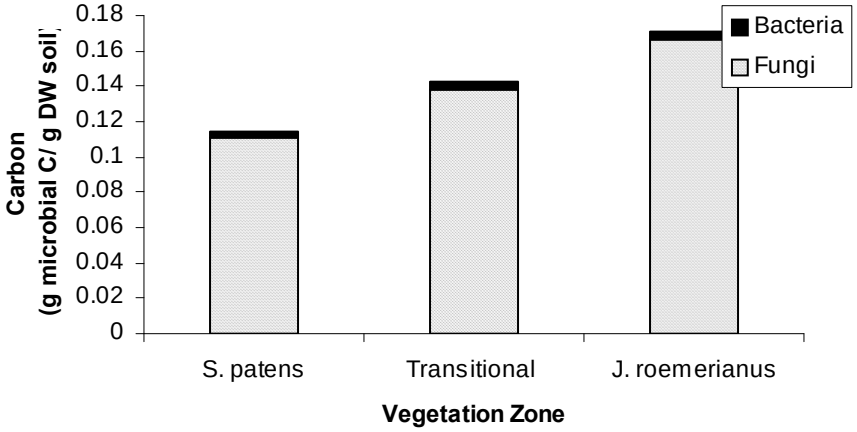


Table 3.5. Fungal carbon content of soil samples as a percent of total microbial carbon.

Site	Total Microbial Carbon	Percent Fungal Carbon
S. patens	0.1145263	96.70%
Transitional	0.1427135	96.53%
J. roemerianus	0.1717085	96.90%
Low	0.15408788	97.06%
Mid	0.1527662	96.42%
High	0.1221607	96.74%

Figure 3.13. Live fungal data collected at five points along the 6 m cross-vegetation transect. Each point represents the average of three soil cores, one from each transect in that marsh region. Error bars represent + and – 1 standard error.

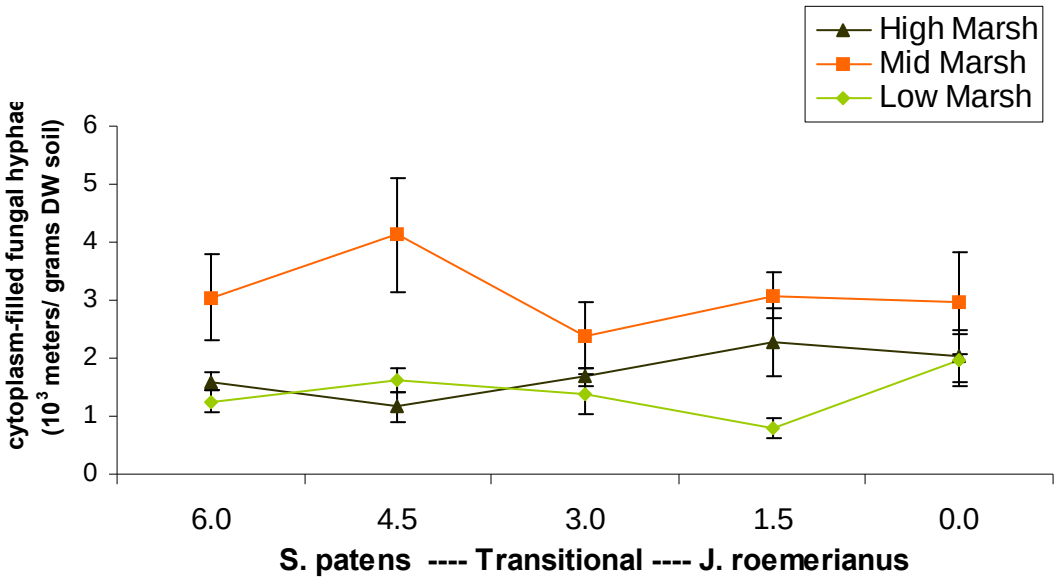
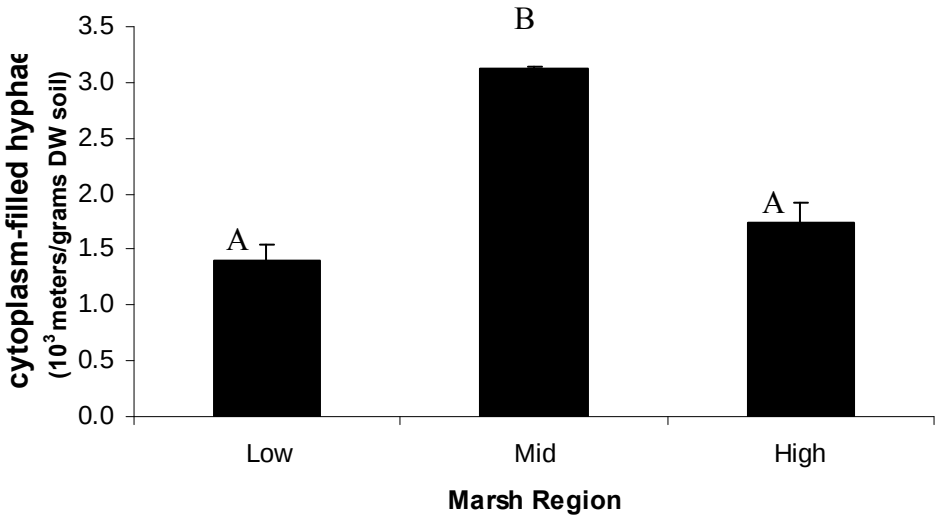


Figure 3.14. Main effects of marsh region (a) and vegetation zone (b) on cytoplasm-filled (live) fungal abundance. Bars with letters in common are not significantly different. Error bars represent + 1 standard error.

(a)



(b)

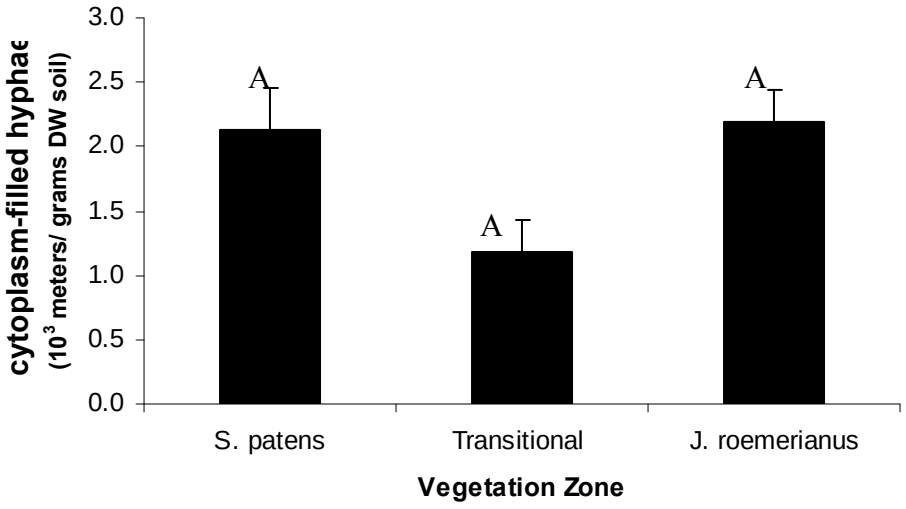


Figure 3.15. Cytoplasm-filled (live) fungal abundance results were compared between each marsh region-plant zone combination. Bars with letters in common are not significantly different. Error bars represent +1 standard error.

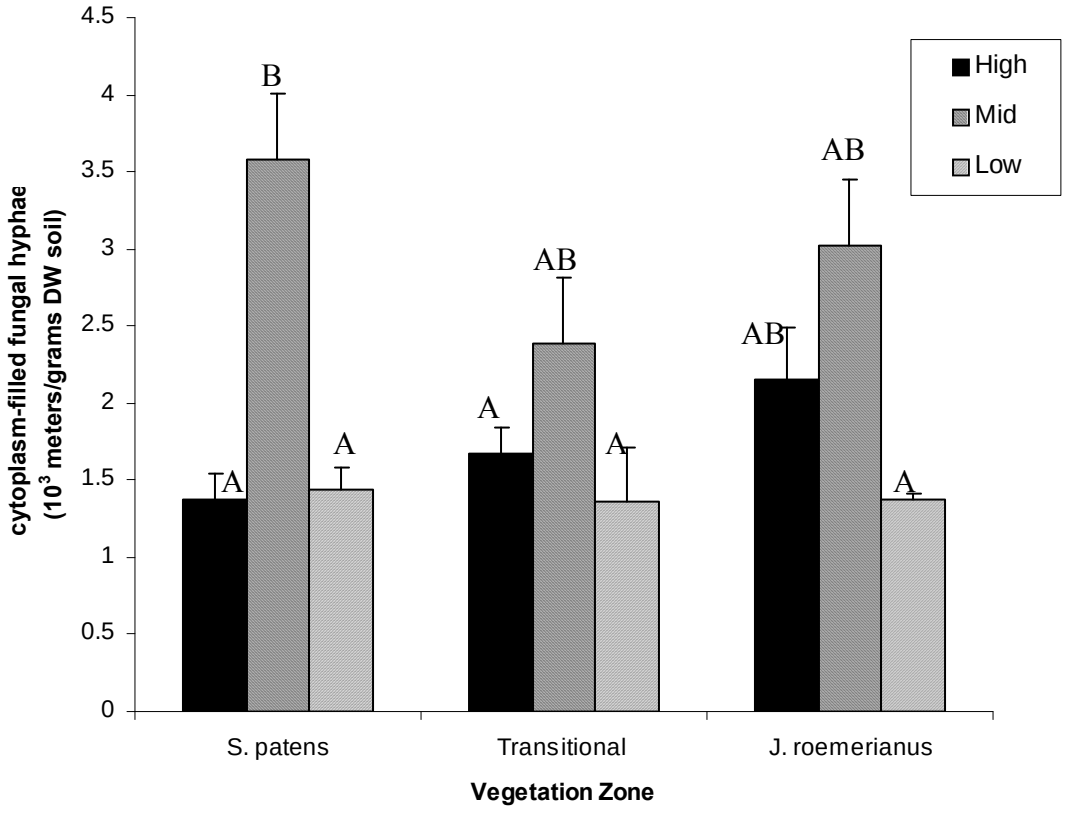
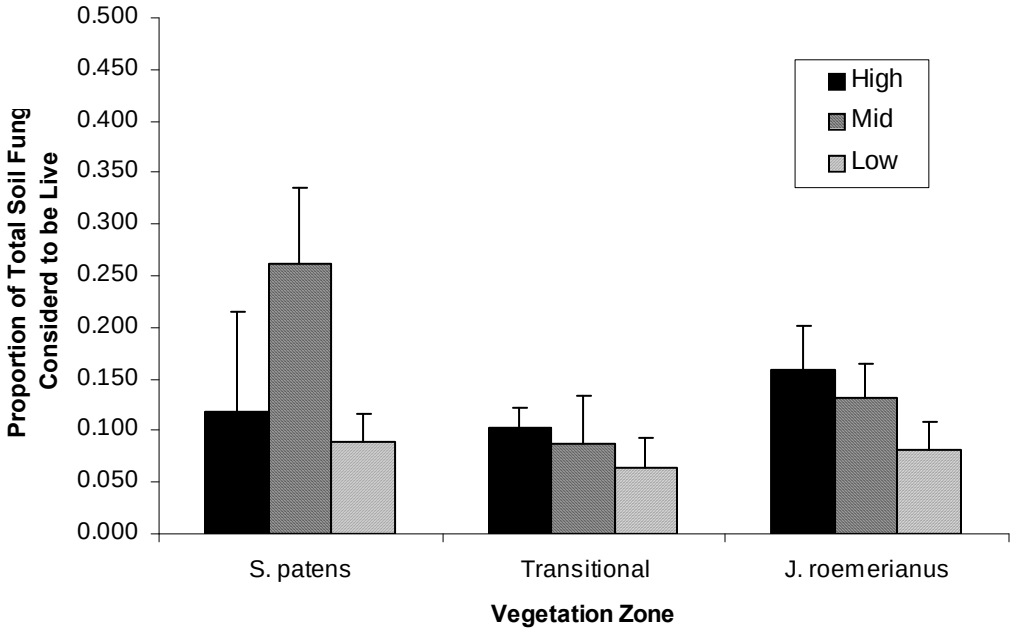


Table 3.6. ANOVA results for cytoplasm-filled fungal hyphae. Main effects of marsh region and plant zone were tested in SAS proc GLM, and the interaction effect of region x zone was tested in proc MIXED. Significance (*) is indicated at the 95% confidence level.

Source	DF	Type III SS	Mean Squared Error	F Value	Pr>F
region	2	8.87E+08	4.43E+08	0.33	0.7185
zone	2	2.47E+10	1.24E+10	16.18	<.0001*
region*zone	4	-	-	1.28	0.2961

Figure 3.16. Proportion of total soil fungi that were live. No bars were significantly different at the 95% confidence level. Error bars represent +1 standard error.



3.2 Environmental Influences

In marsh soil samples, total fungal abundance was correlated with total bacterial cell counts, as well as with several of the abiotic parameters measured. Live fungal abundance was significantly correlated with soil moisture and soil organic matter. MANOVA tables (Table 3.15 and 3.16) and Pearson's Correlation tables (Table 3.17 and 3.19) summarize these findings.

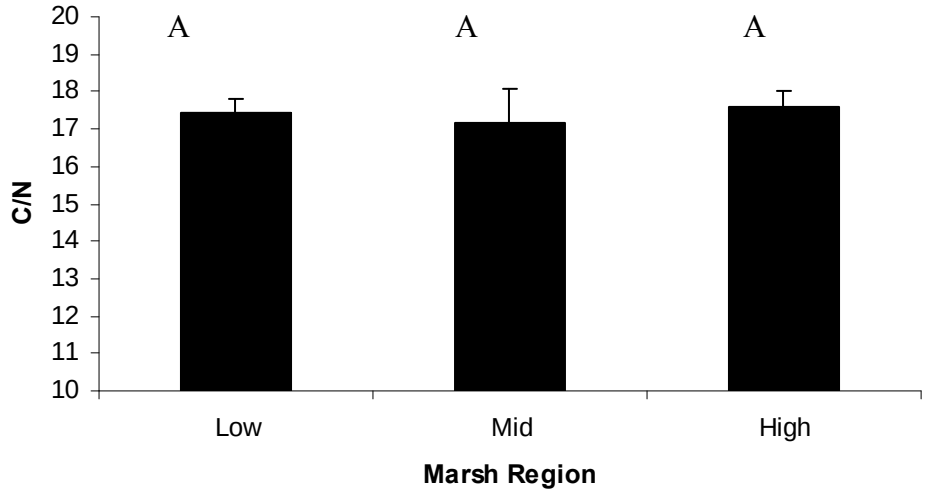
3.2.1 Soil Characteristics

In 2004, there was a significant plant effect $F(1, 58) = 4.38$, $p < 0.05$, but no marsh region effect, $F(2, 57) = 0.14$, $p< 0.05$, on soil C/N. The *S. patens* soil had the highest carbon to nitrogen ratio, averaging 18.1 while *J. roemerianus* soil averaged 16.7 (Fig. 3.17). Similar results were obtained in 2005. The transitional zone yielded soil C/N that was intermediate to *S. patens* and *J. roemerianus* soils (Fig. 3.18). A region x zone interaction was present in both 2004 and 2005 (Tables 3.7 and 3.8).

Soil fungal abundance was plotted against three measured soil properties for all samples cores. There was a weak, positive correlation between fungal abundance and soil moisture: $r(190) = 0.3894$, $p < 0.05$, but no correlation was detected between abundance and either soil organic matter or soil C/N (Fig. 3.19).

Figure 3.17. Soil C/N data from August 2004 cores were grouped according to marsh region (a) and vegetation zone (b). Pair-wise comparisons were performed and bars with letters in common are not significantly different. Error bars represent +1 standard error.

