

## THE EFFECTS OF DRAINAGE AND NITROGEN ENRICHMENT ON *PHRAGMITES AUSTRALIS*, *SPARTINA ALTERNIFLORA*, AND THEIR ROOT-ASSOCIATED MICROBIAL COMMUNITIES

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**Abstract:** Salt marshes along the northeastern coast of the United States are increasingly subject to changes in hydrology and enrichment with nitrogen as a result of human activities. We conducted a greenhouse experiment to determine the response of *Phragmites australis*, *Spartina alterniflora*, and their root-associated microbial communities to these environmental perturbations. Two sets of treatments were compared: 1) saturated versus drained hydrology under low N enrichment and 2) low versus high N enrichment under drained hydrologic conditions. Unvegetated sediments were planted with either *Phragmites australis* or *Spartina alterniflora*, and after one growing season, sediment characteristics, macrophyte biomass, and sediment microbial community structure, as described by phospholipid fatty acids (PLFAs), were analyzed. Under all conditions tested, *Spartina* root production was significantly greater than *Phragmites*. While *Spartina* invested more biomass in roots, *Phragmites* invested proportionally more biomass in shoots and rhizomes, and *Phragmites* response to drained hydrology or to an increase in N also differed from that of *Spartina*. Under N enrichment, the rate of *Phragmites* stem production doubled, and under drained conditions the ratio of *Phragmites* shoot:root biomass increased, while *Spartina* biomass ratios remained unchanged. Although *Spartina* root biomass was significantly greater than that of *Phragmites*, under drained conditions the *Spartina* sediment PLFA diversity was significantly lower than the PLFA diversity in both *Phragmites* and unvegetated sediments. Under saturated conditions, vegetated sediments exhibited greater PLFA diversity, while no diversity differences were seen in unvegetated sediments under the two hydrologic conditions. Six PLFAs were responsible for 80% of the separation seen within the Principal Components Analysis ordination space. Significant differences in these PLFAs were due to hydrology when comparing saturated vs. drained sediments, and predominantly due to the plant species when comparing N treatments under drained conditions. Our results suggest that macrophyte root association can influence the structure of estuarine sediment microbial communities, but that saturated hydrological conditions may override the plant influences.

**Key Words:** estuarine, invasive species, microbial diversity, nitrogen, PLFA, rhizosphere, salt marsh, sediment, Simpson diversity index, wetland

### INTRODUCTION

*Spartina alterniflora* Loisel (hereafter *Spartina*) is the dominant macrophyte species in estuarine salt marshes along the Atlantic Coast of North America (Howes et al. 1986, Koch et al. 1990). *Spartina* tall form is found along low marsh creek banks and *Spartina* short form and/or *Spartina patens* occupy saturated higher marsh elevations between the creek bank and the terrestrial edge (Niering and Warren 1980, Howes et al. 1981, Roman et al. 1984, de la

Cruz et al. 1989). In undisturbed estuarine marshes, *Phragmites australis* (Cav.) Trin. ex. Steud. (hereafter *Phragmites*) is found at the upper bounds of the high marsh adjacent to the upland interface. This monospecific zonation of salt marsh vegetation results from plant species interactions with complex environmental factors. One of the primary abiotic factors influencing plant zonation is the duration and frequency of flooding (Chambers et al. 2003). The transitions from low to high marsh, and from high marsh to terrestrial border, have been corre-

lated with mean high water levels (Niering and Warren 1980).

*Phragmites* spread has been extensive under brackish conditions (salinity < 18 ppt), and this plant has become emblematic of tidal wetland alteration (Chambers et al. 1999, Rice et al. 2000, Bertness et al. 2002). *Phragmites* is known to thrive in disturbed estuarine systems (Niering and Warren 1980) and has been incorporated into treatment wetlands due to its ability to remove nutrients from sediments (Kadlec and Knight 1996). In numerous locations, environmental and/or genetic factors have favored an expansion of *Phragmites* into locations previously dominated by *Spartina* species (Roman et al. 1984, Windham and Lathrop 1999, Meyerson et al. 2000, Rooth and Stevenson 2000, Saltonstall 2002, Chambers et al. 2003, Minchinton and Bertness 2003).

*Phragmites* encroaches into the lower marsh through vegetative spread (Bart and Hartman 2001). Reduction of tidal flooding and subsequent lowering of the water table has resulted in conversion of *Spartina*-dominated low marsh habitat to monospecific stands of *Phragmites*, whose biomass production has been positively correlated with lower water table height (Hellings and Gallagher 1992). Conversely, removal of tidal restrictions and re-introduction of tidal flooding has resulted in an increase in *Spartina* species in the low marsh, a reduction of *Phragmites* densities and shoot heights, and in some cases, restriction of *Phragmites* to the upland edge (Sinicrope et al. 1990, Roman et al. 2002).

In addition to plant response to changes in water levels, nutrient availability, particularly nitrogen (N) and sometimes phosphorus, can enhance macrophyte growth in brackish estuarine ecosystems (Valiela and Teal 1974, Levine et al. 1998, Mendelssohn et al. 1999, Sundareshwar et al. 2003). Estuarine plants can adjust to sediment fertility by changing their root:shoot ratio (Barko et al. 1991, Otto et al. 1999, Farnsworth and Meyerson 2003), and the direct correlation between available N and biomass varies for different plant species (Howes et al. 1986, Rickey and Anderson 2004). Lower N concentrations have been observed in estuarine porewater associated with *Phragmites* versus *Spartina* species (Chambers 1997, Bart and Hartman 2001, Kohzu et al. 2003, Windham and Ehrenfeld 2003, Windham-Myers 2005). *Phragmites* has been shown to produce greater biomass when urea is added as a fertilizer, while *Spartina pectinata* does not (Rickey and Anderson 2004), and *Phragmites* can exhibit leaf N concentrations more than 2-fold greater than *Spartina* (Farnsworth and Meyerson

2003). These studies suggest that the *in situ* requirement for N may be greater for *Phragmites* than for *Spartina*, and that N concentrations may be a factor in the outcome of interspecific competition (Levine et al. 1998).

The competition for available nutrients between plants and sediment microbes can affect nutrient cycling processes, as evidenced by rhizosphere microbial community response to various organic substrates (Ravit et al. 2006). In samples obtained from undisturbed low marsh creek banks, microbial mineralization of N-containing amino acid substrates was found to be more rapid in *Phragmites* versus *Spartina* or unvegetated sediments (Ravit et al. 2006), suggesting plant-associated differences in microbial community composition and functionality. In undisturbed estuarine sediments, microbial functional activities in vegetated versus unvegetated sediments have been found to differ (Ravit et al. 2003, 2005), as have sediment biogeochemical processes associated with plants from saturated versus drained zones, as evidenced by enzyme activity (Mendelssohn 1981) and substrate utilization patterns (Bagwell and Lovell 2000, Bergholz et al. 2001). Sediment microbial community structure may also vary in saturated versus drained portions of a marsh as evidenced by 16S rRNA gene sequence analysis (Bagwell et al. 2001, Leaphart et al. 2003), and between plant species, as demonstrated using *in situ* hybridization probes (Burke et al. 2002). Conversely, studies analyzing the presence of specific bacterial gene sequences responsible for nitrogen fixation show little differences in root-associated *nifH* genes under drained versus saturated marsh conditions (Piceno et al. 1999, LaRocque et al. 2004), or between *Phragmites* and *Spartina* species (Burke et al. 2002), and Otto et al. (1999) saw no differences in microbial community function in response to N additions.

