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Interactions among Plant Species and Microorganisms in Salt Marsh Sediments

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The interactions among *Spartina patens* and sediment microbial populations and the interactions among *Phragmites australis* and sediment microbial populations were studied at monotypic sites in Piermont Marsh, a salt marsh of the Hudson River north of New York, N.Y., at key times during the growing season. Arbuscular mycorrhizal fungi (AMF) effectively colonized *S. patens* but not *P. australis*, and there were seasonal increases and decreases that coincided with plant growth and senescence (17 and 6% of the *S. patens* root length were colonized, respectively). In sediment samples from the *Spartina* site, the microbial community and specific bacterial populations were at least twice as large in terms of number and biomass as the microbial community and specific bacterial populations in sediment samples from the *Phragmites* site, and peak values occurred during reproduction. Members of the domain *Bacteria*, especially members of the α-, γ-, and δ-subdivisions of the *Proteobacteria*, were the most abundant organisms at both sites throughout the growing season. The populations were generally more dynamic in samples from the *Spartina* site than in samples from the *Phragmites* site. No differences between the two sites and no differences during the growing season were observed when restriction fragment length polymorphism analyses of *nifH* amplicons were performed in an attempt to detect shifts in the diversity of nitrogen-fixing bacteria. Differences were observed only in the patterns generated by PCR or reverse transcription-PCR for samples from the *Spartina* site, suggesting that there were differences in the overall and active populations of nitrogen-fixing bacteria. Regression analyses indicated that there was a positive interaction between members of the δ-subdivision of the *Proteobacteria* and root biomass but not between members of the δ-subdivision of the *Proteobacteria* and macroorganic matter at both sites. In samples from the *Spartina* site, there were indications that there were bacterium-fungus interactions since populations of members of the α-subdivision of the *Proteobacteria* were negatively associated with AMF colonization and populations of members of the γ-subdivision of the *Proteobacteria* were positively associated with AMF colonization.

Coastal salt marshes are among the most productive ecosystems in North America, and productivity that is tidally exported is an important source of carbon and energy for estuarine waters (37, 53). Thus, salt marsh plant communities can have a profound effect on estuarine productivity. These communities are largely dominated by C_4 grasses, such as *Spartina alterniflora* and *Spartina patens* (12), which form different vegetation zones depending on environmental conditions such as salinity, soil anoxia, and nitrogen availability (6, 29). Changes in soil physicochemical conditions that occur because of nutrient enrichment or increasing disturbance due to human activities can alter the pattern of plant zonation. Increased levels and availability of nitrogen, for example, might facilitate the growth of invasive plant species like the highly competitive C_3 grass *Phragmites australis*, which is capable of exploiting enhanced nutrient resources (2), and result in the displacement of native plant species, such as *S. patens*.

In salt marsh systems, below-ground biomass can exceed above-ground productivity as much as 10-fold, making soil microbial communities important primary consumers of plant productivity (51). Estimates of below-ground biomass for Piermont Marsh (New York, N.Y.) have revealed that *S. patens* and *P. australis* produce comparable amounts of biomass over similar surface areas at similar depths (56). However, *S. patens* tissue contains more nitrogen per unit of carbon than *P. australis* tissue contains (56). In addition, the *S. patens* biomass is generally restricted to depths of less than 40 cm and consists of proportionally more roots that provide more labile carbon than the biomass of *P. australis*, which consists of proportionally more thick rhizomes. These rhizomes do not act as absorptive organs for soil nutrients and do not supply large amounts of carbon to the soil except through turnover (9, 56). The presence of plant species with distinct patterns of growth and resource allocation could lead to differences in the proportion of modified bulk soil present and thus could result in different populations or degrees of activity for soil microorganisms (48).

The objective of this study was to analyze the interactions among *S. patens* and sediment microbial populations and the interactions among *P. australis* and sediment microbial populations at key times during the growing season that coincided with major plant phenological stages that previously have been linked to alterations in the soil microbial community (44, 52). In our study, we emphasized analysis of arbuscular mycorrhizal fungi (AMF) (10, 22) that effectively increase the nutrient supply to the host plant (1, 17) and can affect specific soil microbial populations (30). In addition, in situ hybridization combined with image analysis was used to determine the num-

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bers and biomass of organisms at the domain level, as well as specific members of the domain *Bacteria*, such as the α-, β-, γ-, and δ-subdivisions of the *Proteobacteria*, in the sediment. Since many of these bacteria are able to fix molecular nitrogen (3, 4, 14), we attempted to analyze plant-related differences and seasonal shifts in the diversity of nitrogen-fixing bacteria using *nifH*, the structural gene for the key enzyme nitrogenase reductase, as a target for PCR-based typing (restriction fragment length polymorphism [RFLP]) protocols (55).