(a)



(b)

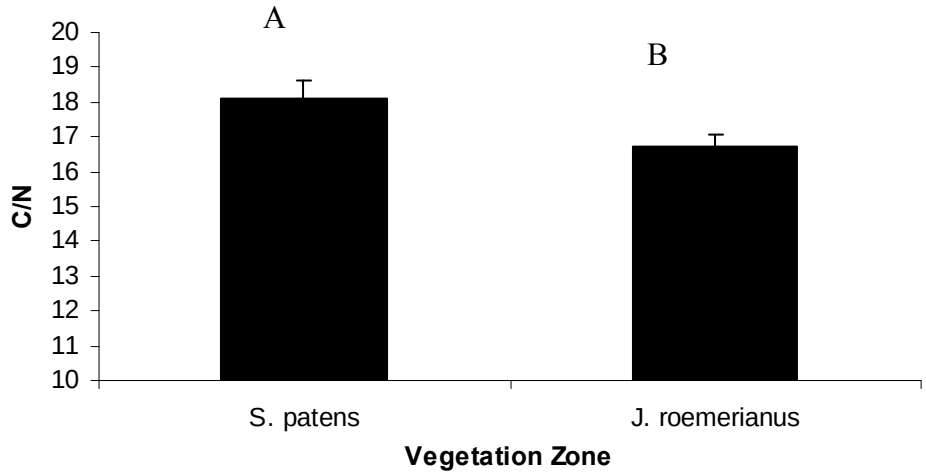
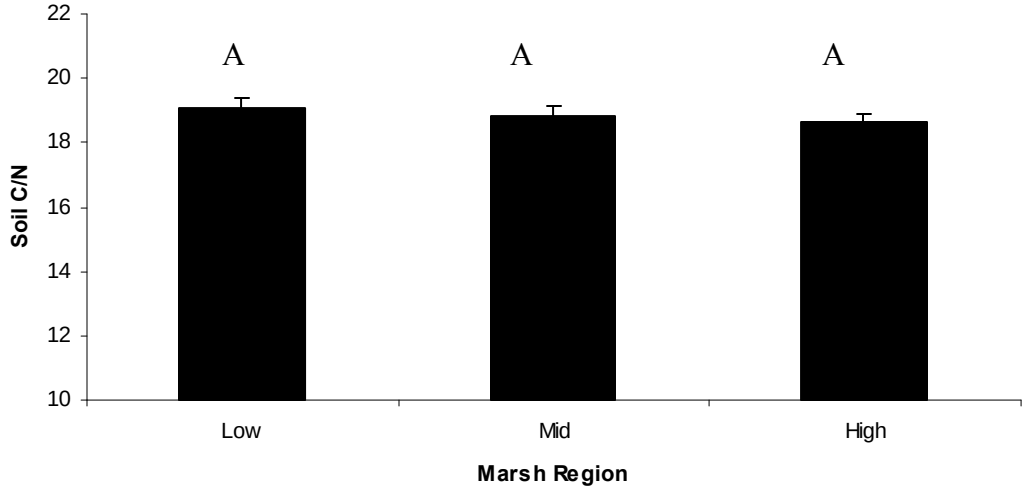


Table 3.7. ANOVA results for soil C/N in August 2004. Significance (*) is indicated at the 95% confidence level.

Source	DF	Type III SS	Mean Squared Error	F Value	Pr>F
region	2	2.06	1.03	0.14	0.8677
zone	1	29.09	29.09	4.38	0.0408*
region*zone	2	-	-	12.76	<.0001*

Figure 3.18. Soil C/N data from July 2005 cores were grouped according to marsh region (a) and vegetation zone (b). Pair-wise comparisons were performed and bars with letters in common are not significantly different. Error bars represent +1 standard error.

(a)



(b)

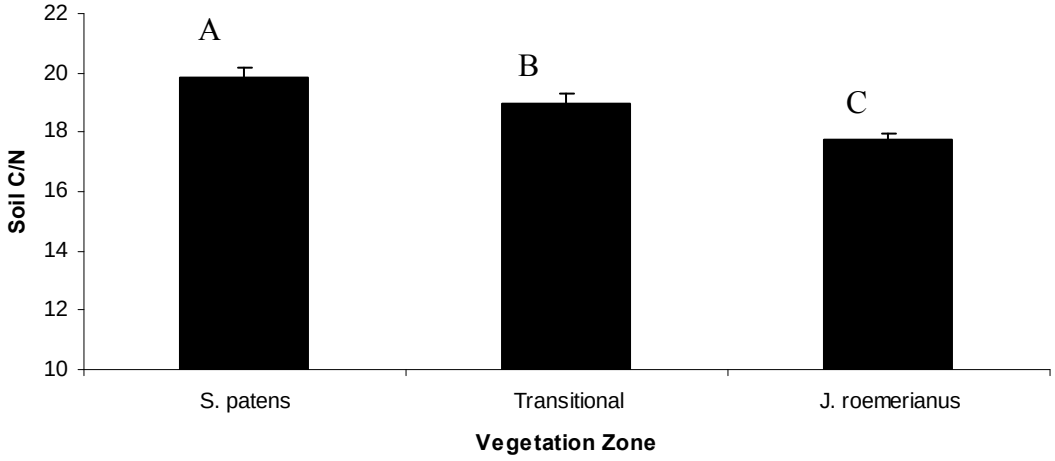
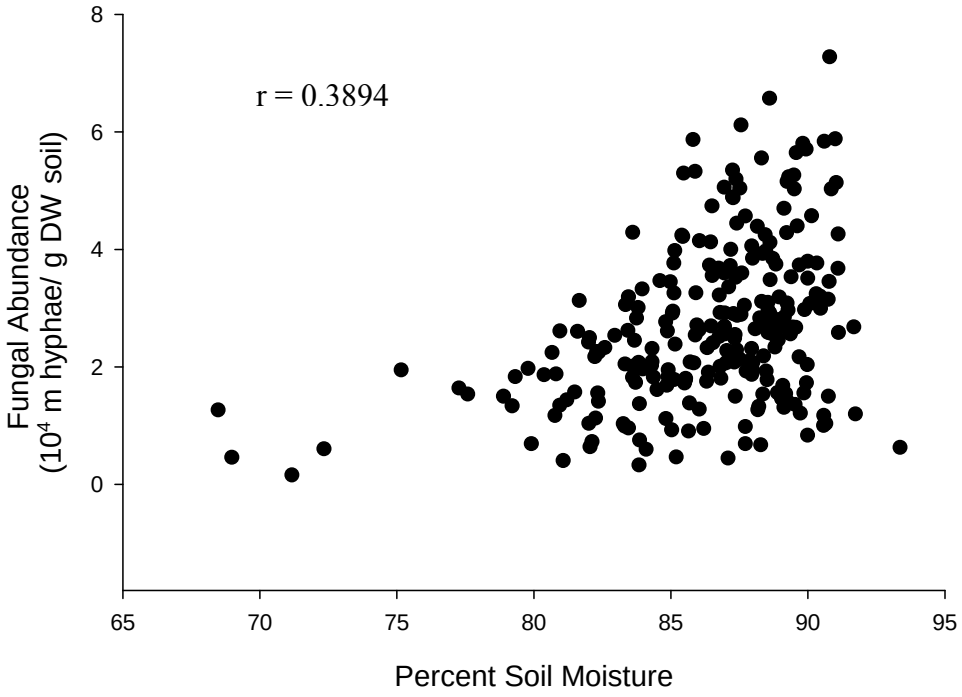


Table 3.8. ANOVA results for soil C/N in July 2005. Significance (*) is indicated at the 95% confidence level.

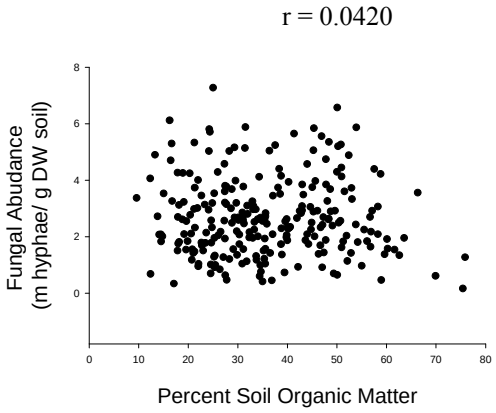
Source	DF	Type III SS	Mean Squared Error	F Value	Pr>F
region	2	5.62	2.81	0.48	0.6194
zone	2	152.02	76.01	14.98	<.0001*
region*zone	4	-	-	4.09	0.0034*

Figure 3.19. Soil fungal abundance was plotted against three measured soil properties for all sample cores: (a) soil moisture, (b) soil organic matter and (c) soil C/N.

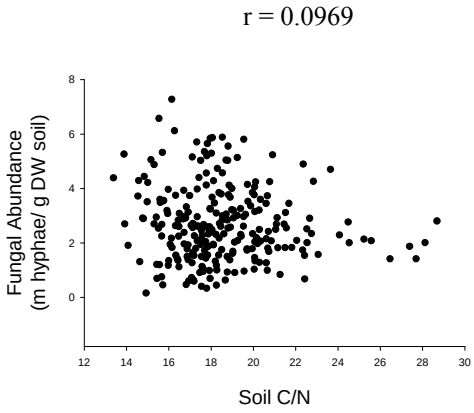
(a) Soil Moisture



(b) Soil Organic Matter



(c) Soil C/N



3.2.2 Aboveground Biomass and C/N

Live aboveground biomass differed significantly between plant zones, $F(2, 24) = 4.43$, $p < 0.05$, but no effect of marsh region was observed. No significant interaction between zones was found. The same was true for dead aboveground biomass (zone: $F(2, 24) = 7.02$, $p < 0.05$). Thus, both live and dead aboveground biomass varied significantly across plant zone (Fig. 3.20), but not across marsh region (data not included). Live aboveground biomass was greatest in the *J. roemerianus* and transition zones (291.2 and 298.4 g m^{-2}, respectively) Biomass was significantly lower in the *S. patens* (143.6 g m^{-2}). Dead aboveground biomass showed similar patterning to live biomass, but the transition zone was not significantly different from either distinct plant zone (Fig. 3.20, Tables 3.9 and 3.10).

Aboveground C/N was determined from live biomass samples that were separated by plant type. *Spartina alterniflora* was only found in the *S. patens* and transition zones of the low marsh transects. Overall, C/N values were generally highest in the high marsh region and showed no clear trend across plant zones (Fig. 2.19). The total amount of nitrogen (g) in aboveground standing stock did not differ significantly between marsh regions or plant zones, although there was consistently more nitrogen in the low marsh for all plant zones (Fig. 3.22).

3.2.3 Belowground Root Mass and C/N

Belowground biomass was quantified in the first 5cm of the marsh soil. There was no effect of marsh region or the interaction term on biomass belowground, but there was a significant vegetation zone effect, $F(2, 27) = 4.82$, $p < 0.05$ (Table 3.11). The transition

Figure 3.20. Aboveground biomass was analyzed across plant zones, live and dead, and significant differences are indicated by different letters. There was no significant difference between marsh regions. Error bars represent +1 standard error.

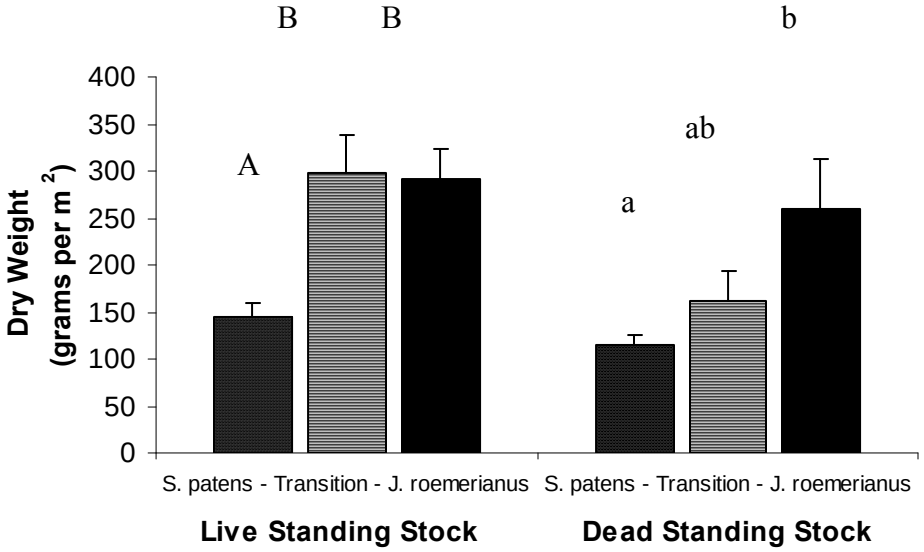


Table 3.9. ANOVA results for live aboveground biomass.

Source	DF	Type III SS	Mean Squared Error	F Value	Pr>F
region	2	2.71E+03	1.36E+03	2.25	0.1267
zone	2	4.62E+03	2.31E+03	4.43	0.0231*
region*zone	4	-	-	0.41	0.797

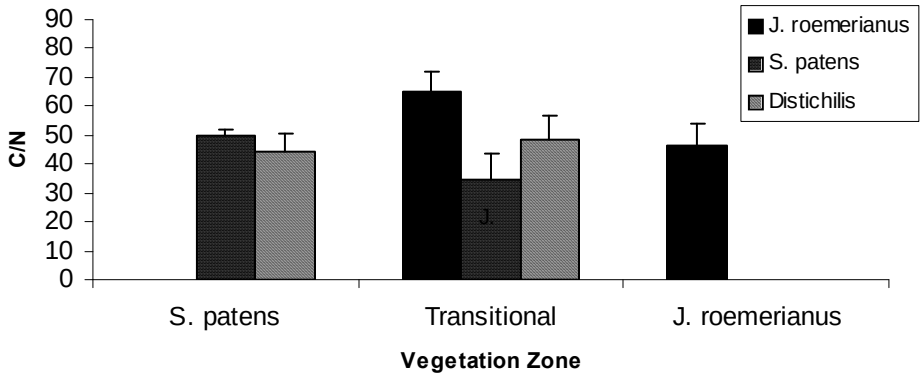
Table 3.10. ANOVA results for dead aboveground biomass.

Source	DF	Type III SS	Mean Squared Error	F Value	Pr>F
region	2	1.75E+03	8.75E+02	0.79	0.4668
zone	2	1.05E+04	5.25E+03	7.02	0.004*
region*zone	4	-	-	0.38	0.8224

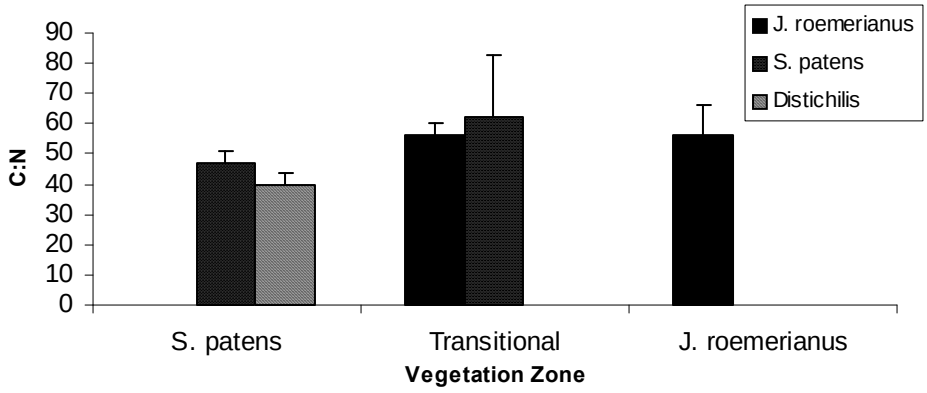
* indicates significance at the $\alpha = 0.05$ level

Figure 3.21. Aboveground C/N results in each marsh region were generally highest in the high marsh region, and showed no clear trends across plant zones. Error bars represent + 1 standard error when multiple samples were available.