Under field conditions in estuarine marshes, variations in abiotic factors (hydroperiod, salinity fluctuations, nutrient loadings) make it difficult to separate the effects of specific environmental factors on emergent plants. Similarly, fluctuating abiotic factors make it difficult to determine the effect of plant roots in structuring sediment microbial communities. To determine the response of *Spartina*, *Phragmites*, and their associated sediment microbial communities to drained hydrology and N enrichment under brackish wetland conditions, a greenhouse experiment was conducted. We tested four null hypotheses: 1) Drained hydrology will have no effect on *Spartina* or *Phragmites* biomass production or allocation to root, shoot, or rhizome structures, 2) Drained hydrology will have no effect on

sediment microbial community composition, 3) N enrichment will have no effect on *Spartina* or *Phragmites* biomass production or allocation to root, shoot, or rhizome structures, and 4) N enrichment will have no effect on sediment microbial community composition.

This study allowed us to control the hydroperiod, the salinity level, and the concentration of N in floodwaters, and to focus on the major disturbance factors in estuarine marshes – drainage and N enrichment. We focused on the effects of drained hydrology because *Phragmites* is not typically found in the low marsh zone of undisturbed marshes, and on N because nitrogen is considered to be the major limiting nutrient in estuarine systems and is a salient feature of human-dominated regions (Bertness *et al.* 2002). Because we chose to study plant and microbial response to drainage and N enrichment, we did not test N enrichment under saturated conditions.

## METHODS

Tidal mesocosms were used to simulate three different “Treatments:” 1) saturated and 2) drained hydrology with low concentrations of N, and 3) drained hydrology with high concentrations of N. The low N concentrations were similar to porewater N levels found in creek bank sediments from two undisturbed (Mullica and Maurice) northeastern estuaries (Ravit unpublished data), and the high N concentrations were similar to porewater N concentrations found in creek bank sediments from the anthropogenically affected Saw Mill Creek system in the Hackensack Meadowlands (Ravit unpublished data). After one growing season the experimental units were destructively sampled, and sediment characteristics, macrophyte biomass, and root-associated bacterial community structure as described by sediment PLFAs were analyzed.

### Tidal Mesocosms

Three tidal mesocosms were built to replicate environmental conditions as described previously. “Drained” mesocosms were inundated 2–3 cm above the sediment surface for 1 hr twice daily and allowed to completely drain after each flood event. The sediment surface in the “Saturated” mesocosm was inundated twice daily for one hour on five consecutive days in a 14-day “tidal cycle;” for the remaining nine days brackish water flushed the mesocosm tank, but the water table remained 2–4 cm below the sediment surface (Nuttle 1988). Three individual water reservoirs (Figure 1) supplied

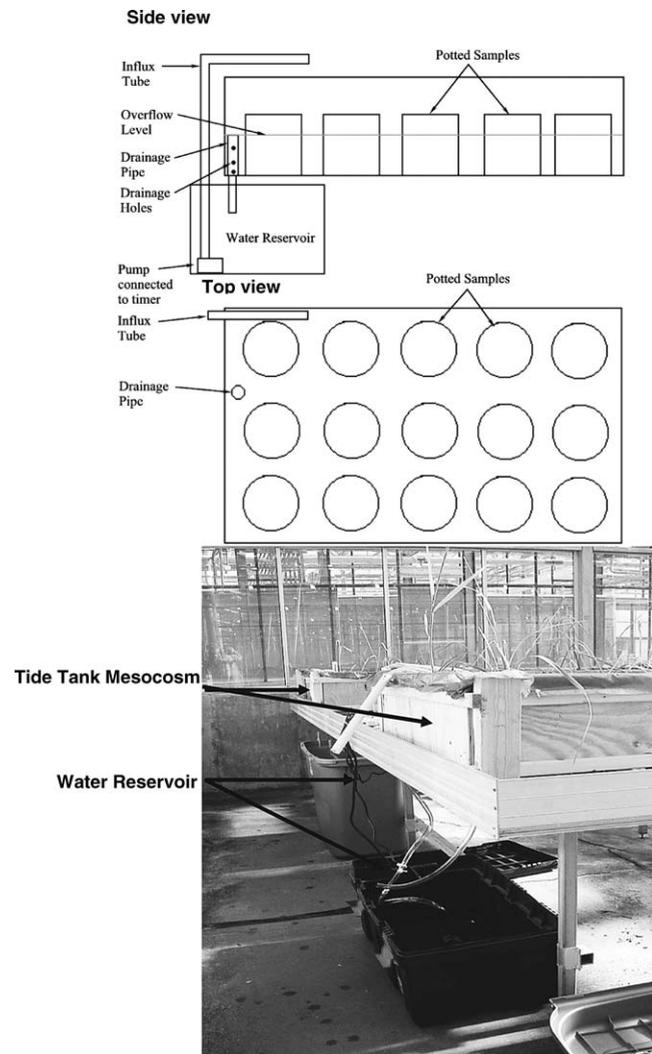


Figure 1. Greenhouse mesocosm system used to controlled conditions of hydroperiod, salinity and nitrogen water concentrations: a) schematic diagram and b) the actual tanks and water reservoirs.

water to flood each mesocosm tank. Reservoir water salinity was maintained at 10 ppt by the addition of Instant Ocean (Aquarium Systems, Sarrebourg, France), which is nitrate and phosphorus free. Reservoir water N levels were maintained by addition of a Miracle Gro (Scotts, Marysville, OH) solution (N:P:K = 15:30:15); low N concentrations equaled  $0.45 \text{ mg NO}_3^- \text{-N L}^{-1}$ ; high N concentrations equaled  $15 \text{ mg NO}_3^- \text{-N L}^{-1}$ .

*Phragmites* rhizomes 10 cm long containing at least two nodes with a new bud tip formation (Bart and Hartman 2001) and *Spartina* tall-form shoots (3–5 cm tall) were obtained from a salt marsh creek bank in Cheesequake State Park, New Jersey, in March and May 2002, respectively. Rhizomes were stored in the dark under moist conditions for two months at  $4^\circ\text{C}$  until planting. Unvegetated intertidal

sediment (TKN content 0.018 mg/g) was obtained in May 2002 from tidal marshes in the Glades Wildlife Preserve, which is located on Delaware Bay in Fortescue, New Jersey, USA. This site, like our Mullica and Maurice River field sites, is distant from industrial land-use and is among the least disturbed tidal marshes in the region.