(a) High



(b) Mid



(c) Low

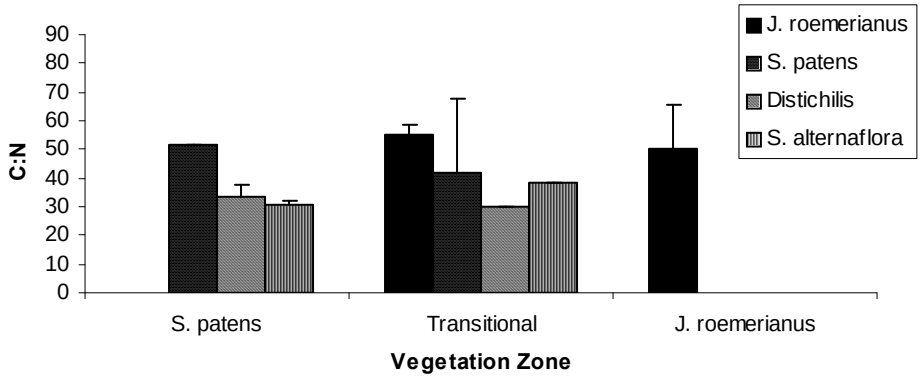


Figure 3.22. Total grams nitrogen from above ground standing stock did not differ significantly between marsh regions or vegetation zones, although there was consistently more nitrogen in the low marsh in each vegetation zone. Error bars represent +1 standard error.

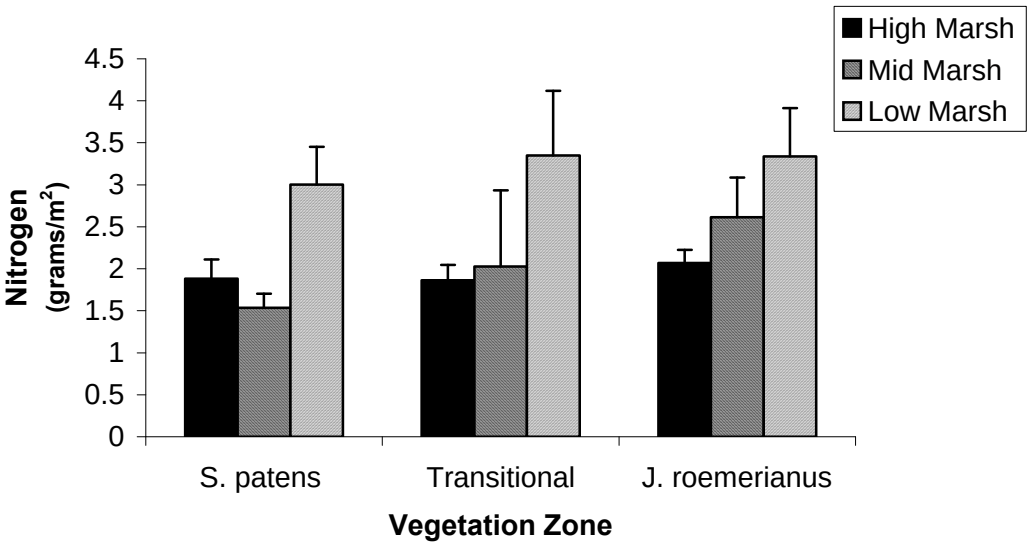


Figure 3.23. Belowground root mass was determined as ash-free dry weight of live and dead roots to a depth of 5cm. Comparisons were made within these groups, and bars with letters in common are not significantly different. Error bars represent + 1 standard error.

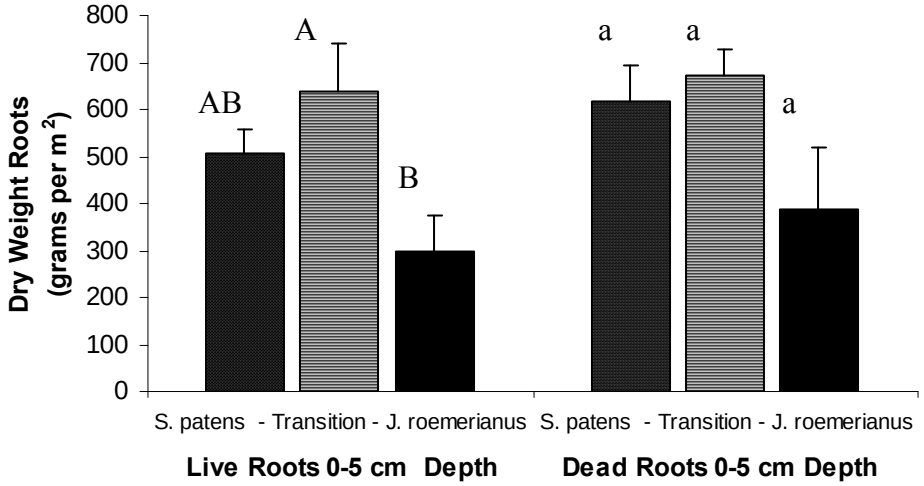


Table 3.11. ANOVA results for live belowground biomass.

Source	DF	Type III SS	Mean Squared Error	F Value	Pr>F
region	2	0.24	0.12	0.19	0.8248
zone	2	4.28	2.14	4.82	0.0173*
region*zone	4	-	-	1.93	0.1484

Table 3.12. ANOVA results for dead belowground biomass.

Source	DF	Type III SS	Mean Squared Error	F Value	Pr>F
region	2	0.29	0.15	0.25	0.7845
zone	2	0.15	0.07	0.12	0.8852
region*zone	4	-	-	1.62	0.2137

* indicates significance at the $\alpha = 0.05$ level

Figure 3.24. Root mass C/N content was determined separately for hand-sorted live and dead roots. Pair-wise comparisons were performed within each viability category, and bars with letters in common are not significantly different (uppercase letters analyzed separately from lower case letters). Error bars represent +1 standard error.

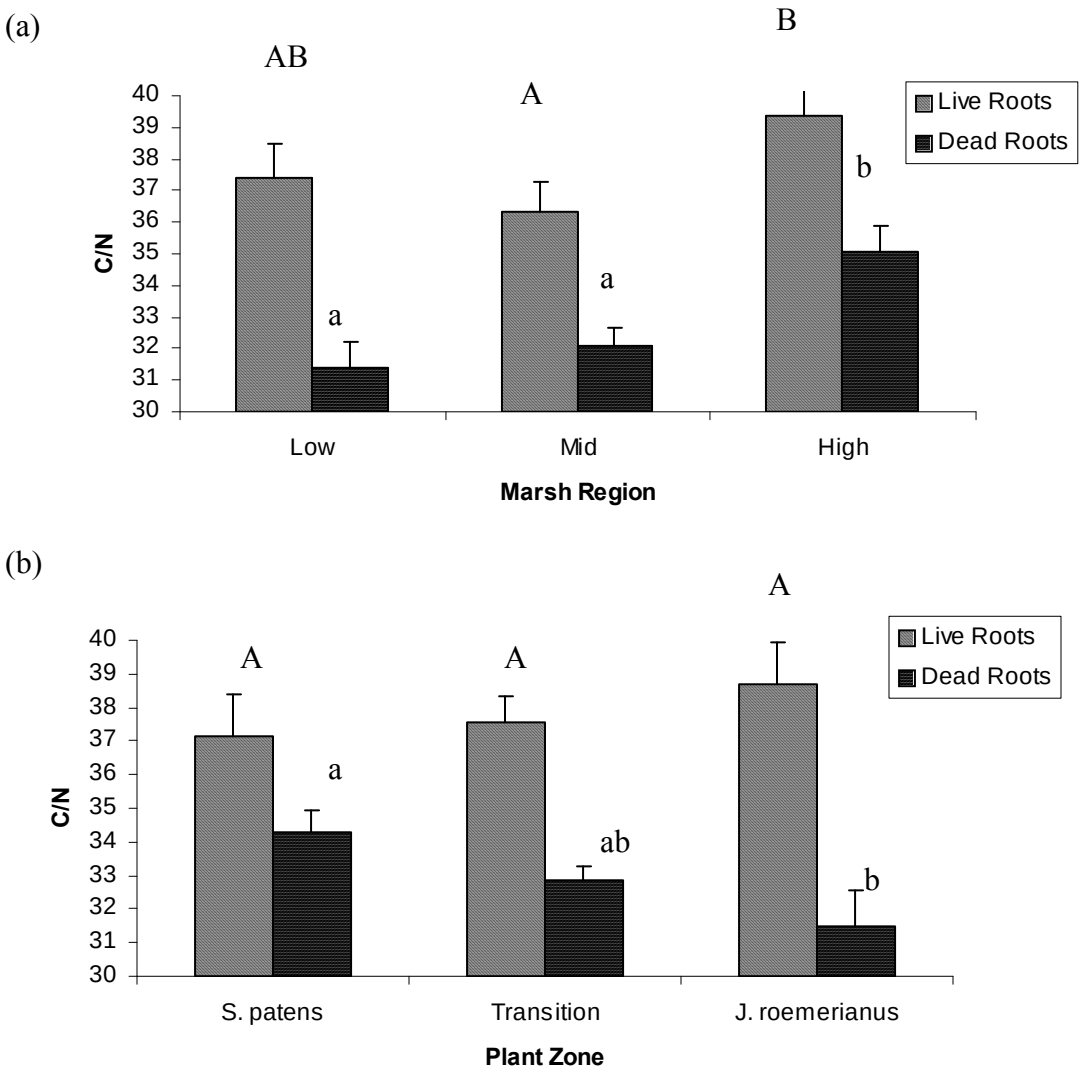


Table 3.13. ANOVA results for live root mass C/N.

Source	DF	Type III SS	Mean Squared Error	F Value	Pr>F
region	2	57.13	28.57	1.35	0.2779
zone	2	17.04	8.52	0.37	0.6924
region*zone	4	-	-	0..62	0.6571

Table 3.14. ANOVA results for dead root mass C/N.

Source	DF	Type III SS	Mean Squared Error	F Value	Pr>F
region	2	68.98	34.49	2.72	0.0859
zone	2	36.12	18.06	1.29	0.2944
region*zone	4	-	-	0.62	0.6571

zone had the greatest mass of live roots (x = 640.2 g DW of roots m^{-2}, and the *J. roemerianus* had the least (x = 299.8 g DW roots m^{-2}) (Fig. 3.23). There was no effect of region, zone, or the interaction term on the mass of dead roots (Table 3.13).

Live and dead roots were separated from 20-cm deep soil cores and C/N was determined for live and dead roots. Live root C/N was significantly different between high and mid marsh regions, but did not vary across plant zones. Dead root C/N was significantly higher in the high marsh than in either the low and mid marsh regions (Fig. 3.24, Table 3.13 and 3.14).

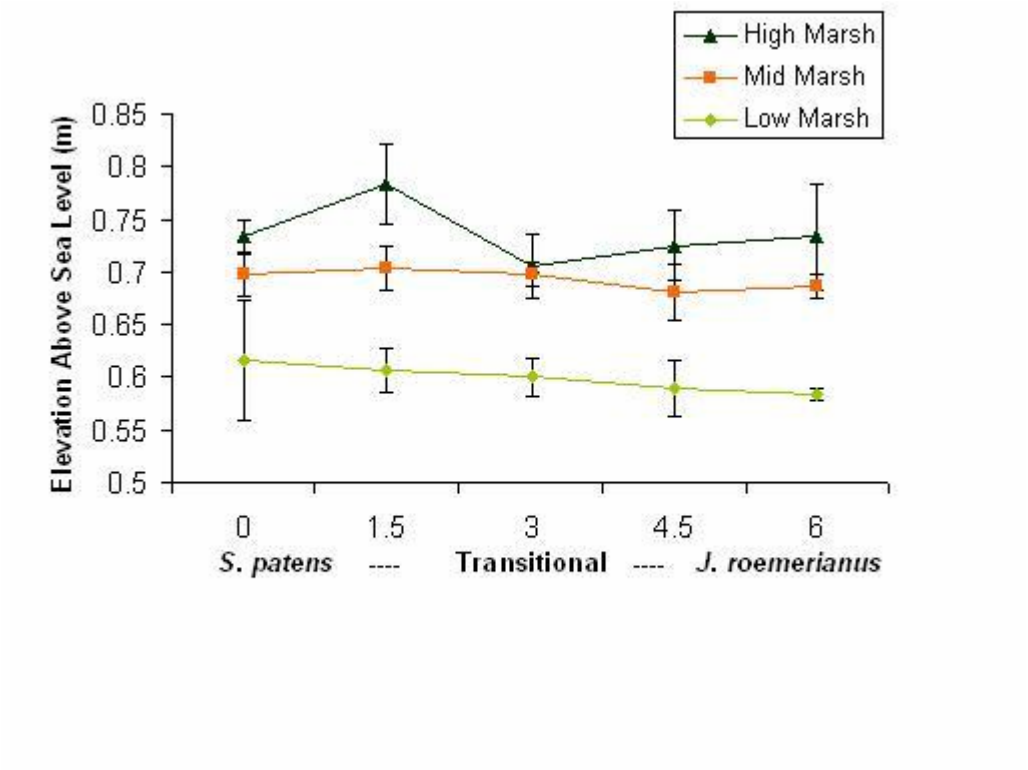
3.2.4 Marsh Elevation

Marsh elevation above mean sea level was highest in the high marsh (x = 0.705m – 0.783m), slightly lower in the mid marsh (x = 0.703m – 0.680m), and significantly lower in the low marsh (x = 0.583m – 0.617m). Along each transect, elevation was higher in the *S. patens* zone than in the *J. roemerianus* zone and more variable in the high marsh than in the mid or low marsh regions (Fig. 3.25).

3.2.5 MANOVA

Using MANOVA analysis, fourteen dependent variables (see Table 3.15 for variables) were tested against the independent variables vegetation zone and marsh region. The main effect of vegetation was significant for all variables tested. The main effect of marsh region was significant in all cases except soil C/N and the mass of live and dead roots. The interaction term was significant in all cases except for fungal abundance and soil moisture. SAS supplies four statistics for the test of significant

Figure 3.25. Marsh elevation above mean sea level was measured in August 2004 at five points along each experimental transect. Each line plotted represents an average of three transects in that marsh region, and error bars represent + or - 1 standard error.



differences among groups: Wilk's lambda, Pillai's trace, Hotelling-Lawley trace, and Roy's greatest root. All are based on the eigenvectors (linear combinations of all the dependent variables) or eigenvalues (the amount of variation explained by each eigenvector). Specifically, the first three statistics are based on either the sum or the product of all eigenvalues while Roy's greatest root is the first eigenvalue (and is considered an upper bound). When MANOVA test statistics differ, preference should be given to either Pillai's trace or Roy's greatest root. The former has been shown to be the more robust to violations of assumptions, whereas the later has the greatest power (Scheiner and Gurevitch, 2001). The MANOVA statistics, their F-values, and their significance are reported in Table 3.16. In all cases, the null hypothesis of 'no effect' was rejected.

2.3.6 Correlations Coefficients

Pearson's correlation coefficients for the dependent variables including total microbial abundance, soil properties, elevation, and above and belowground root mass characteristics are reported in Table 2.16. Soil fungal abundance was strongly, positively correlated with bacterial abundance, $r(190)=0.425$, $p < 0.05$, and soil moisture, $r(190)=0.473$, $p < 0.05$. Fungal abundance was negatively correlated with live belowground root mass, $r(190) = -0.279$, $p < 0.05$, and belowground C/N, $r(190) = -0.260$, $p < 0.05$. Somewhat weaker correlations exist between fungi and marsh elevation, $r(190) = -0.236$, $p < 0.05$, and fungi and soil C/N, $r(190) = -0.200$, $p < 0.05$ (Table 3.17). A second Pearson's Correlation analysis included live fungal abundance and found this parameter significantly correlated with soil moisture and soil organic

matter. Both of these are strong, positive relationships at 0.420 and 0.308, respectively. Live fungal abundance was not significantly correlated with total fungal or total bacterial abundance (Table 3.18).

Table 3.15. MANOVA results as indicated by Pr>F for 2005 data are reported in tables (a) and (b). N = 192. Significance (*) is indicated at the 95% confidence level.

(a)

Effect	DF	Fungal AODC	Bacterial AODC	Soil Moisture	Soil Organic Matter	Total Microbial Carbon	Soil C/N	Soil N
Overall	8	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*
Zone	2	<.0001*	<.0001*	0.0003*	0.0215*	<.0001*	<.0001*	0.0007*
Region	2	0.0022*	<.0001*	<.0001*	<.0001*	0.0016*	0.6772	<.0001*
Region x Zone	4	0.0529	0.0126*	0.062	0.0419*	0.0472*	0.0049*	<.0001*

(b)

Effect	DF	Live Below-ground Biomass	Dead Below-ground Biomass	Live Below-ground C/N	Above-ground. Biomass	Live Above-ground C/N	Total Live Above-ground Nitrogen	Elevation
Overall	8	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*
Zone	2	<.0001*	<.0001*	0.0022*	<.0001*	<.0001*	<.0001*	<.0001*
Region	2	0.6446	0.0574	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*
Region x Zone	4	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*

Table 3.16. MANOVA test criteria and F approximation for H_o of no overall marsh region effect (a), no overall plant zone effect (b), and no region*plant interaction effect. Significance (*) is indicated at the $\alpha = 0.05$ level

(a)

Statistic	Value	F Value	Num DF	Den DF	Pr>F
Wilks' Lambda	0.05841055	37.88	28	338	<.0001*
Pillai's Trace	1.48535991	35.05	28	340	<.0001*
Hotelling-Lawley Trace	6.81073791	40.9	28	293.86	<.0001*
Roy's Greatest Root	4.91767916	59.71	14	170	<.0001*

(b)

Statistic	Value	F Value	Num DF	Den DF	Pr>F
Wilks' Lambda	0.03669721	50.94	28	338	<.0001*
Pillai's Trace	1.60904793	49.98	28	340	<.0001*
Hotelling-Lawley Trace	8.65345596	51.97	28	293.86	<.0001*
Roy's Greatest Root	5.3869166	65.41	14	170	<.0001*

(c)

Statistic	Value	F Value	Num DF	Den DF	Pr>F
Wilks' Lambda	0.12398933	50.94	28	338	<.0001*
Pillai's Trace	1.50415307	49.98	28	340	<.0001*
Hotelling-Lawley Trace	3.16387638	51.97	28	293.86	<.0001*
Roy's Greatest Root	1.93765218	23.81	14	172	<.0001*

Table 3.17. Pearson's Correlation Coefficients for all variable combinations, including Acridine Orange Direct Counts (AODC) of fungi and bacterial, soil moisture, organic matter, aoil C/N, and belowground (BG) and aboveground (AG) characteristics. Significance (***bold**) is indicated at the 95% confidence level.

	Fun AODC	Bac. AODC	Soil Moisture	Soil OM	Soil C/N	Live BG Biomass	Live BG C/N	Live AG Biomass	Live AG C/N	Live AG Nitrogen	Elevation
Fun AODC	1	0.425 <.0001* 192	0.473 <.0001* 191	-0.038 0.6003 192	-0.200 0.0054* 192	-0.279 <.0001* 192	-0.260 <.0001* 192	0.189 0.0086* 192	0.013 0.8558 192	0.071 0.3253 192	-0.236 0.0010* 192
Bac. AODC		1	0.649 <.0001* 191	0.029 0.6855 192	-0.148 0.0399* 192	-0.103 0.1548 192	0.014 0.8507 192	0.172 0.0170* 192	0.188 0.0089 192	-0.026 0.7227 192	-0.152 0.0359* 192
Soil Moisture			1	-0.105 0.1501 191	0.079 0.2767 191	0.039 0.5949 191	0.007 0.9220 191	0.153 0.0347* 191	0.124 0.0868 191	0.035 0.6282 191	-0.231 0.0013* 191
Soil OM				1	-0.054 0.4574 192	-0.040 0.5846 192	-0.073 0.3176 192	0.120 0.0976 192	-0.151 0.0369 192	0.212 0.0031 192	-0.236 0.0010* 192
Soil C/N					1	0.350 <.0001* 192	0.043 0.5567 192	-0.128 0.0775 192	-0.074 0.3071 192	0.003 0.9709 192	-0.031 0.6715 192
Live BG Biomass						1	0.314 <.0001* 192	0.022 .07618 192	0.259 0.0003 192	0.043 0.5537 192	0.101 0.1618 192
Live BG C/N							1	0.114 0.1155 192	0.225 0.0017* 192	0.089 0.2197 192	0.356 <.0001* 192
Live AG Biomass								1	0.075 0.3044 192	0.891 <.0001* 192	-0.505 0.3570 192
Live AG C/N									1	-0.146 0.0438 192	0.380 <.0001* 192
Live AG Nitrogen										1	-0.626 <.0001* 192
Elevation											1

Table 3.18. Pearson's Correlation Coefficients for all variable combinations, including total microbial abundance (AODC), live fungal abundance (FDA), and belowground (BG) and aboveground (AG) characteristics. Significance (*) is indicated at the 95% confidence level. N = 45.

	Fungal AODC	Bacterial AODC	Fungal FDA	Soil Moisture	Soil OM	Soil C/N	Live BG Biomass	Live BG C/N	Dead BG Biomass	Dead BG C/N	Live AG Biomass	Live AG C/N	Elevation
Fungal AODC	1.0000	**0.6655**	-0.0461	**0.4552**	-0.0356	-0.1668	-0.0903	-0.2458	-0.0117	-0.0737	**0.3223**	0.1463	-0.0913
		<.0001*	0.7638	**0.0017***	0.8167	0.2734	0.5551	0.1037	0.9390	0.6306	**0.0309***	0.3376	0.5511
Bacterial AODC		1.0000	0.0153	**0.5828**	-0.0408	-0.0342	-0.2022	-0.2119	-0.0271	**-0.3807**	**0.3978**	0.1588	**-0.4614**
			0.9205	**<.0001***	0.7900	0.8235	0.1829	0.1623	0.8599	**0.0099***	**0.0068***	0.2975	**0.0014***
Fungal FDA			1.0000	**0.4197**	**0.3084**	-0.0262	0.0226	-0.0151	0.0066	-0.0676	-0.1833	0.1795	0.1992
				0.0041*	**0.0393***	0.8644	0.8829	0.9216	0.9655	0.6590	0.2282	0.2380	0.1896
Soil Moisture				1.0000	0.0803	0.0762	-0.1491	-0.2661	0.0256	-0.2638	0.1509	-0.0631	**-0.4348**
					0.6003	0.6187	0.3284	0.0772	0.8676	0.0799	0.3223	0.6805	**0.0028***
Soil OM					1.0000	0.2175	0.1615	0.1223	0.1485	0.2546	**-0.3550**	0.2636	**0.6233**
						0.1513	0.2893	0.4235	0.3304	0.0915	**0.0167***	0.0802	**<.0001***
Soil C/N						1.0000	0.2292	-0.1126	0.0564	0.0705	**-0.3848**	**-0.3061**	0.0603
							0.1299	0.4616	0.7128	0.6455	**0.0091***	**0.0408***	0.6942
Live BG Biomass							1.0000	**0.3209**	0.0987	**0.3738**	-0.2140	0.0190	0.2764
								0.0316*	0.5191	**0.0114***	0.1581	0.9016	0.0660
Live BG C/N								1.0000	**0.4819**	**0.4277**	0.1023	0.1349	0.1764
									0.0008*	**0.0034***	0.5036	0.3768	0.2465
Dead BG Biomass									1.0000	**0.4058**	0.1087	-0.0266	0.0652
										0.0057*	0.4771	0.8623	0.6706
Dead BG C/N										1.0000	-0.2102	0.0534	**0.4619**
											0.1658	0.7274	**0.0014***
Live AG Biomass											1.0000	**0.3119**	**-0.5155**
												0.037*	**0.0003***
Live AG C/N												1.0000	0.2013
													0.1849
Elevation													1.0000

3.3 Fungal Community Composition

Concentrations of extracted soil DNA were consistent across all sampling locations. However, fungal community patterns as described by T-RFLP were not. The relative genetic dissimilarities between samples, calculated using the Jaccard coefficient, can be displayed in Mantel tables, NMDS plots and semi-variogram parameters. These methods combine to describe a pattern of fungal community structure that is influenced by multiple biotic and abiotic factors in the salt marsh soil.

3.3.1 Concentration of Soil DNA

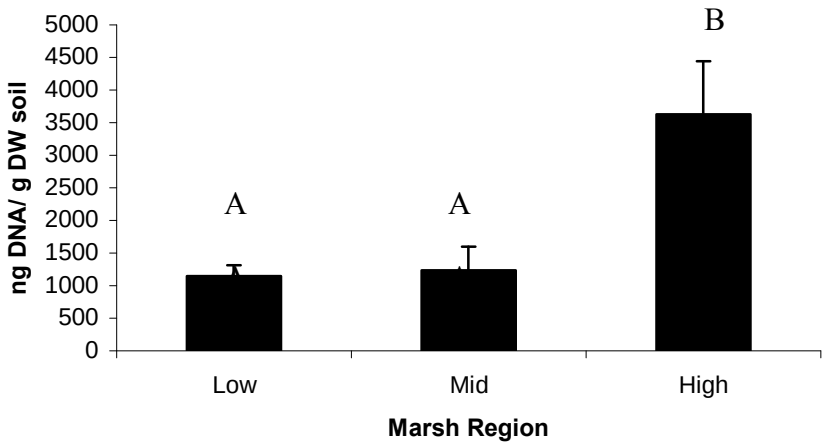
There was no marsh region or plant zone effect on the concentration of DNA extracted from the soil (Fig. 3.26, Table 3.19). However, post ANOVA comparisons indicated a significantly higher concentration of soil DNA extracted in the high marsh (3.62 μg DNA g^{-1} DW soil) than in the mid or low marsh regions (1.23 and 1.14 μg g^{-1} DW soil, respectively). Soil DNA concentration was highest in the *J. roemerianus* zone (2.61 μg DNA g^{-1} DW soil), lower in the transitional zone (1.19 μg DNA g^{-1} DW soil), and lowest in the *S. patens* zone (1.50 μg DNA g^{-1} DW soil), but none of these comparisons were significant (Fig. 3.26). The interaction effect of region x zone was also not significant (Table 3.19).

3.3.2 Mantel Tests

Seven Mantel tests were performed using the community dissimilarity matrix, with each one comparing the community dissimilarity against a measured environmental variable or combination of variables. A Monte Carlo procedure using 1000 permutations

Figure 3.26. Concentration of DNA in a gram of soil (dry weight) was determined across marsh region (a) and plant zone (b). Bars without letters in common are significantly different. Error bars represent +1 standard error.

a) Soil DNA across Marsh Region (n = 10 low marsh; n = 5 mid marsh; n = 12 high marsh).



b) Soil DNA across Plant Zone (n = 10 *J. roemerianus*; n = 7 Transition; n = 10 *S. patens*).

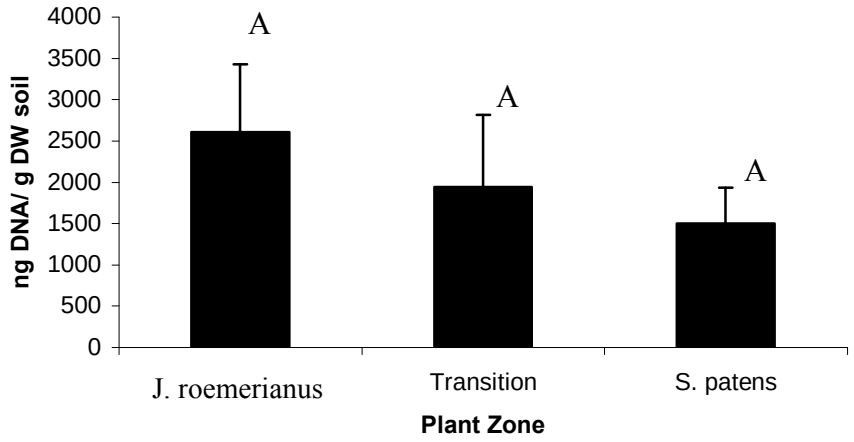


Table 3.19. Extracted soil DNA concentration ANOVA results. Main effects of marsh region and plant zone were tested in SAS proc GLM, and the interaction effect of region x zone was tested in proc MIXED. Significance (*) is indicated at the 95% confidence level.

Source	DF	Type III SS	Mean Squared Error	F Value	Pr>F
region	2	4.26E+06	2.13E+06	0.51	0.607
zone	2	3.75E+07	1.88E+07	6.65	0.005*
region*zone	4	-	-	0.9	0.985

evaluated the significance of the Mantel test statistic and revealed several significant comparisons (Table 3.20). An alpha level of a = 0.007 was used to indicate 95% confidence after Bonferroni adjustment (alpha = 0.05 divided by 7 comparisons).

Not surprisingly, the results of the Mantel tests reveal a strong link between nutrients (soil OM and C/N, and above and belowground plant C/N) and fungal community composition, although we are unable to discern between influential and causal relationships at this time. Additionally, marsh region, but not soil moisture, compared significantly with the community matrix, indicating that some facet of tidal inundation other than moisture is influencing fungal community composition.

Table 3.20. Mantel Test results for the comparisons of Fungal Community composition against environmental variables (n =109). Significance is indicated at the 95% confidence level with an asterisk.

Variable	ρ	p-value	ά-value	Number of iterations
"plant zone" model matrix	0.057	0.004*	0.007	999
"marsh region" model matrix	0.221	<0.001*	0.007	999
Soil Organic Matter	0.082	0.009*	0.007	999
Soil Moisture	0.060	0.053	0.007	999
Soil C:N and Belowground Biomass	0.097	0.003*	0.007	999
Aboveground Biomass	0.030	0.227	0.007	999
Above and Belowground C:N	0.199	<0.001*	0.007	999

3.3.3 Non-metric Multidimensional Scaling

In (non-metric) multidimensional scaling (as in Factor Analysis) the origination of the axes used to express the underlying 'distance' between samples are truly arbitrary. This distance, defined here by the relative fungal genetic dissimilarity between samples, is minimized when samples are grouped by plant zone. More specifically, communities in the high, mid, and low marsh are all unique (Fig. 3.27a), however, there is also a strong effect of plant on fungal community composition (Fig. 3.27b).

3.3.4 Semi-variogram Analysis

Semi-variograms (variograms) were useful in describing variance in both microbial abundance and relative fungal genetic dissimilarity across the experimental transects. Variograms were constructed based on marsh region for each of the three dependent variables – soil fungal abundance, soil bacterial abundance, and fungal community relative genetic variability. Community data were also examined by plant zone for each transect. Model fits for each variogram were solved using SigmaPlot (SYSTAT, San Jose, CA). Model parameters are given in Table 3.20. When combined with the nugget effect (discontinuity at the origin), one of three models has been noted as adequate for most data sets: the linear model, the exponential model, or the Gaussian model. However, the Gaussian model is not favored due to recent warnings against the risk of numerical instability associated with it (Goovaerts 1998).