Prior to planting in the unvegetated sediment, roots and rhizomes were soaked in a 5% hypochlorite bleach solution and then thoroughly rinsed with tap water. Shoots and rhizomes were planted in 20 cm diameter pots perforated on the sides and bottom, which allowed free flow of water. The plants and unvegetated sediment controls ("Species") were maintained in the mesocosm tide tanks for one growing season (five months) under the saturated or drained conditions. Five replicates of each species were maintained in each tank ( $N = 3$  species  $\times$  5 replicates  $\times$  3 tidal tanks = 45). Greenhouse ambient temperatures were maintained at 24°C and daily light energy averaged 158 micro-Einsteins  $m^{-2} sec^{-1}$ .

Six weeks after planting (July 8, 2002), shoots were 25–35 cm tall. During the growing season, weekly measurements of sediment temperature, pH, and  $E_h$  were taken using a portable field probe. Vegetation stem number and stem height were also measured on a weekly basis. At the end of one growing season (October 2002), plants were destructively sampled and separated into shoot, root, and rhizome components, which were then dried to a constant weight. The initially planted *Phragmites* rhizome was removed prior to drying. To determine moisture and organic matter content, sediments were dried to a constant weight prior to being ground to a fine powder. Total organic matter content was determined by loss on ignition after ashing in a 500°C muffle furnace. Microbial community composition was characterized by phospholipid fatty acid (PLFA) analysis. Sediment PLFAs were extracted from each sample using the method of White et al. (1979) as previously described (Ravit et al. 2003).

#### Statistical Analyses

All analyses of variance (ANOVA) and contrast analyses were conducted using SAS GLM (SAS Software, Version 9.1). Two-way ANOVAs [(TREATMENT factor saturated vs. drained or low N vs. N enrichment)  $\times$  (SPECIES factor *Phragmites* and *Spartina*) with  $n = 5$  for each combination of treatment and species] were conducted to test for differences in root, rhizome, and shoot biomass. Two-way ANOVAs [(TREATMENT factor satu-

rated vs. drained or low N vs. N enrichment)  $\times$  (SPECIES factor *Phragmites*, *Spartina*, and unvegetated) with  $n = 5$  for each combination of treatment and species] were conducted to test for differences in sediment moisture and organic matter. When Treatment  $\times$  Species interactions were observed, differences within each treatment were tested using contrast analysis. Repeated measures ANOVAs (Species  $\times$  Treatment  $\times$  Time) were used to test sediment temperature, pH, and  $E_h$  differences. Relative growth rates of plant stem height (RGR<sub>SH</sub>) and stem number (RGR<sub>SN</sub>) were calculated using the following formula (Hunt 1990):  $G = (\text{Log}_e N_2 - \text{Log}_e N_1)/t$ , where  $G$  = the mean rate of increase over the time interval,  $N$  = either the number of stems or the average height of stems in cm, and  $t$  = time (63 days). Growth rates were compared using two-way ANOVAs as above. Post hoc tests of the means were conducted using Tukey's HSD method, and the statistical power of the plant biomass analyses was tested using SAS Factorial ANOVA Power Analysis (SAS Software Version 9.1).

A multivariate data set consisting of 62 PLFAs recovered from the sediment samples was examined using Principal Components Analysis (PCA) to detect patterns among the various treatment-species combinations. PLFA diversity was calculated using the Simpson Index of diversity (PC-Ord, Version 4), and two-way ANOVAs as described above were conducted to test PLFA diversity differences among samples. The six PLFAs responsible for ~80% of the variance in the ordination space of the PLFA loading plot (PC 1) were arcsine square root transformed (PC-Ord, Version 4) prior to using two-factorial ANOVA to test for treatment and species differences.

## RESULTS

### Effects of Hydrology: Drained versus Saturated Sediments (Low N)

*Sediment Characteristics.* Although all experimental units began with the same unvegetated sediment, all sediment parameters differed significantly 93 days after planting as a result of a time  $\times$  treatment interaction. Sediment moisture and organic matter were higher under saturated conditions (Table 1). Redox level ( $E_h$ ) and pH (Table 2) were lower in saturated sediments ( $F_{7,24} = 13.38$ ,  $p < 0.0001$ ;  $F_{7,24} = 2.31$ ,  $p = 0.0198$ , respectively), while temperature in the saturated sediment was 2–4°C higher ( $F_{7,24} = 38.77$ ,  $p < 0.0001$ ).

*Plant Biomass.* Under both drained and saturated conditions, *Spartina* root biomass (Figure 2) was

Table 1. Effect of hydrology and nutrient inputs on sediment characteristics of moisture and organic matter under drained low N (D-LN), saturated low N (S-LN), or drained high N (D-HN) conditions (means and SE). All experimental units were established using unvegetated sediment ("Sediment pre-experiment" = Day 0 characteristics). Means were tested (D-LN compared with S-LN and D-LN compared with D-HN) using two-factorial ANOVA ( $df$ ) = 7, 24. Significant factors are shown and significant differences are indicated by asterisk:  $\alpha \leq 0.05$  = \*;  $\alpha \leq 0.01$  = \*\*;  $\alpha < 0.001$  = \*\*\*.

Treatment	Vegetation	Moisture (%)	Organic Matter (%)
D-LN	<i>Spartina</i>	78 ± 10	8 ± 1.1
	<i>Phragmites</i>	94 ± 24	9 ± 2.5
	Unvegetated	105 ± 9	13 ± 3.9
S-LN	<i>Spartina</i>	142 ± 4.5	15 ± 0.3
	<i>Phragmites</i>	141 ± 16.7	13 ± 1.7
	Unvegetated	156 ± 18.7	15 ± 1.3
Significant	Factor(s)	Treatment*, F = 4.84 Species*, F = 2.87	Treatment*, F = 3.91
D-HN	<i>Spartina</i>	168 ± 6.9	17.1 ± 0.62
	<i>Phragmites</i>	141 ± 12.8	13.3 ± 2.58
	Unvegetated	176 ± 6.5	16.7 ± 0.23
Significant	Factor(s)	Treatment × Species* F = 3.34	Treatment** F = 6.39
Sediment	Pre-experiment	87 ± 6.7	8.7 ± 0.62

significantly greater than that of *Phragmites* ( $F_{3,15} = 4.6$ ,  $p = 0.0178$ ). Under drained conditions, *Spartina* biomass was greater than *Phragmites* for all structures except rhizomes, and these differences were statistically significant at the  $\alpha = 0.10$  level. *Spartina* produced more biomass under saturated hydrology than under drained hydrology [root ( $F = 7.73$ ,  $p = 0.0166$ ), shoot ( $F = 7.35$ ,  $p = 0.0189$ ), total belowground ( $F = 8.16$ ,  $p = 0.0144$ ), total biomass ( $F = 8.95$ ,  $p = 0.0112$ )]. *Phragmites* biomass production for all compartments was higher under drained versus saturated conditions, although these differences were only significant at the  $\alpha = 0.10$  level.

A comparison of the proportion of plant biomass devoted to shoot, rhizome, and roots (Figure 3) shows that *Phragmites* invested proportionally more biomass in shoots and rhizomes, while *Spartina* produced proportionally more roots. *Phragmites*

shoot:root ratio was three-fold greater than *Spartina* under drained hydrology. The shoot RGR<sub>SH</sub> was not significantly different between the two plants (Figure 4). Under drained conditions, the rate of *Spartina* stem production ( $0.08 \pm 0.01$  stems day<sup>-1</sup>) was almost three-fold greater than *Phragmites* ( $0.03 \pm 0.01$  stems day<sup>-1</sup>). Conversely, under saturated conditions both plants produced new stems at the same rate ( $0.04 \pm 0.01$  stems day<sup>-1</sup>).

**Microbial Community Structure.** Principal Components Analysis of sediment PLFAs showed drained versus saturated sediments were clearly separated within the ordination space (Figure 5a), and the PLFA variance reflected in PC1 was due to hydrology ( $F_{4,40} = 45.91$ ,  $p < 0.0001$ ). In vegetated sediments, PLFA diversity was significantly higher under saturated conditions ( $F_{5,40} = 3.73$ ,  $p = 0.0074$ ), but no diversity differences were seen

Table 2. Effect of hydrology and nitrogen inputs on sediment characteristics of temperature, pH, and E<sub>h</sub> based on nine measurements taken over the course of the growing season (July through October). Repeated Measures ANOVA comparisons (significant statistic = Wilks' Lambda) were made between species for the different treatments: drained versus saturated sediments under low N (Hydrology), and low versus high N under drained conditions (Nitrogen). The factor responsible for significant differences between samples is shown and the asterisks indicate p-values at the  $\alpha$  levels ( $< 0.05$  = \*;  $< 0.01$  = \*\*;  $< 0.001$  = \*\*\*). P = *Phragmites*, S = *Spartina*, U = Unvegetated, D = Drained, St = Saturated, HN = High N, LN = Low N.

Sediment Characteristic	Hydrology (Drained vs. Saturated)	Nitrogen (Low N vs. High N)
Temperature	St > D; S = P = U $F_{8,17} = 38.77$ , Time × Treatment***	HN > LN; S = P = U $F_{8,17} = 28.42$ , Time × Treatment***
Redox (E <sub>h</sub> )	St < D; S = P = U $F_{5,20} = 13.38$ , Time × Treatment***	HN < LN; S = P = U $F_{5,20} = 17.26$ , Time × Treatment***
pH	St < D; S = U = P $F_{16,34} = 22.38$ , Time × Treatment**	HN < LN; S < P = U $F_{8,17} = 2.23$ , Time × Species × Treatment*

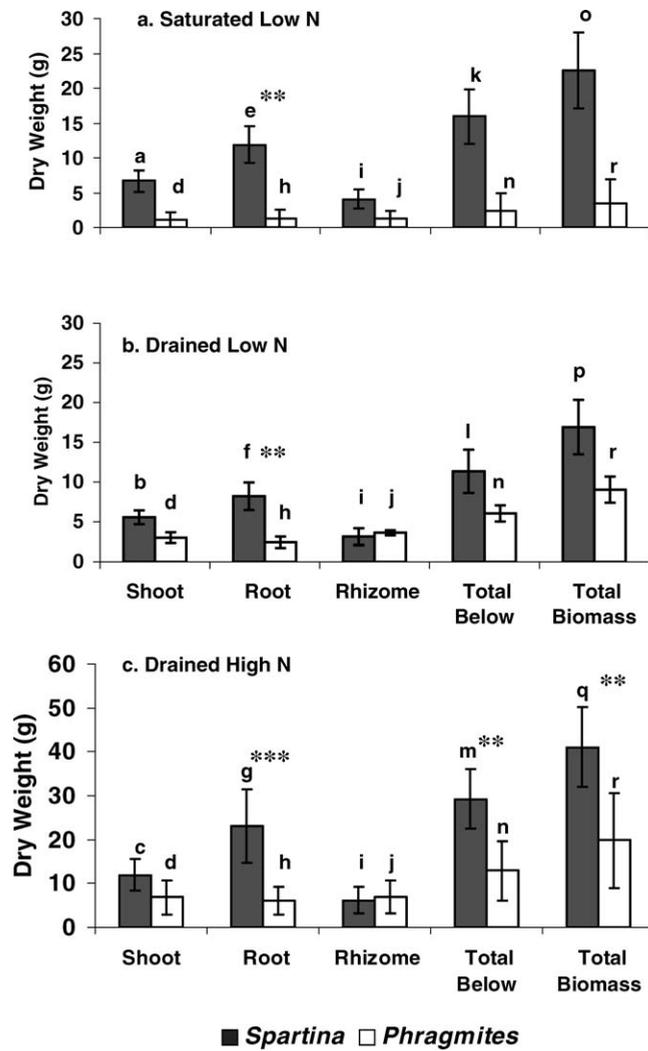


Figure 2. Effects of draining and nitrogen inputs on *Spartina* and *Phragmites* biomass. A comparison of means and (SE) under a) saturated low N, b) drained low N, or c) drained high N treatments. Significant differences within a given treatment between *Spartina* and *Phragmites* at the  $\alpha = 0.05$  (\*),  $\alpha = 0.01$  (\*\*),  $\alpha < 0.0001$  (\*\*\*) level are indicated by asterisks. Significant differences within each species between treatments are indicated with a letter. Note the value of the Y-axis in Fig. 2C is 2-fold greater than the Y-axis in Figs. 2A and 2B.

between the two treatments in unvegetated samples (Figure 6). The loadings of individual PLFAs on PC axis 1 (not shown) indicated that approximately 80% of the difference between samples was associated with six PLFAs (14:0, 15:0 ISO, 15:0 Ante ISO, 16:0, 16:1  $\omega$ 7c, 18:1  $\omega$ 7c). In four cases (PLFAs 15:0 Ante ISO, 16:0, 16:1  $\omega$ 7c, 18:1  $\omega$ 7c), these differences were solely due to hydrology; for only one PLFA (15:0 ISO) was the difference due to the species (Table 3, Figure 7).