The model that best describes the majority of the microbial abundance variograms was the linear model, indicating that spatial correlation among samples taken from these locations will not be reached (because no sill is reached). Two exceptions were high

marsh fungal abundance, described by the pure nugget effect model, and high marsh bacterial abundance, described by the exponential model. A pure nugget effect indicates that local variation is occurring at a spatial scale smaller than the sampling interval (10cm). As a model, the nugget effect shows constant variance at all ranges (Legendre and Legendre, 1998). The exponential model indicates that beyond a particular distance (the range) samples are no longer spatially correlated. This function is described by

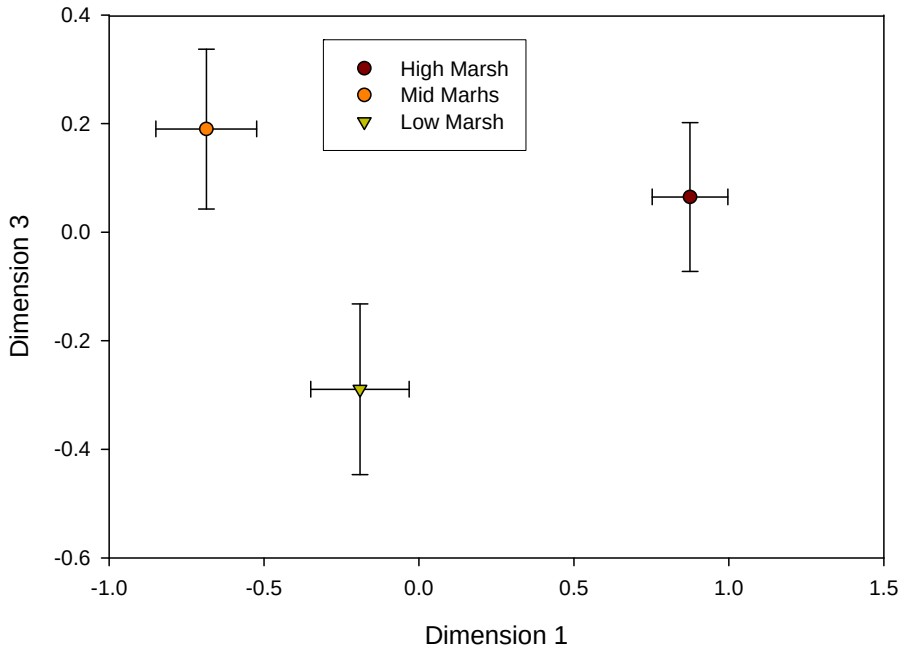
$$f(x) = C_0 + C_1(1-\exp(-3x/a))$$

where C_0 is the nugget, C_1 added to C_0 determines the sill, and a indicates the range. Models were chosen based on R^2 values obtained from each fit (Fig. 3.28 and 3.29).

Variograms for relative fungal genetic dissimilarity (constructed from T-RFLP binary data subjected to Jaccard dissimilarity conversions) were all best described using the exponential model. While these three variograms differ in shape – and, most notably, range – each contains a high nugget value (0.502 – 0.693) and a strong R^2 value (0.573 - 0.760) (Fig. 3.30). When variogram data was separated by marsh region and plant zone R^2 values were even higher, with one exception – the mid marsh *J. roemerianus* zone (R^2 = 0.549) (Fig. 3.30 – 3.33). Nugget values were lowest in the low marsh *S. patens* and highest in the mid marsh *J. roemerianus*.

Figure 3.27. Fungal community composition by marsh region (a) and plant zone (b) using Non-metric Multidimensional Scaling (NMDS) (n = 109). Error bars represent + or – 1 standard error.

(a) Marsh Region



(b) Plant Zone

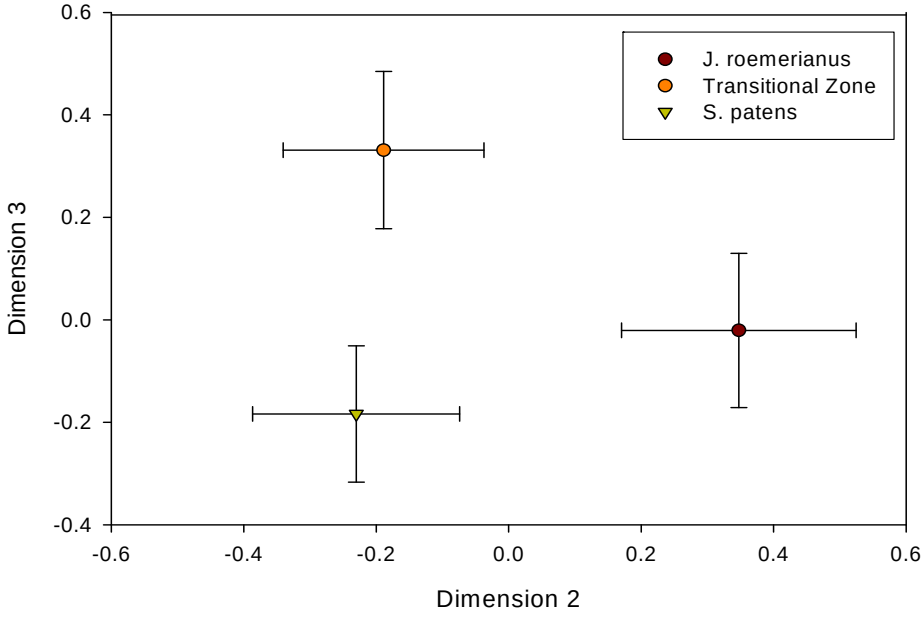
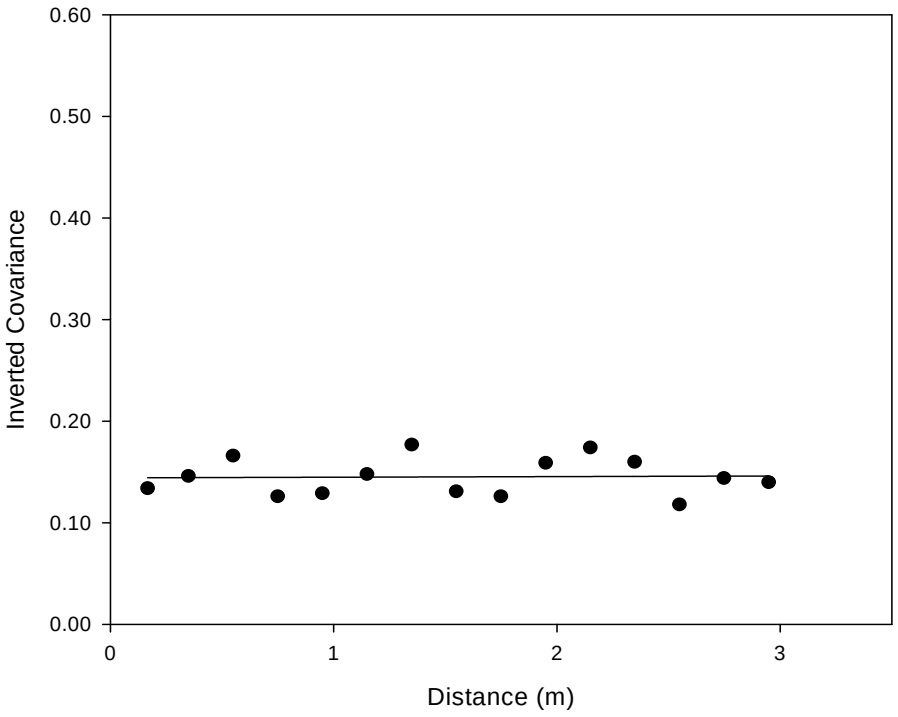
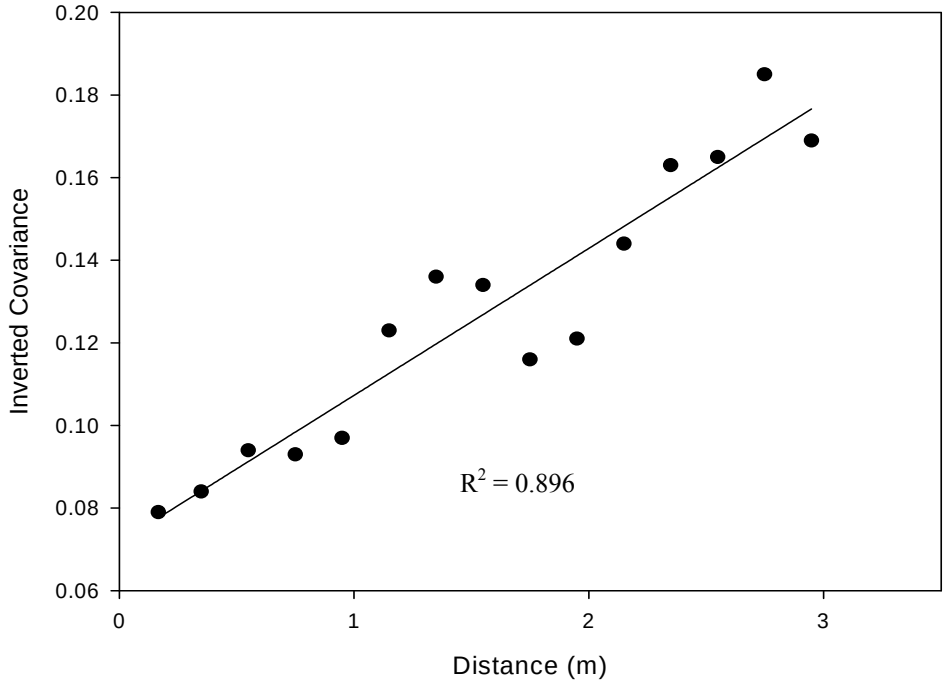


Figure 3.28. Variograms used to model spatial autocorrelation in fungal community abundance in each marsh region – a) high b) mid and c) low. The 4 m sampling transect spanned the *J. roemerianus*, transitional, and *S. patens* plant zones.

a) High Marsh – Pure Nugget Effect (n = 40)



b) Mid Marsh – Linear Model (n = 40)



c) Low Marsh – Linear Model (n = 40)

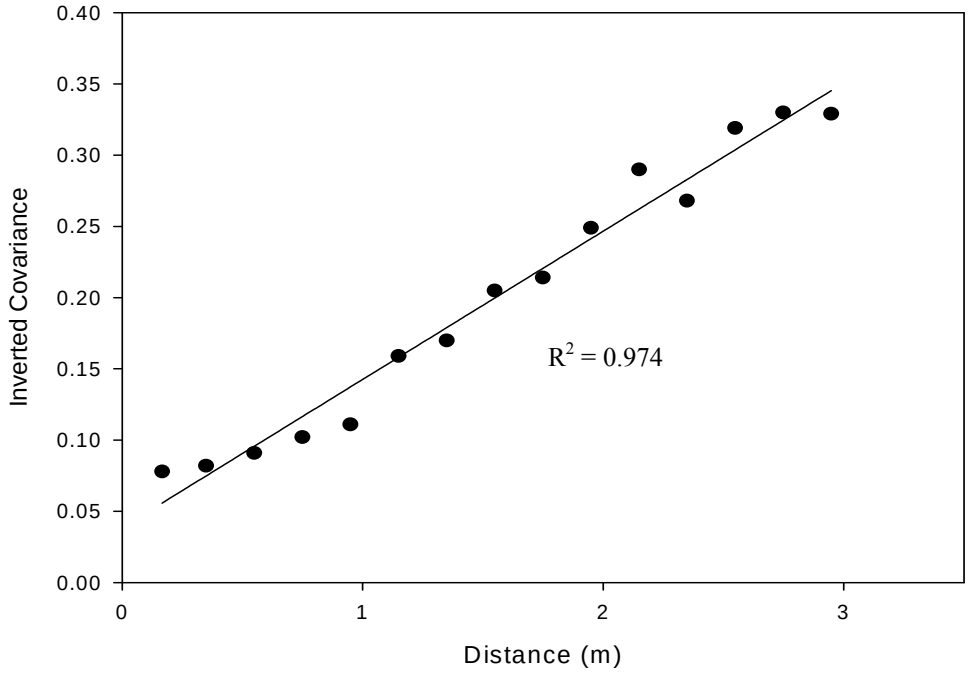
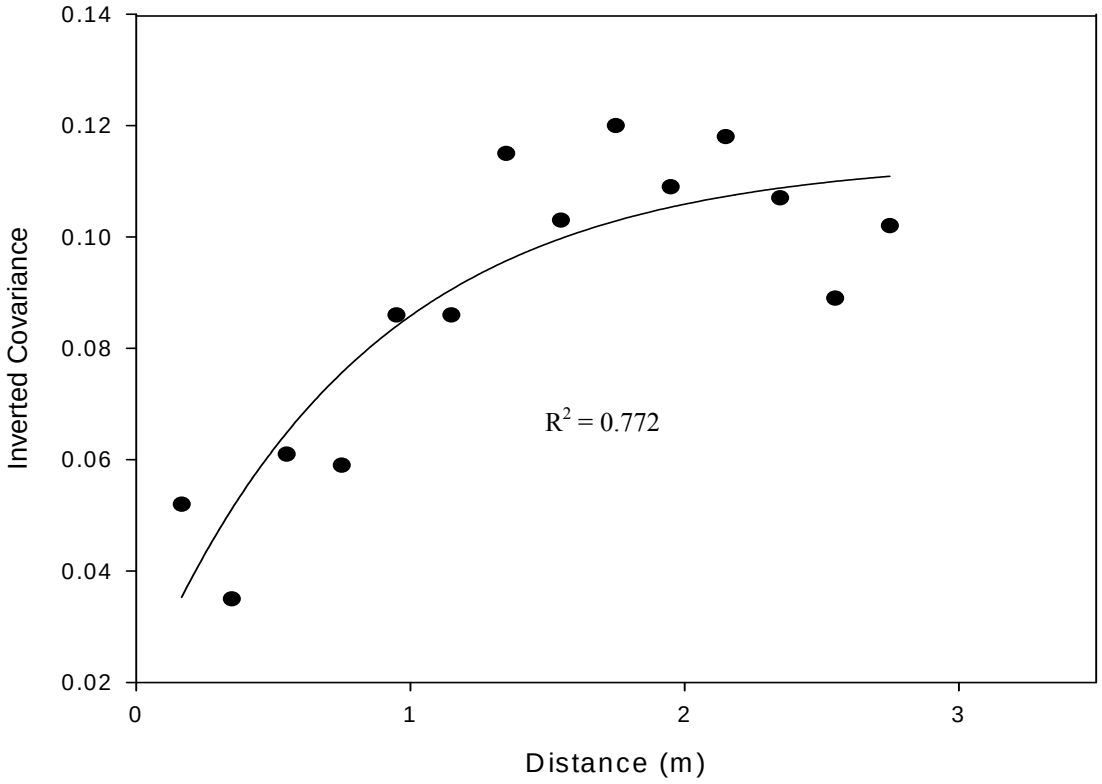
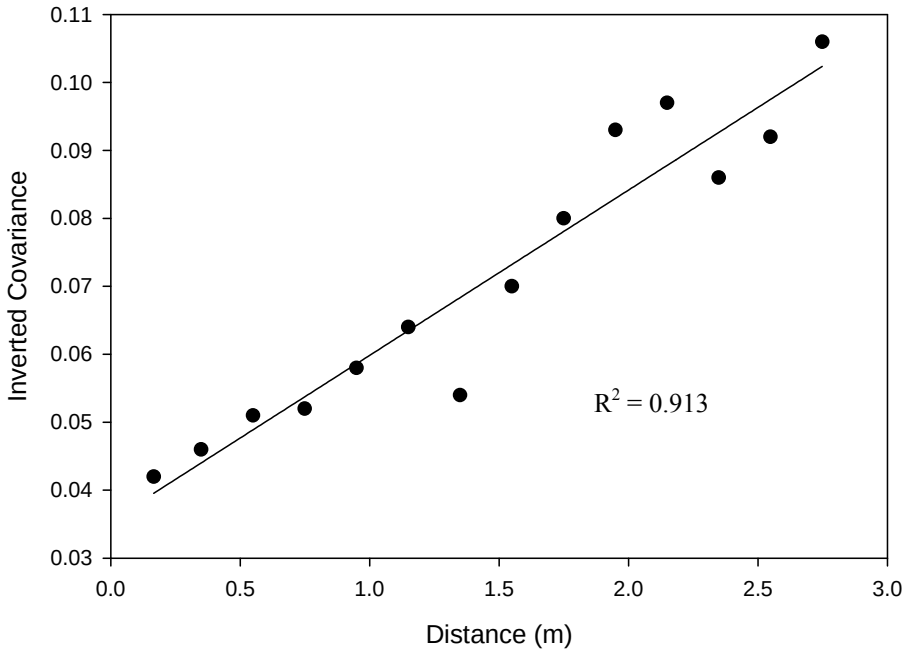


Figure 3.29. Variograms used to model spatial autocorrelation in bacterial community abundance – a) high b) mid and c) low. The 4 m sampling transect spanned the *J. roemerianus*, transitional, and *S. patens* plant zones.

a) High Marsh – Exponential Model (n = 40)



b) Mid Marsh – Linear Model (n = 40)



c) Low Marsh – Linear Model (n = 40)