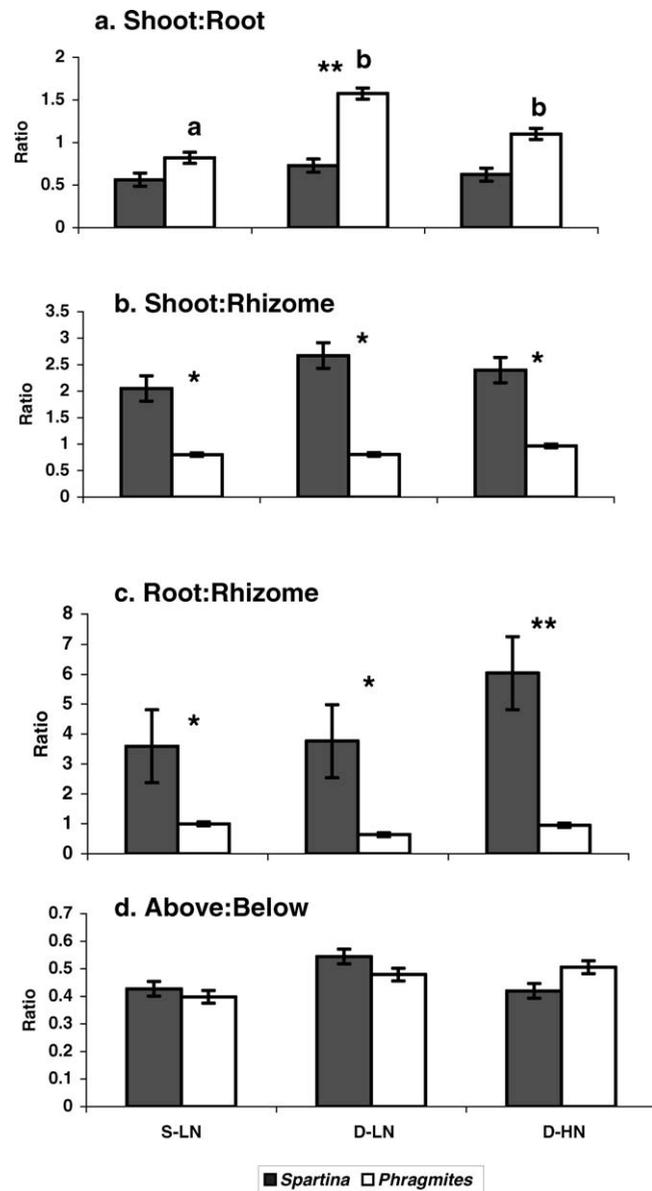


Figure 3. Effects of draining and nitrogen on plant allocation to a) shoot:root, b) shoot:rhizome, c) root:rhizome, and d) above:belowground biomass ratios. Significant differences between *Spartina* and *Phragmites* within a given treatment at the  $\alpha = 0.05$  (\*),  $\alpha = 0.01$  (\*\*) level are indicated by asterisks. Significant species differences between treatments are indicated by letters.

Effects of Nitrogen: Low versus Enriched N (Drained Conditions)

*Sediment Characteristics.* Although all experimental units began with the same unvegetated sediment, 93 days after planting sediment moisture and organic matter were significantly greater in the enriched N treatment (Table 1). The largest plant-associated difference between the two treatments was in the *Spartina* sediments, where moisture and

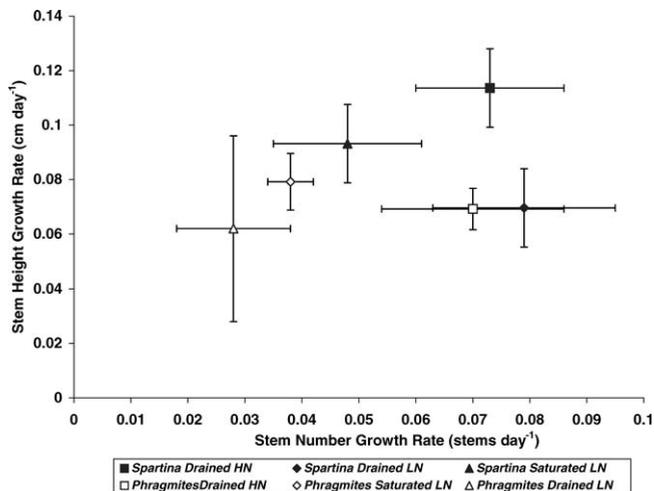


Figure 4. Effects of draining and nitrogen inputs on *Spartina* and *Phragmites* relative stem height growth (RSG<sub>SH</sub>) and relative stem number growth (RSG<sub>SN</sub>). Means and (SE) under drained nitrogen enrichment (HN), drained low nitrogen (LN), and saturated low nitrogen (LN) treatments.

organic matter doubled under N enrichment. Sediment temperature (Table 2) was significantly higher in the enriched N treatment ( $F_{8,17} = 28.42$ ,  $p < 0.0001$ ), while  $E_h$  was significantly lower ( $F_{5,20} = 17.26$ ,  $p < 0.0001$ ). In both cases, the differences in sediment characteristics were due to a Time  $\times$  Treatment interaction. The pH under enriched N was significantly lower than the pH under low N, and the pH in *Spartina* sediments was lower than *Phragmites* and unvegetated pH due to a Time  $\times$  Treatment  $\times$  Species interaction ( $F_{8,17} = 2.23$ ,  $p = 0.024$ ).

**Plant Biomass.** In the enriched N treatment, *Spartina* biomass (Figure 2c) was significantly greater than that of *Phragmites* [two-factorial ANOVA (df = 3,15): root ( $F = 15.15$ ,  $p < 0.0001$ ); total belowground ( $F = 13.27$ ,  $p = 0.0002$ ); total biomass ( $F = 12.59$ ,  $p = 0.0002$ )]. A comparison of the proportion of plant biomass devoted to shoot, rhizome, and roots (Figure 3) shows that *Phragmites* invested proportionally more biomass in shoots, while *Spartina* produced significantly more roots. The *Phragmites* shoot:root ratio was 1 to 1.5, which was two- to three-fold greater than *Spartina*.

The relative growth rate in the number of stems produced by the two plants was equal under N enrichment ( $0.07 \pm 0.01$  stems day<sup>-1</sup>) because *Phragmites* RGR<sub>SN</sub> more than doubled (Figure 4). The increase in RGR<sub>SH</sub> under enriched versus low N was significantly greater for both plant species ( $F_{3,15} = 3.19$ ,  $p = 0.05$ ).

**Microbial Community Structure.** Although sediment PLFAs (Figure 5b) were not as clearly separated in the Principal Components Analysis ordination space as the hydrology samples, when PC1 was analyzed using two-factorial ANOVA, *Spartina* sediment PLFAs were found to differ significantly ( $F_{5,33} = 7.78$ ,  $p < 0.0001$ ) from the *Phragmites* and unvegetated sediments due to both the plant species ( $F_2 = 9.00$ ,  $p = 0.0008$ ) and the N treatment ( $F_1 = 17.60$ ,  $p = 0.0002$ ). The *Spartina* sediment PLFA diversity patterns also differed ( $F_{5,33} = 5.16$ ,  $p = 0.0014$ ) from those of *Phragmites* or unvegetated sediments (Figure 6). Under N enrichment, *Phragmites* sediment PLFAs were more diverse than *Phragmites* low N sediments ( $F_1 = 7.27$ ,  $p = 0.01$ ). However, N enrichment had no effect on *Spartina* sediment diversity ( $F_1 = 8.63$ ,  $p = 0.001$ ), and thus under N enrichment, *Spartina* sediments were less diverse than the other sediments. An analysis of the PLFA loading plot for PC 1 (not shown) indicates approximately 80% of the variation observed between the samples was due to the same six PLFAs responsible for PC 1 variations in the hydrology experiment (14:0, 15:0 ISO, 15:0 Ante ISO, 16:0, 16:1  $\omega 7c$ , 18:1  $\omega 7c$ ). Significant species differences (Table 3, Figure 7) were observed in five of the six PLFAs (15:0 ISO, 15:0 Ante ISO, 16:0, 16:1  $\omega 7c$ , and 18:1  $\omega 7c$ ), and significant differences due to the N enrichment were seen in three of these dominant PLFAs (15:0 ISO, 16:0, and 18:1  $\omega 7c$ ). Four PLFAs (15:0 ISO, 15:0 Ante ISO, 16:1  $\omega 7c$ , and 18:1  $\omega 7c$ ) were significantly higher in *Spartina* sediments.

## DISCUSSION

Both drainage and N enrichment differentially affected the growth and biomass allocation patterns of *Spartina alterniflora* and *Phragmites australis*, as well as the community profile of their root-associated bacteria. Biomass production of both plants was more responsive to N enrichment than to hydrological alteration, but under both conditions *Spartina* biomass was consistently greater than that of *Phragmites* for all plant compartments except rhizomes. A *post hoc* analysis of the plant biomass results (Table 4) revealed that the experimental design of this study may not have provided sufficient analytical power for the biomass analysis. Given this caveat, the biomass patterns indicate that *Spartina* shoot, total below, and total biomass would be higher than *Phragmites* under both hydrologic conditions, and that *Phragmites* biomass would be higher under N enrichment versus low N. The two plants differed in their allocation of biomass to root,

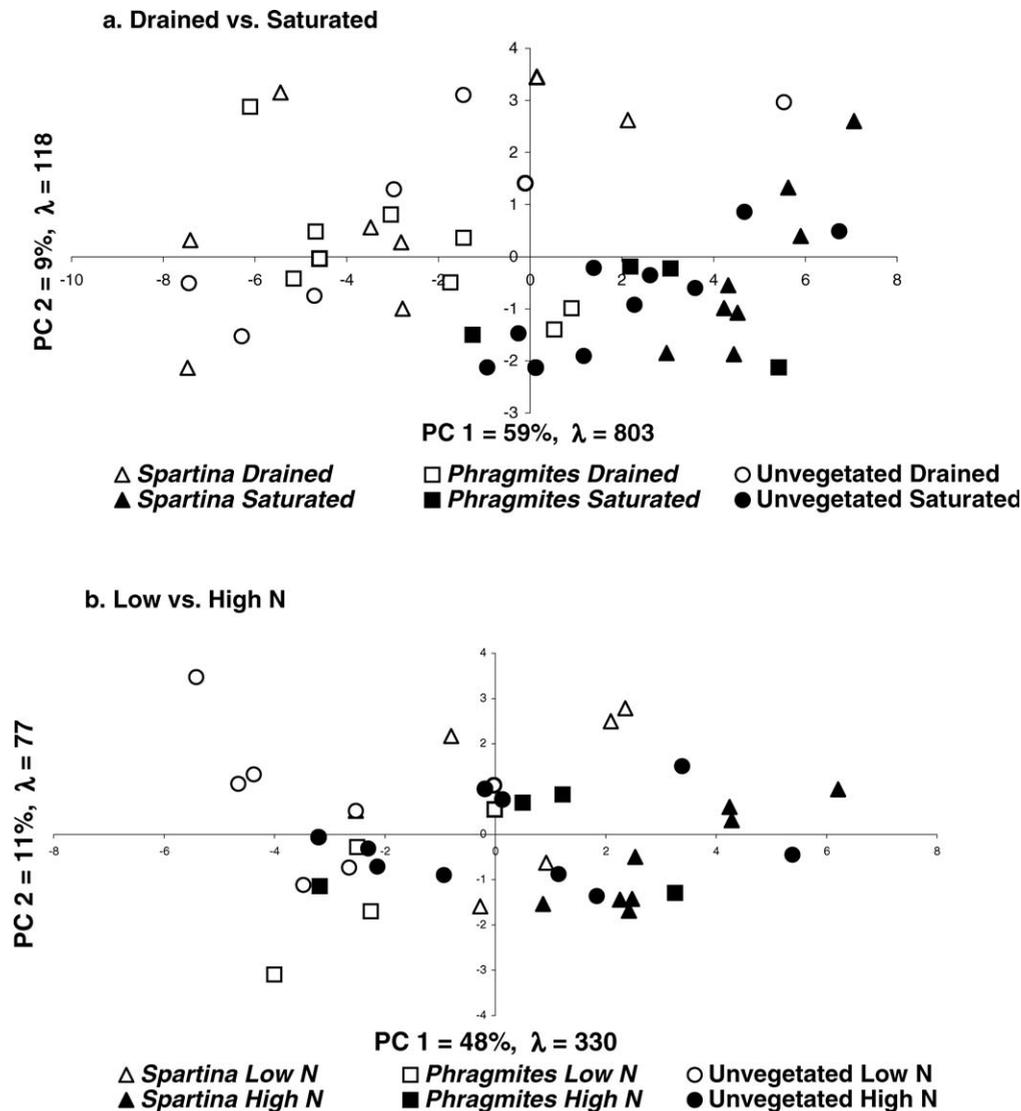


Figure 5. Principal Components Analysis of PLFAs extracted from *Spartina*, *Phragmites*, and unvegetated sediments under a) drained versus saturated hydrology, and b) under low versus high nitrogen treatments. The variation explained is indicated on each axis.

shoot, and rhizome structures. Under drained conditions, *Phragmites* invested proportionally more biomass in shoots, which were produced at more than twice the rate as under saturated conditions. Conversely, *Spartina* biomass ratios were similar under all treatments, suggesting that *Phragmites* may exhibit greater versatility than *Spartina* in responding to environmental conditions.

Under N enrichment, the increase in *Spartina* root biomass did not correspond to an increase in the PLFA diversity of the root-associated bacteria, which was the opposite of the PLFA diversity patterns in *Phragmites* and unvegetated sediments. Freshwater marsh experiments (Otto et al. 1999) have also documented differences in plant response to N enrichment without a corresponding difference

in expression of sediment microbial community functions. These results suggest that while plants may influence the structure of estuarine rhizosphere bacteria under drained hydrologic conditions, the effects of the macrophyte root zone can be overwhelmed by saturated hydrology.