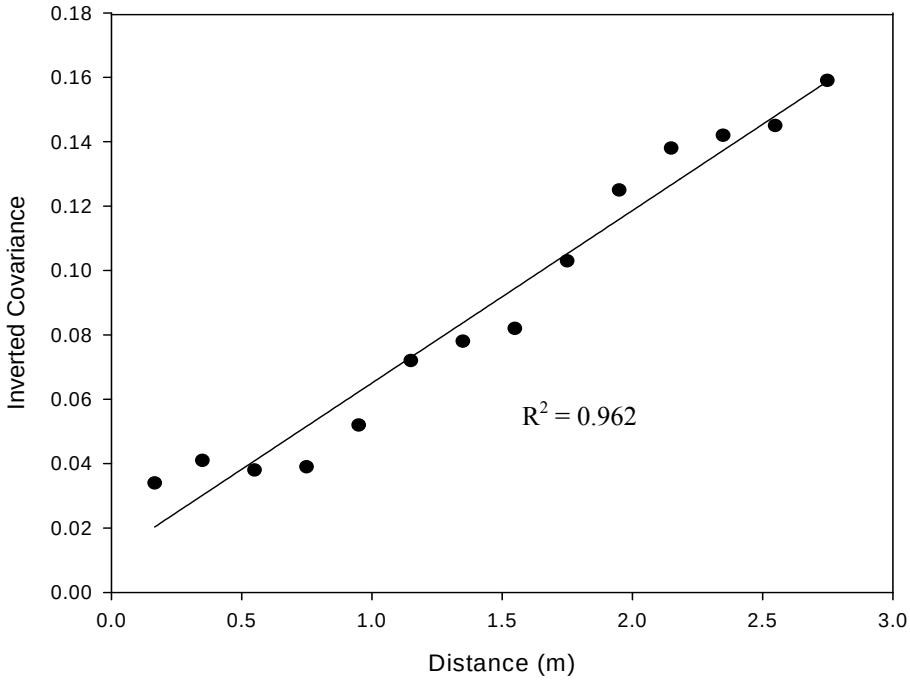
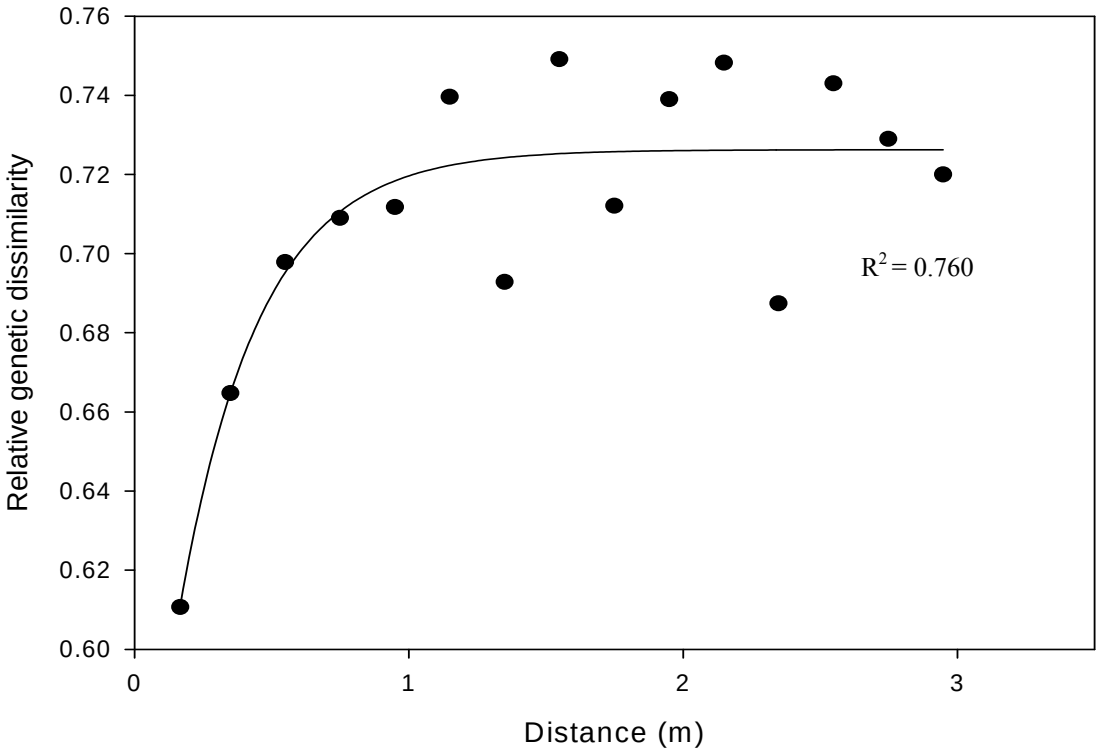
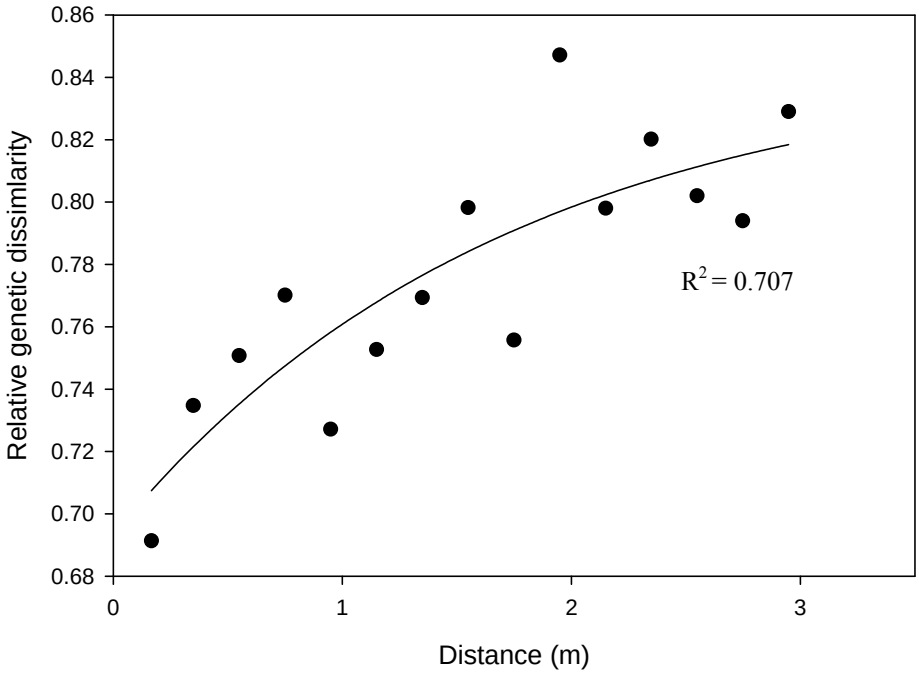


Figure 3.30. Variograms used to model spatial autocorrelation in fungal community relative genetic dissimilarity and for the determination of fungal community patch size. Data is organized by sampling region – a) high marsh b) mid marsh and c) low marsh. The 4 m sampling transect in each region spanned the *J. roemerianus*, transitional, and *S. patens* plant zones.

a) High Marsh – Exponential Model (n = 36)



b) Mid Marsh – Exponential Model (n = 37)



c) Low Marsh – Exponential Model (n = 32)

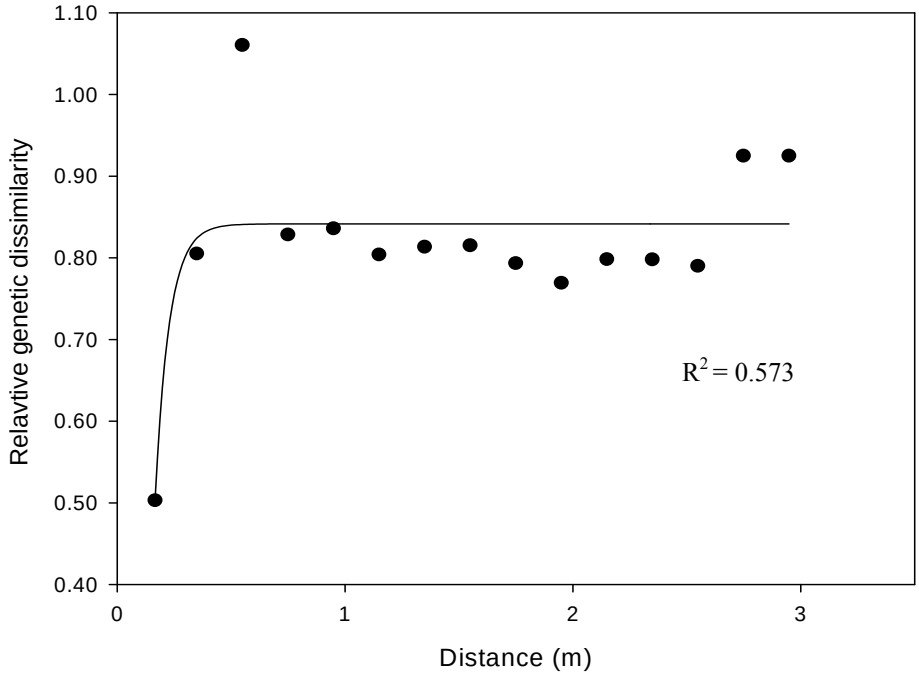
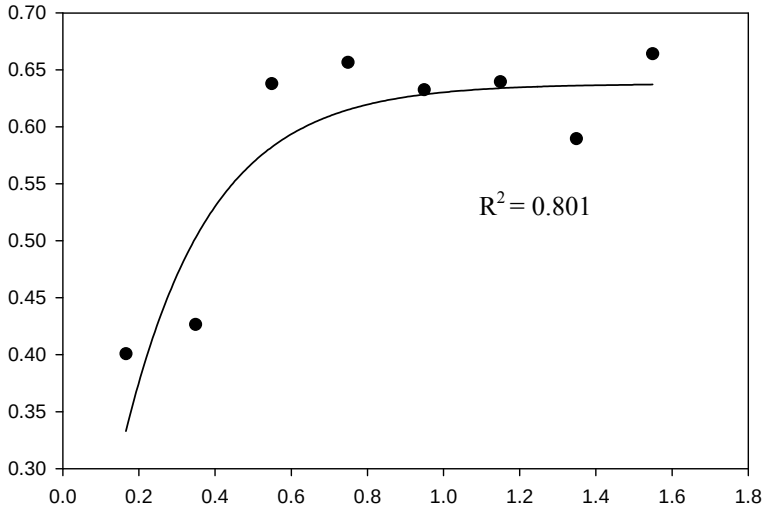


Table 3.21. Summary of model parameters from fitting the best fit equation to the experimental variograms.

Parameter	Model	Marsh Region	Nugget (C_0)	Sill ($C = C_0 + C_1$)	Relative Nugget Effect (C_0/C_1)	Spatial Dependence (C_1/C)	R^2	Range (cm)
Fungal Abundance (AODC)	nugget effect	High	0.144	0.144	---	---	0	---
	linear	Mid	0.072	---	---	---	0.896	---
	linear	Low	0.039	---	---	---	0.974	---
Bacterial Abundance (AODC)	exponential	High	0.018	0.114	0.18	0.85	0.772	245
	linear	Mid	0.036	---	---	---	0.913	---
	linear	Low	0.011	---	---	---	0.962	---
Difference in fungal community structure (TRFLP)	exponential	High	0.523	0.726	2.57	0.28	0.760	88
	exponential	Mid	0.693	0.846	4.55	0.18	0.707	515
	exponential	Low	4.063	8.967	0.83	0.55	0.573	186

Figure 3.31. Variograms constructed to model spatial autocorrelation in fungal community genetic dissimilarity in the **high marsh** region. Data is separated by plant zone – a) *Juncus roemerianus* and b) *Spartina patens*.

a) J. roemerianus – Exponential Model



b) S. patens – Exponential Model

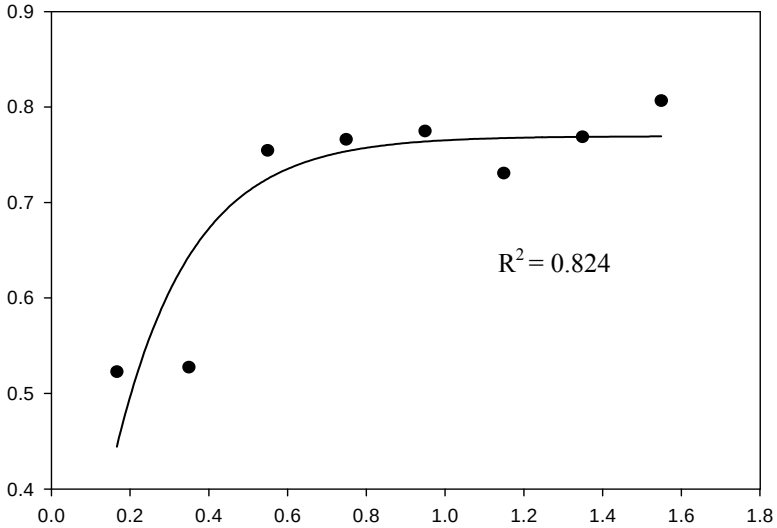
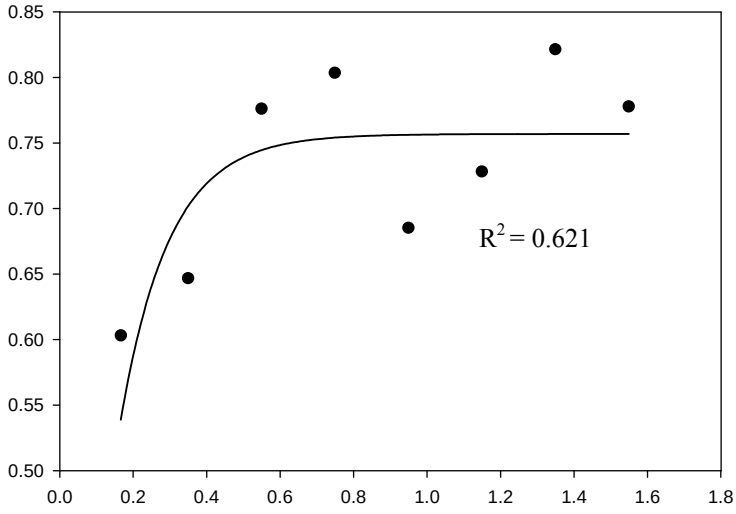


Figure 3.32. Variograms constructed to model spatial autocorrelation in fungal community genetic dissimilarity in the **mid marsh** region. Data is separated by plant zone – a) *Juncus roemerianus* and b) *Spartina patens*.

a) J. roemerianus – Exponential Model



b) S. patens – Exponential Model

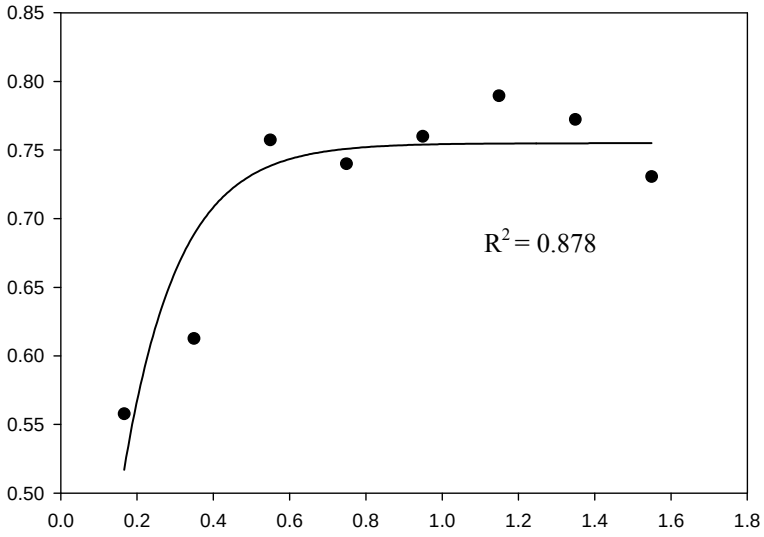
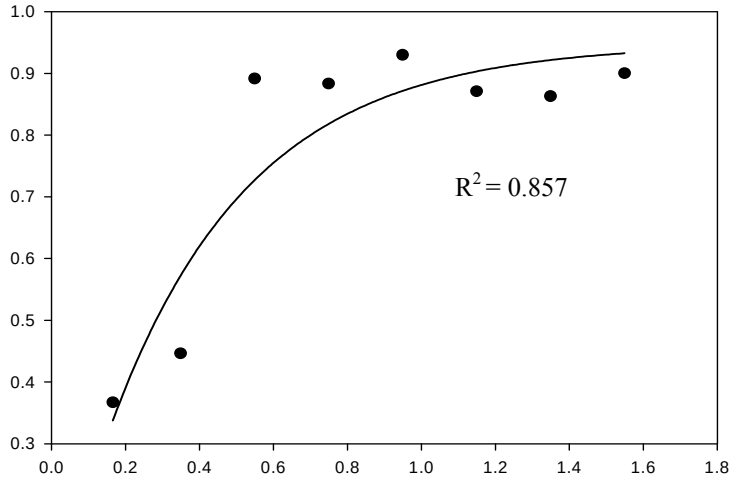