*Sediment Characteristics.* Over the time course of the experiment (one growing season), key sediment characteristics (moisture, organic matter percent, temperature,  $E_h$ , and pH) diverged from their starting conditions under saturated hydrology or N enrichment, which in both cases resulted in an increase in sediment temperatures. Flooding and subsequent drainage results in fluctuating sediment temperatures (Li et al. 2006). It is quite probable

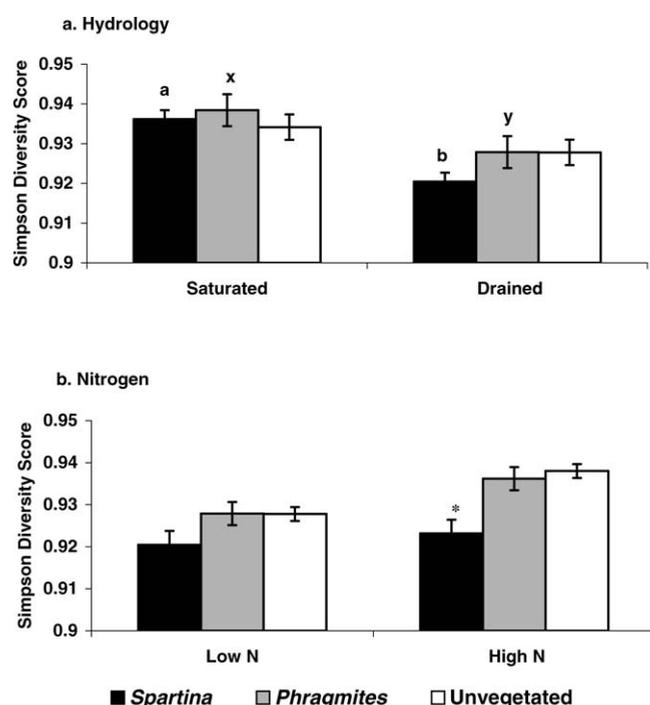


Figure 6. Simpson Diversity Index scores (Mean and SE) for *Spartina*, *Phragmites*, and unvegetated sediments under a) saturated versus drained hydrology, and b) under low versus high nitrogen treatments. Significant differences within a given treatment between *Spartina*, *Phragmites*, and unvegetated sediments at the  $\alpha = 0.05$  level are indicated by asterisks. Significant species differences between treatments are indicated with letters.

that the greater water content seen in the saturated and high N treatments insulated the sediments and stabilized the temperature at a higher level (Coleman and Crossley 2003). Redox levels were lower under saturation and enriched N. This lower  $E_h$  could be related to the higher water content, which precludes

the movement of oxygen into the sediments (Nuttle 1988). The lower  $E_h$  may also be due to the higher amounts of organic material, which upon microbial degradation would increase the biological oxygen demand. Unlike Koretsky *et al.* (2005), who found  $E_h$  to be lower in vegetated sites due to such an increase in organic material, we saw no  $E_h$  differences between vegetated and unvegetated sediment. Conversely, Kostka *et al.* (2002) did not discern geochemical differences between vegetated and unvegetated tidal creek banks, and suggests that plant roots are not the primary factor determining redox state. We note that the organic content of unvegetated sediments was as high as that seen in the vegetated sediments under saturated and high N treatments. It is possible that this was due to the presence of algae and/or microalgae, but since we did not test for the presence of eukaryotes, we can only speculate as to the source of this organic material.

In this experiment, plant species was a factor affecting only one sediment characteristic (pH), which was lower in *Spartina* than *Phragmites* or unvegetated sediments under N enrichment. Field experiments have demonstrated the presence of organic acids, specifically acetate (300–600  $\mu\text{M}$ ) and malonate (25  $\mu\text{M}$ ), associated with *Spartina* growing on drained creek banks (Kostka *et al.* 2002). The release of organic acids from the extensive *Spartina* root system, or the production of acetate by the microbial community (see *Microbial Community Structure* following), may be factors in producing the lower pH seen in this experiment.

*Plants.* *Phragmites* responded positively to N enrichment by almost doubling its number of new shoots, thus increasing the plant's ability to photosynthesize organic compounds that support the

Table 3. Effect of hydrology and nitrogen inputs on sediment microbial community PLFA composition as compared by two-way ANOVAs ( $df = 5,39$ ) of the six PLFAs responsible for 80% of the PCA variation (PC 1) in PLFAs recovered from mesocosm sediments. Drained sediments are compared with saturated sediments under low N conditions (Hydrology), and low versus high N treatments are compared under drained conditions (Nutrient). Factors responsible for significant differences are shown and asterisks indicate p-values at the  $\alpha$  level ( $< 0.05 = *$ ;  $< 0.01 = **$ ;  $< 0.001 = ***$ ). S = *Spartina*, P = *Phragmites*, U = Unvegetated, LN = Low N, HN = High N, D = Drained, St = Saturated.

PLFA	Hydrology (Drained vs. Saturated)	Nutrient (Low vs. High N)
14:0	NS	NS
15:0 Iso (Gram +)	NS	F = 9.11, Species** S > P = U
15:0 Ante Iso (Gram +)	F = 3.43, Species* S = U > P F = 4.40, Treatment* D > St	F = 11.36, Treatment** LN > HN F = 6.17, Species** S = U > P
16:1 $\omega$ 7c (Gram -)	F = 30.37, Treatment***	F = 10.82, Species** S > P = U
16:0 (Ubiquitous)	F = 12.32, Treatment** D > St	F = 13.22, Treatment** LN > HN F = 5.79, Species** S = P > U
18:1 $\omega$ 7c (Anaerobic Gram -)	F = 17.05, Treatment** D > St	F = 7.9, Treatment** LN > HN F = 7.41, Species** S = P > U

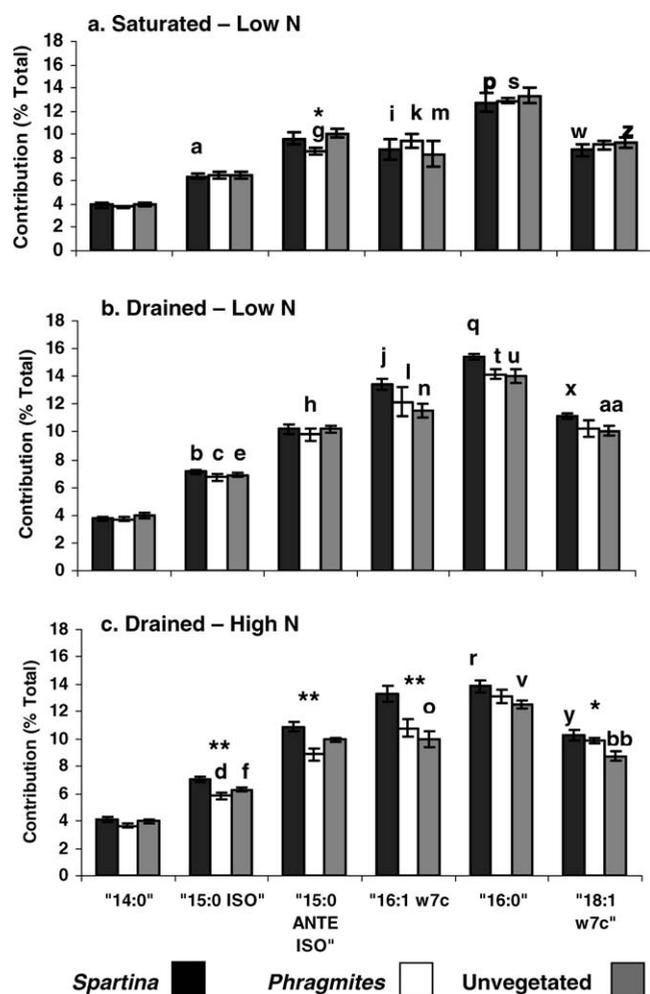


Figure 7. Effects of draining and nitrogen inputs on the six sediment PLFAs responsible for ~80% of the variation within the PCA loading plot ordination space (PC1 – not shown). A comparison of means and (SE) under a) saturated low N, b) drained low N, or c) drained high N conditions. Significant differences within a given treatment between *Spartina* and *Phragmites* and unvegetated sediments are at the  $\alpha = 0.05$  (\*) or  $\alpha = 0.01$  (\*\*) level are indicated by asterisks. Significant differences within each species between treatments are indicated with a letter.