Figure 3.33. Variograms constructed to model spatial autocorrelation in fungal community genetic dissimilarity in the **low marsh** region. Data is separated by plant zone – a) *Juncus roemerianus* and b) *Spartina patens*.

a) J. roemerianus – Exponential Model



b) S. patens – Exponential Model

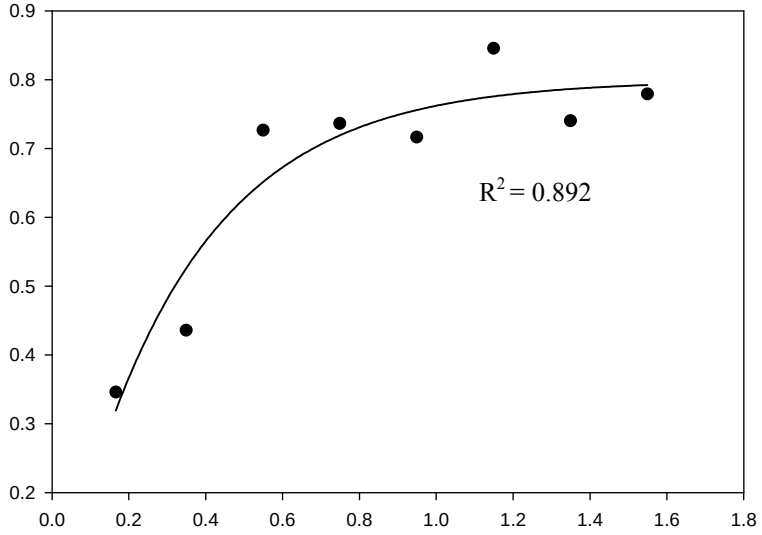


Table 3.22. Summary of model parameters from fitting the exponential model to the plant zone relative genetic dissimilarity experimental variograms.

Parameter	Model	**Plant Zone**	**Nugget (C_0)**	**Sill ($C = C_0 + C_1$)**	**Relative Nugget Effect (C_0/C_1)**	**R^2**	**Range (cm)**
High Marsh Fungal Community Structure	exponential	*J. roemerianus*	0.157	0.649	0.32	0.779	84
	exponential	*S. patens*	0.319	0.796	0.67	0.741	108
Mid Marsh Fungal Community	exponential	*J. roemerianus*	0.430	0.772	1.26	0.549	76
	exponential	*S. patens*	0.355	0.770	0.85	0.795	81
Low Marsh Fungal Community Structure	exponential	*J. roemerianus*	0.161	1.243	0.15	0.849	84
	exponential	*S. patens*	0.001	0.795	0.00	0.892	98

CHAPTER FOUR: DISCUSSION

Soil microorganisms mediate many processes such as nitrification, denitrification, and methanogensis that regulate ecosystem functioning. These processes are of particular interest in wetland ecosystems where nutrient cycling is highly responsive to fluctuating hydrology and nutrients. Processes generating soil gases may be sensitive to climate warming, and vegetation health and soil stability affect how the marsh may respond to rising sea levels. While some previous work has been done indicating the importance of fungi and other microorganisms at the surface of estuarine sediments - particularly that fungi outweigh bacteria in their contribution to microbial biomass at these swallow depths (Rublee, 1982), there is a disconnect between relative bacterial and fungal abundance and the breath of investigation given to these organisms. Bacteria are by far the most studied salt marsh microbe, but are rarely studied in this environment alongside fungi (Buchan *et al*., 2003 and references therein). Work on general soil fungal community composition is sparse, and in wetlands even more rare (Gutknecht *et al.*, 2006).

Due to the high salinity, frequently saturated environment of the salt marsh soil, scientists have not expected to observe thriving fungal communities there (Mentzer *et al.*, 2006). Interestingly, this study finds bacteria comprising less than 4% of the total soil microbial carbon of Upper Phillips Creek salt marsh, while fungi made up the overwhelming remainder (Fig. 3.12). Furthermore, my results indicate that fungi are not only abundant in the first few centimeters of the salt marsh soil, but they are also alive and very active, responding to differences in environmental conditions, vegetation patterns and probably even soil bacterial influences. Data revealed the most cytoplasm-filled (live) hyphae in the mid marsh region, and the highest percentage of live hyphae in

the mid marsh *Spartina patens*, followed by the high marsh *S. patens* and *Juncus roemerianus.* Genetic community profiling via T-RFLP produced strong NMDS groupings of relative fungal community genetic similarity by both vegetative zone and marsh region. This now leads to questions of fungal residency in the salt marsh soil, or, perhaps continual inoculation via standing dead fungal communities, tidal waters or airborne forest spores. Clearly, several environmental influences are likely involved in structuring the size, richness and diversity of this thriving soil community.

4.1 Fungal Abundance in the Salt Marsh Soil

Even though fungi are aerobic organisms not expected to thrive in saturated, high salinity environments, large soil communities were found in all regions of Upper Phillips Creek (UPC) salt marsh (Fig. 3.4). Fungi residing in the regularly flooded low and mid marsh regions are clearly adapted to very moist environments, while conditions in the high marsh, where the soil frequently stands exposed from tidal waters, may be too dry to allow soil fungal communities to thrive. Fungal abundance and elevation (a parameter related to tidal inundation) were inversely related across marsh region and plant zone, but not significantly correlated (Table 3.18), implying that higher ground may be a negative factor influencing fungal abundance.

In a pastureland study by Wichern *et al*. (2006), soil respiration and soil microbial biomass decreased with increasing levels of salinity. While clearly adapted to pore water salinities of 25 ppt or greater across UPC marsh (Blum and Christian, 2004), fungi were found in greatest abundance in the mid marsh for both the *S. patens* and the transitional vegetation zones. The only exception is the *J. roemerianus* soils, which have the greatest

fungal abundance in the low marsh region (Fig. 2.6). The mid marsh of Upper Phillips Creek has the lowest measured pore water salinity (Blum and Christian, 2002) and consequently the most hospitable environment for these microorganisms. Clearly, soil fungal abundance in UPC is influenced by one, or several, factors that vary from low to high marsh, potentially moisture and salinity as delivered by tidal inundation.

4.1.1 Fungal Abundance and Vegetation

The chemical composition and quantity of plant inputs to soil are primary factors controlling the size and structure of the soil microbial community. Plants can influence the soil biota directly through providing a source of carbon energy via exudation and root turnover inputs to the detrital pool and indirectly by altering soil structure and hydrology, thereby influencing the microclimate experienced by soil organisms (Wolters *et al.*, 2000). The current study indicated similar patterning in soil fungal abundance, live standing stock and total above ground nitrogen (Fig. 3.1, 3.3 3.20 and 3.22). It is not surprising that soil microbial communities would be influenced by the dominant vegetative species at the sampling location when their decomposition and nutrient cycling activities are so closely tied to substrate supply.

In salt marsh systems, belowground root mass can exceed aboveground productivity as much as10-fold, making soil microbial communities important consumers of plant productivity (Valiela *et al*., 1976). I observed a significant effect of plant zone on both fungal and bacterial soil abundance, with significant (negative) correlations between fungal abundance, root biomass and root C/N (Table 3.18). The appearance of sparser fungal communities in areas of greater plant production and plant nutrient concentration

may be due to the possible, although rare, situation where plants are able to out compete fungi for soil nutrients and pose a potential feedback pressure that dictates the composition of the soil fungal community (Eom *et al*., 2000). Additionally, a negative interaction between live root biomass and fungi might be an indication that the fungi I observed were mycorrhizal. This follows from the assumption that mycorrhizal plants make fewer roots because the fungal symbiont takes the place of roots, especially under low phosphorus conditions. It has been noted that, in Upper Phillips Creek marsh, phosphorus decreases from low to mid to high marsh (Blum and Christian, 2005).

4.1.2 Fungal Abundance and Soil Characteristics

In salt marshes, environmental influences of primary concern to the soil microbial community are substrate availability, moisture, salinity, pH, soil nutrients and organic matter. The current study concentrated on relating soil fungal abundance to patterns of soil C/N, soil organic matter and soil moisture. Pang and Mitchell (2005) found that the spatial distribution of soil C and N, and soil microbial biomass C, N, and P were very similar in the profiles in three types of wetlands. This indicates the importance of the soil environment in structuring the microbial community, and alludes to the tight food web found in soil systems. In Upper Phillips Creek salt marsh, soil fungal biomass was negatively correlated with soil C/N, unassociated with soil organic matter and positively correlated with soil moisture (Fig. 3.19).

In forest soils, soil C/N was found to be strongly (negatively) correlated with soil fungal abundance (Mabuhay *et al.*, 2004). On the contrary, Talley *et al.* (2002) provides evidence of a positive relationship between soil fungal abundance and soil nitrogen, a

result likely due to the competitive edge that fungi have over other microorganism in areas of lower nutrient availability or resource gaps. In the current study, soil C/N was inversely related to soil fungal abundance, with a weak, non-significant, correlation (Table 3.18).

Schnurer *et al.* (1985) found that, in an array of agricultural soils, mean hyphal diameters and mean bacterial cell volumes decreased with decreasing soil organic matter content, and microbial biomass is known to be positively correlated with the lability of soil organic matter across multiple land-use types and ecosystems (Alvarez and Alvarez, 2000; Wardle, 1992). In the current study, soil organic matter content and fungal abundance were not correlated, possibly indicating an abundance of lable organic matter available for carbon energy. Further investigation into the carbon profile of UPC salt marsh soils may be desired to specifically correlate fungal abundance with soil carbon structure.

Additionally, this study found a significant, positive correlation between soil fungal abundance and soil moisture content (Table 3.18), implying that moisture, not soil nutrients or carbon energy may be the limiting variable in the first few centimeters of the salt marsh soil. Terrestrial fungi do not typically prefer conditions that are frequently flooded. For example, microbial communities in agricultural soils had the highest proportion of fungal biomass under the driest conditions (Drenovsky *et al*., 2004). Conversely, in UPC salt marsh, the high marsh region has the lowest soil moisture content and also the lowest soil fungal abundance, perhaps indicating that soil fungi observed are not local marsh residents but may be delivered by, and adapted to, marine waters (see discussion below).

4.1.3 Fungal Abundance verses Bacterial Abundance

Stable trophic pathways and healthy nutrient cycling requires both fungal and bacterial decomposition pathways to be present and thriving in the soil (Moore *et al.*, 2005). The soil microbial community composition in terms of bacterial and fungal abundance is known to be influenced by carbon input (Brant *et al.*, 2006), and the pH and C/N of the decomposing substrate (Hogberg *et al.*, 2007). In a recent study by Wichern *et al.* (2006), the addition of organic matter to acidic pastureland soils led to a spatial differentiation of the microbial community in the soil, with bacteria dominating the surface of the substrate, yet the fungi appeared to be much better adapted to osmotic stress as displayed by a shift towards fungal dominance under elevated salt conditions.

In the current study, total soil bacterial abundance and total soil fungal abundance were found to be positively correlated across marsh region and vegetation zone. However, when total bacterial biovolume was compared to live fungal abundance no relationship was found between the two, indicating a lack or data (n=45), a need for live bacterial data, or simply no correlation between the abundance of these two types of microorganisms.

Although plant diversity has been found to affect the relative abundance of fungi and bacteria in the soil microbial community (Zak *et al.*, 2003), the number of marsh grasses present at each sampling location (greatest at the transition zone) did not appear to affect this ratio. Although rare in recent studies of microbial communities (see **Chapter One**), the positive correlation between the two types of microorganisms observed in Upper Phillips Creek salt marsh soils may be explained by the fungal role of making carbon substrate available to bacteria, as in the case of lignin. While bacteria do

not degrade lignin as efficiently as fungi, some bacteria are capable of using at least part of the degradation intermediate of lignin produced by fungi (Ruttimann *et al*., 1991; Hodson and Moran 1989). This would allow bacteria to thrive more readily in the presence of a large fungal community, and supports the work of Newell *et al.* (1988), which indicates that, interactions between fungal and bacterial saprophytes on standing dead marsh grasses may be based on temporal resource partitioning. In Upper Phillips Creek marsh, Blum and Christian (2005) found that *Spartina alterniflora* always decayed more slowly than *Juncus roemerianus* even though the two plant species' roots had similar percentages of lignin (30.9 ± 3.4 and 29.6 ± 2.1 of ash-free dry weight, *J. roemerianus* and *S. alterniflora*, respectively).

4.2 Incidence of Live Fungi in the Salt Marsh Soil

Translocation of fungal cytoplasm, and thus, nutrients, through the mycelium makes these organisms well adapted to growth in the heterogeneous soil environment. Because saprophytic fungi in soils are non-discrete organisms (Carlisle, 1994), it is now recognized that most fungal hyphal lengths in soil do not contain active cytoplasm (Boreman *et al.*, 1996; Ingham and Horton, 1987; Klein *et al.*, 1996; Stahl and Parkin, 1996). The ability to transfer cytoplasm based on resource availability implies that the hyphal structure may empty (either temporarily or as an artifact) at the time of microscopic investigation. This differentiation in mycelial structure is readily observed in culture (Deacon, 1980) and has been observed in soil and decomposing litter as early as the 1970s (Frankland, 1975). Frankland *et al.* (1990) suggests that fungi must “be

specified as living, dead or total" in order to give an accurate portrait of the microbial community structure.

4.2.1 Environmental Influences on Live Fungi

The abundance of live (cytoplasm filled) hyphae is known to be influenced by the location of fungi in relationship to energy sources such as the root (Norton and Firestone, 1991), the presence of added nitrogen (Klein *et al.*, 1989) and whether they appear in early or late successional soils (Klein *et al.*, 1995). In this study, live fungal abundance was significantly, but weakly, correlated with soil moisture and soil organic matter in Upper Phillips Creek salt marsh (Table 3.18), but uncorrelated with soil C/N, root biomass and root C/N, indicating that plant community effects are not strong factors in determining the abundance of live soil fungal hyphae. Conversely, marsh location, including distance from the creek bank, may be influencing the abundance of live fungi through variations in flooding, salinity and soil type.

A theoretical model of fungal growth response to carbon and nitrogen availability by Paustian and Schnurer (1987), produced model behavior such that in relation to substrate supply active cytoplasm filled hyphae show a positive correlation to substrate availability, while total hyphal length shows an inverse response. These results are similar to mine only in the relationship between the live and total hyphae, not in the potential mechanism. In Upper Phillip's Creek salt marsh, total fungal abundance and the proportion of live fungal abundance follows opposite trends across marsh region (with only one exception – the mid marsh *S. patens* zone). However, the organic material measured in Upper Phillip's Creek salt marsh may be a measure of unavailable material

(after labile material has decayed), not an indicator of substrate ability. In that case, the negative relationship that I saw between live fungal abundance and soil organic matter would indicate a limited energy source for fungal growth. Further investigation into the carbon molecules present in these soil samples would elucidate this.