growth of new plant biomass (Dai and Wiegert 1997, Wigand et al. 2004), and improving *Phragmites* ability to shade out competitors. Under N enrichment *Phragmites* rhizome production also more than doubled. This structure allows the plant to spread via clonal growth, transport O<sub>2</sub> over long distances, store carbohydrates and amino acids for future energy needs, and support new shoot growth (Hocking 1989, Armstrong et al. 1996). The ability to proportionally increase production of shoot and rhizome biomass gives *Phragmites* a decided advantage in drained ecosystems with high nutrient inputs (Levine et al. 1998), and increased *Phragmites*

biomass under high nutrient conditions has been documented in various field studies (Hocking 1989, Mendelssohn et al. 1999, Čížková et al. 2001, Šantrůčková et al. 2001, Farnsworth and Meyerson 2003, Rickey and Anderson 2004, Saltonstall and Stevenson 2007).

Based on the single growing season in this study, it is not possible to know if *Phragmites* shoot and rhizome growth would continue through multiple growing seasons, but our results support the hypothesis that the plant is more likely to survive along drained creek banks, a pattern that has been observed in *Phragmites* invasion of the Hackensack Meadowlands (Bart and Hartman 2001). Additional *in situ* factors that we did not measure, such as disturbance (Minchinton and Bertness 2003), or an introduced genotype (Saltonstall and Stevenson 2007), may also enable *Phragmites* to out-compete *Spartina*. However, the fact that *Spartina* biomass was consistently greater than *Phragmites* biomass under drained hydrology and high N would indicate that these two conditions alone are not the only decisive factors in *Phragmites* ability to invade coastal low marshes.

**Microbial Community Structure.** *Spartina* production of significantly greater root biomass, an observation consistent with results obtained from our field experiments (Ravit et al. 2003), provides both a larger surface area for microbial colonization and potentially more organic material available to this community. Yet under drained conditions typical of low marsh creek banks, *Spartina* sediment PLFAs were less diverse than *Phragmites* or unvegetated sediments, regardless of the level of N input. This suggests that factor(s) associated with the *Spartina* root system may favor the dominance of certain microbial species. If this is the case, then under drained conditions the *Spartina* root zone may contribute to structuring sediment microbial populations, resulting in communities that differ from those of *Phragmites* or unvegetated sediments.

The estimates of rhizosphere N-fixation (supported by macrophyte root carbon exudates) in *Spartina* coastal marshes range from 2.6–18.3 g m<sup>-2</sup> yr<sup>-1</sup> (Hocking 1989, Herbert 1999, Tyler et al. 2003), and *Spartina* rhizosphere microbial communities are known to contain a significant number of nitrogen fixing organisms, which appear to be very stable under varying environmental conditions, including high and low nutrient inputs (de Souza and Yoch 1997, Bagwell et al. 1998, Piceno and Lovell 2000 a,b, Lovell et al. 2001). *Spartina* rhizosphere sediments have significantly higher microbial numbers, biomass, and activity of

Table 4. Power analysis of the Tukey's HSD statistical tests used to examine differences between plant species and treatments in the plant biomass analyses.

Variable	Hydrology		Nitrogen	
	$\alpha = .05$	$\alpha = 0.1$	$\alpha = .05$	$\alpha = 0.1$
Shoot				
Treatment	0.173	0.273	0.93	0.969
Vegetation	0.525	0.662	0.73	0.836
Vegetation*Treatment	0.052	0.102	0.135	0.223
Root				
Treatment	0.386	0.524	0.955	0.982
Vegetation	0.874	0.936	0.955	0.999
Vegetation*Treatment	0.054	0.105	0.613	0.741
Rhizome				
Treatment	0.196	0.303	0.634	0.759
Vegetation	0.11	0.188	0.075	0.137
Vegetation*Treatment	0.061	0.117	0.051	0.102
Total Below				
Treatment	0.323	0.455	0.988	0.996
Vegetation	0.4	0.538	0.956	0.982
Vegetation*Treatment	0.05	0.1	0.461	0.601
Total Biomass				
Treatment	0.283	0.409	0.988	0.996
Vegetation	0.446	0.585	0.942	0.975
Vegetation*Treatment	0.05	0.1	0.37	0.507

nitrogen fixing nifH genes compared to *Phragmites* sediments (Burke et al. 2002), and so *Spartina* microbial communities may be less responsive to a short-term N enrichment such as that utilized in this study.

The PLFAs responsible for 80% of the Loading Plot ordination separation are the same PLFAs we have observed as being dominant in creek bank sediments from brackish field sites (Ravit et al. 2006). These six PLFAs are commonly found in Gram negative proteobacteria (Ratledge and Wilkinson 1988), which under estuarine conditions suggest sulfate reducing bacteria (SRB). Sulfate reduction rates have been shown to correlate with *Spartina* biomass production (Hines et al. 1999) and  $\delta$ -proteobacteria SRB may account for 20–30% or more of sediment microbial biomass (Hines et al. 1999, Klepac-Ceraj et al. 2004).

PLFAs 15:0 ISO and 15:0 Ante ISO are also found in Gram positive bacteria (Ratledge and Wilkinson 1988), which in anaerobic sediments suggests the presence of acetogens (Drake 1994), important participants in organic carbon mineralization under anaerobic conditions. These PLFAs were significantly higher in *Spartina* sediments under N enrichment. Genes associated with acetogens have been shown to be present in the rhizosphere of *Spartina*, but not in the rhizosphere of other salt

marsh plants examined (Leaphart et al. 2003). Both SRBs (Nielsen et al. 2001) and acetogens (Drake 1994) have the ability to fix nitrogen, and although it is possible that SRBs, acetogens, or other N-fixing organisms may dominate the *Spartina* rhizosphere community (Gandy and Yoch 1988), accounting for the lower PLFA diversity seen under drained conditions, further molecular studies are needed to understand the interaction between abiotic conditions and the structure and functional activities of rhizosphere sediment bacteria.

*Conclusions.* There is a complex interaction between abiotic conditions, which contribute to structuring sediment biochemical characteristics, salt marsh plants, and their associated sediment microbial communities. *Phragmites australis* growth may be enhanced by drainage and enriched levels of N, but these factors also cause *Spartina alterniflora* to thrive, indicating there are additional factors related to *Phragmites'* ability to invade low marsh elevations. Under high N conditions, *Spartina* sediments exhibited lower bacterial diversity than *Phragmites* or unvegetated sediments, the greatest differences in species-associated microbial communities seen in this study. Further research is needed to determine what effect, if any, lower bacterial diversity has on sediment biogeochemical cycling processes in *Spartina* versus *Phragmites* sediments.

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