4.2.2 Live verses Total Fungi

Live verses total fungal abundance is best assessed by the use of microscopy and vital stains such as FDA, which will fluoresce when enzymatically cleaved (Klein *et al.*, 1998). From the microscopic counts performed in this study, it was found that the greatest live fungal abundance was in the mid marsh region and that abundance did not vary significantly across plant zones. These finding are not in accord with the total fungal abundance data collected, which increased from the high to the low marsh and from the *S. patens* to the *J. roemerianus* plant zones.

The lack of correlation between these two measurements may be due to differing rates of decay and decomposition of the evacuated hyphal lengths across marsh regions, and even between plant zones. Ideally, the chemical composition of the hyphal structure, the potential decomposer community, and the environmental factors influencing the decomposition must be considered. Many types of fungi produce melanin, a complex polymer pigment and precursor to humic acids, which is more difficult to decompose than other fungal cell components such as cellulose and even lignin (Butler and Day, 1998). While melanin seems to prolong the life of fungal propagules, and is produced in many fungi, it is produced in varying quantities and with differing regulatory pathways across fungal species (Takano *et al*., 1997). Understanding what factor(s) limit the rate of

decomposition of fungal hyphae in Upper Phillips Creek salt marsh (or of the melanin producing species), as well as the structure and activity of the bacterial and mesofaunal decomposer community, might provide insight into the relationship between live and total fungi observed in this study.

4.3 Fungal Community Patterns in the Salt Marsh Soil

Diversity is defined as the number of species (richness) and their relative abundance (evenness) in a given sample, community or area. Waldrop *et al.* (2006) summarizes the two current hypotheses concerning the factors regulating fungal diversity in soil. The first states that higher levels of plant detritus production increases the supply of limiting resources (i.e. organic substrates) thereby increasing fungal diversity. Alternatively, greater plant diversity increases the range of organic substrates entering the soil (via roots and litter), thereby increasing the number of niches to be filled by a greater array of heterotrophic fungi. Their study concluded that plant diversity was unrelated to fungal diversity, but fungal diversity was a unimodel function of resource supply. In the current study, microbial community structure as determined by relative genetic fingerprinting was clearly grouped by plant community, with effects likely due the differences in litter and soil characteristics across plant species. Additionally, genetic fingerprints showed strong groupings according to marsh region, indicating influences of tidal frequency and/or salinity on fungal community structure.

An important note for this study is that the method for the analysis of soil fungal community composition relies on extraction of DNA from the members of the soil fungal community. Amplification of the DNA and analysis by T-RFLP provides information

about only the most abundant organisms in a sample even though microbial DNA has been known to persist in the environment even after cells have lost their viability (Nocker and Camper, 2006). Additionally, the diversity of soil fungi is unrelated to patterns of soil fungal abundance, except in resource-limited environments where fungal species are unable to meet their minimum resource requirement (Chase and Ryberg, 2004; Rajaniemi, 2003). Thus, DNA extraction and genetic fingerprinting techniques like T-RFLP only provide information about the composition of the soil fungal community.

4.3.1 Influence of Soil and Vegetation on Community Composition

Fungal community composition is correlated with marsh region, likely due to soil effects (or dispersal, see below), but community composition is also correlated with vegetation type, likely due to the impact of substrate type (or mycorrhizal association, see below) on the fungal community. This finding suggests that the factors controlling fungal community structure in wetlands are the same as those in forest, grassland and cultivated soil.

In a study of soil from forest, grassland and arable ecosystems, multivariate regressions indicated that incubation temperature, pH and soil organic matter content were all important in predicting the soil fungal community profile. The study concluded that vegetation type, management practices that control soil pH and mediate dissolved organic matter availability were important predictors of microbial community composition (Cookson *et al*., 2007). Additionally, several recent studies have used genetic analysis to conclude that soil fungal community diversity patterns resemble

vegetation type, specific plant species, and plant present/absence (Mathimaran *et al.*, 2007; Potthoff *et al.*, 2006; Wallenstein *et al.*, 2007).

Several taxa of saprotrophic fungi have a high degree of plant litter host affinity (Paulus *et al.*, 2006), and are often highly specific to certain plant tissues (Kumar and Hyde, 2004; Lee *et al.*, 2005; Yanna and Hyde, 2001, 2002). Evidence by Lyons *et al.* (2005) suggests that few species-specific associations, either positive or negative, exist between bacterial and fungal members of the *Spartina alterniflora* decomposer community during initial colonization. However, automated ribosomal intergenic spacer analysis (ARISA) used to examine the composition of fungal communities associated with four temperate salt marsh plants, including *Juncus roemerianus* and *Distichlis spicata*, showed clear host/substrate specificity for the fungal communities (Torzilli *et al.*, 2006).

Microbial communities in soils are also largely structured by the supply of growth limiting substrates, which enter the soil via plant detritus and root exudation (Zak *et al.*, 1994; Zak *et al.*, 2003). Plants use exudates as symbiotic signs to microbes involved in nutrient procurement and, and low nutrient environments also employ these exudates in other ways, such as for increasing iron availability through celation and to solubilize unavailable soil Ca, Fe and Al phosphates (Dakora and Phillips, 2002). It is possible that fungal community groupings by plant type are related to the release of inhibitory root exudates that would vary between plant species (Bais *et al*., 2006). Furthermore, one explanation for the presence of a unique fungal community in the transition zone would be a group of fungi that were resistant to the inhibitory compounds produced by both *S. patens* and *J. roemerianus*. On the other hand, the ability of fungi to use specific carbon

compounds might be limited by the stress of living in a salty, low O_2 environment and not by the antagonistic efforts of these grasses.

4.3.2 Microbial Community Patch Size

One of the few studies to use geostatistics to examine fungal community size, Lilleskov *et al*. (2004) concluded that most of the dominant fungal taxa in forest soils had variogram ranges of less than 3 m (with some up to 17 m) and that they were not necessarily affected by proximity to the primary autotrophs (trees). Burndrett and Abbott (1995) measured fungal infectiveness in soil cores sampled from an Australian forest and found a high degree of spatial variability; variability between adjacent cores was as great as that from cores taken 15 m apart.

In a comprehensive study of small-scale spatial variability in bacterial abundance and community structure in Upper Phillips Creek salt marsh, Franklin et al. (2002) determined the community patch size by identifying the range reach in experimental variograms of relative genetic similarity. They estimated the soil bacterial community patch size in the marsh to be 17 cm in the vertical direction and 35 cm in the horizontal direction. The percent of variability explained by the spatial separation between samples, as indicated by the spatial dependence parameter (the sill minus the nugget, divided by the sill), ranged between 11 – 22% for community composition and 60-73% for abundance data. However, this work can be directly compared to mine, as it was done in bare sediments (i.e. no plants).

I was unable to determine a correlation length scale for fungal and bacterial abundance in the mid and low marsh regions due to the linear nature of these data (a sill

will not be reached). In the high marsh, the fungal abundance data was fit to the pure nugget model, indicating that variations in abundance are occurring at a spatial scale smaller than the sampling interval (10cm). Fungal community relative genetic similarity revealed patch sizes of 88 cm, 515 cm and 186 cm, in the high, mid and low marsh regions, respectively, and the percent variability explain by distance between samples (spatial dependence) was determined to be 28%, 18% and 55%, respectively (Fig. 3.29, Table 3.21). Thus, the mid marsh not only contains the greatest abundance of live fungal hyphae but also the largest community patch size, indicating that fungal abundance may not be correlated with community species richness. Additionally, this was the region where the smallest amount of community variation could be attributed to the distance between samples, indicating that environmental influences may be affecting the fungal community at a scale smaller than the plant zones, which were relative large along the experimental transect.

4.3.3 Arbuscular Mycorrihzal or Saprophytic Fungi?

Symbiotic fungi associated with plant roots - arbuscular mycorrizhal fungi (AMF) are known salt marsh residents (Burke *et al*., 2003; Hoefnagles *et al*., 1993; McHugh and Dighton, 2004) and play a key role in facilitating the flow of carbon into the soil microbial community (Krsek and Wellington, 2006). AMF community structure in wetlands has been documented as being influenced by flooding (Ipsilantis and Sylvia, 2007; Paradi and Baar, 2006), salinity (Carvalho *et al.,* 2003) and plant community composition (Smith and Smith, 1997), and perhaps nutrient concentrations and soil pH (Paradi and Baar, 2006). All of the salt marsh grasses encountered in this study (*J.*

roemerianus, S. patens and *D. spicata*) have been observed in mycorrhizal associations in North Carolina estuaries (Hoefnagels *et al*., 1993), and mycorrhizal fungi have been documented as responding to the same variables as the fungi that I observed. For example, Lekberg *et al.* (2007) found that in agricultural soils, soil characteristics and distance between samples accounted for a significant proportion of the variation in AMF community composition among sites, suggesting that both environmental variables and dispersal were important factors shaping fungal communities.

The mycelium networks observed in the soil samples were generally non-septate, although this was not quantified. Lack of septa is a characteristic typical of mycorrhizae, although some non-symbiotic, decomposer members of the Division Ascomycota may also be non-septate. Nevertheless, the processes of these two fungal types are tightly linked, with forest soil studies revealing that complex organic N sources may become accessible to ectomycorrhizal fungi and their host plants following mineralization by saprotrophic fungi, especially when soil C/N ratios are particularly low (Wu *et al*., 2005). In a study of the vertical profile of these two groups in forest soils, saprotrophic fungi were primarily confined to relatively recently (<4 yr) shed litter components on the surface of the forest floor, where organic carbon was mineralized while nitrogen was retained. Mycorrhizal fungi dominated in the underlying, more decomposed litter and humus, where they apparently mobilized N and made it available to their host plants. Their observations clearly show that the degrading and nutrient-mobilizing components of the fungal community are spatially separated (Lindahl *et al*., 2007). I can conclude, due to morphology and the strong correlation between fungal abundance and distance

from the creek bank, that at least some of the fungi observed in this study were not mycorrhizal.

A clear understanding of how saprotrophic and mycorrhizal fungi interact to re-circulate carbon and nutrients from plant litter and soil organic matter in the salt marsh is limited by poor understanding of their spatiotemporal dynamics. In wooded meadows, the ectomycorrhizal fungal community consisted of 172 observed species, due to diverse soil conditions and host trees (Tedersoo *et al*., 2006). As many species may be present across the tidal inundation gradient of Upper Phillips Creek salt marsh and between marsh grass communities. Sequencing T-RFLP fragments such as the ones collected in this study could elucidate this, and confirm of the identity of the fungal players that were so abundantly observed.

4.3.4 Fungal Transients or Salt Marsh Residents?

While the work presented here is novel in its investigation of the influences affecting salt marsh soil fungal abundance, it is also the first time hyphal abundance was measured in marsh soil. This study showed that live fungal hyphase are abundant in the first few centimeters of the salt marsh soil. Whereas this may be a surprising result for marsh soils, there are thriving fungal communities in neighboring environments. Soil fungal communities are well studied (Korkama *et al*., 2007; Anderson *et al*., 2003), and fungi are common in estuaries and marine waters (Borut and Johnson, 1962; Kohlmeyer and Volkmann-Kohlmeyer 1991). A reasonable expectation is that the fungal species present in the marsh soil share characteristics with marine and forest types (such as salt or drought tolerance) or, perhaps, that neighboring environments could serve as direct

inoculation sources for the salt marsh soil fungal community. Hendrato and Dickinson (1984) isolated common marine sediment species from the salt marsh, particularly near the seaward edge, where tidal coverage was most frequent (suggesting transport into the marsh in tidal waters), while Pugh (1962) collected similar evidence along the edge of the marsh that bordered the forest, suggesting a terrestrial source of fungi.

Compared to other studies that measure fungal abundance by stained hyphal lengths, abundance in the salt marsh soil (as investigated in this study) is equivalent to that found on dead (decaying) marsh vegetation, not other soil environments (Table 4.1). Further work is required to determine whether hyphae observed in the first few centimeters of the marsh soil are artifacts of the standing dead community, devoid of cytoplasm, or if they are a thriving community of their own, perhaps of a different genetic makeup. Evidence for fungi as local residents of the salt marsh is provided by Pugh and Beeftink (1980) who saw community shifts between salt marsh mycoflora in tidal areas to a more general mycoflora community in non-tidal soils, and by Torzilli (1997) on the adaptations of the salt marsh isolate *Aureobasidium pulllulans* to heat and salt shock. However, as either local residents or invaders from neighboring communities, these organisms likely contribute significantly to plant primary productivity, litter decomposition, and the soil organic matter pool, demanding further study from anyone concerned about sea level rise, water quality and marsh health

Table 4.1. Summary of studies reporting fungal hyphal lengths.

Study	**Environment**	**Sample**	**Viewing Method**	**Total Fungi** (m/gDW)
This Study	**Salt Marsh - Soil**	> 5 cm below the marsh surface	Acridine Orange Direct Counting	20,000 - 70,000
Blum et al., 2004	**Salt Marsh - Plant**	standing dead plant material	Acridine Orange Direct Counting	20,000 - 180,000
Klein et al., 1998	**Short-Grass Steppe - Soil**	20cm core depth homogenized	phase contrast and FDA staining	30 - 70
Balser et al., 2005	**Forest - Soil**	10cm core depth homogenized	phase contrast	2.0 - 11
Zhu et al., 1996	**Forest - Soil**	10cm core depth homogenized	FDA staining	3,000 - 4,000
Frey et al., 1999	**Argoecosystem - Soil**	0-5 cm core segment	M2R flouresent stain	400 - 900

4.4 Conclusions

4.4.1 Significance of this study

It is well known that fungi thrive on resource heterogeneity and that their complex network of soil mycelium allows them to transfer nutrients over extended distances (Klein and Paschke, 2004 and references therein). Due to the heterogeneous nature of the marsh with respect to tidal flooding, vegetation zones, and the parameters that vary within, it was expected that soil microbial abundance would follow predictable patterns from low to high marsh and between *Spartina patens* and *Juncus roemerianus* patches. Data revealed a very high abundance of fungi in the first few centimeters of the salt marsh sediment surface, providing for the potential importance of fungi in salt marsh soil nutrient cycling and sediment dynamics. Results also indicated that several environmental variables may be affecting soil fungal abundance in the salt marsh.

Firstly, tidal flooding, the hallmark of the salt marsh environment, was responsible for the changes in salinity and soil moisture that were correlated with the fungal community abundance and composition. Additionally, plant community differences and microbial community structure were correlated, with effects likely due to the varying supply of growth limiting organic substrates from plant detritus and root mass production, and indirectly from differences in elevation. The significance of this study lies in the confirmation of abundant, alive and active fungal communities in the soils of Upper Phillips Creek salt marsh. Soil fungi should no longer be excluded or discounted in investigations of marsh ecosystem dynamics and sustainability.

4.4.2 The Broader Impact

There is compelling evidence that microbial decomposition can contribute appreciably to overall carbon flux from marshes to the atmosphere (Kuehn *et al*., 2004). Additionally, fungi may form a broad base in the marsh food web, providing nutrients for plants and animals. Every attempt to understand terrestrial ecosystems, and those at the interface between uplands and marine ecosystems, including salt marshes must start and end with soil organisms. Even in salt marshes, fungi are the most abundant microbe on a biomass basis. My observations demonstrate that fungi are an important component of the salt marsh soil microbial community, and that they are very likely to play a critical role in organic matter mineralization, nutrient mineralization, and trophic dynamics.

4.4.3 Further Investigations

Environmental factors shown to decrease fungal hyphal lengths in soils include physical disturbance (Gupta and Germida, 1998; Dick, 1993; McGongle and Miller, 1996), mineral nitrogen (Klein *et al*., 1998; Arnold, 1991; Berg *et al*., 1998), pesticides (Anderson *et al*., 1981; Duah-Yentami and Johnson, 1986; Beare *et al*., 1993), earthworms (McLean and Parkinson, 1997; Zhu and Carreiro, 1999), and heavy metals (Nordgren *et al*., 1983). In the salt marsh, additional variables such as consumption by protozoans and nematodes and elevated ambient CO_2 levels may also be influential. Also important in the study of soil dynamics, nutrient cycling and marsh sustainability is an understanding of the vertical profile of the fungal community, its origin (terrestrial, marine or local), and a quantification of mycorrhizal verse saprobic contributions to total fungal biomass.

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