MATERIALS AND METHODS

Site description and soil sampling. Piermont Marsh (41°02′30″N, 73°55′00″W) is a 500-ha salt marsh located along the western bank of the Hudson River approximately 18 km north of New York, N.Y. This marsh experiences a tidal range of 1 m and has a salinity between 5 and 15 ppt and sediments dominated by organic material. *P. australis* is the dominant plant along the marsh margins, but there are large patches of *Typha angustifolia*, mixed stands of *S. patens* and *Distichlis spicata*, and monotypic stands of *S. patens* in the interior. Beginning on 15 March 2000, soil samples were collected from monotypic stands of *S. patens* and *P. australis* situated approximately 50 m from the Hudson River at the site of previous work examining the seasonal flux of nitrogen in these stands (56). At the *Phragmites* site the places from which samples were collected were at least 2 m from any declining *S. patens* tussocks. Soil samples were collected every 10 weeks during the growing season at times corresponding to major plant phenological stages (dormancy, vegetative growth, reproduction, and senescence). Three 2.5-cm-diameter soil cores from each stand were taken to a depth of 5 cm, and the material obtained at a depth of 1.5 to 3.5 cm (referred to below as the 2.5-cm depth) was retained and used for analysis. Each of the samples was divided into five 1-g portions. One portion was used for root and sediment dry weight determinations, and one was used for an analysis of mycorrhizae. A third portion was fixed in 4% paraformaldehyde in phosphate-buffered saline (57) and used for microbial analyses by in situ hybridization, and the last two portions were frozen at −80°C and used for nucleic acid extraction.

Analysis of environmental characteristics. Immediately after core retrieval, pH, conductivity, temperature, and redox potential were measured at a depth of approximately 2.5 cm with an Oakton pH/mV meter (Cole-Parmer Instrument Company, Vernon Hills, Ill.). In addition, soil temperature data loggers (Tidbit Stowaway; Onset Computers, Brookline, Mass.) were placed in both stands to collect soil temperature data every 15 min during the study period. Pore water was collected with DET (diffusive equilibration in thin films) gel probes (28) that were placed in the marsh sediment to a depth of 10 cm. The gel probes were initially cast in 5-ml plastic pipettes, which were subsequently cut and perforated. Prior to deployment, the gel probes were equilibrated in a 5-ppt artificial seawater solution (Instant Ocean; Aquarium Systems, Inc., Mentor, Ohio) for 4 h. The probes were permitted to adjust to soil conditions for 1 week before they were removed, and the 2.5-cm-depth material was retained for analysis. Immediately after collection, the gels were immersed in 1 ml of distilled water, placed on ice, centrifuged for 1 min at 14,000 × *g* to ensure complete gel immersion, and then back equilibrated for 20 h at 4°C. Concentrations of SO_4^{2-} were determined by a barium chloride turbidity method (24). Concentrations of NH_4^+ were determined by UV-visible spectrophotometry employing the indophenol blue method for seawater samples (27).

Root biomass in soil samples was estimated after the samples were washed in distilled water to separate the sediment and root material. Root material was separated from macroorganic matter by additional washing and floating of live root material in the collecting tube. Root material, macroorganic matter, and soil material were then dried for 4 days at 90°C and weighed. The total water content of a sample (the difference between the original wet weight of the sample and the sum of the three dry weights) was used as an estimate of the soil water content.

Analysis of AMF. The roots used for AMF analysis were stained with trypan blue in lactic acid by using a modification of the procedure outlined by Kormanick and McGraw (26) in which incubation in 5% KOH for 6 h at room temperature was used instead of heat for root clearing. Root samples were mounted on microscope slides, and colonization was determined by the slide mount method of McGonigle et al. (32). A total of 100 intersections were scored for each of 24 slides. The root length colonized by mycorrhizal hyphae, arbuscules, and vesicles was determined by the method of Brundrett et al. (7); hyphae were considered mycorrhizal only if they were visually connected to vesicles or arbuscules.

In situ analysis of microbial communities. Loosely attached bulk soil was separated from root material in the samples used for microbial community analysis. The remaining soil on the root material was then dispersed in 1 ml of 0.1% pyrophosphate in a sonication bath (FS20; Fisher Scientific, Suwanee, Ga.) for 5 min. Bulk soil and dispersed rhizosphere soil were centrifuged at 14,000 × *g* for 1 min, and the liquid was discarded. The pellets were combined and fixed with paraformaldehyde in phosphate-buffered saline (57). Fixed soil samples were stored in 96% ethanol at −20°C at a concentration of approximately 50 mg (wet weight) of soil per ml. Before application to slides, 10-μl portions of the soil samples were dispersed in 990 μl of 0.1% sodium pyrophosphate in distilled water by mild sonication in a sonication bath (FS20; Fisher Scientific) for 5 min. Twenty-microliter portions from each fixed and dispersed sample were spotted onto gelatin-coated slides [0.1% gelatin, 0.01% $KCr(SO_4)_2$], dried at room temperature for about 1 h, and subsequently dehydrated in 50, 80 and 96% ethanol (3 min each) (57).

Hybridizations with fluorescent (Cy3-labeled) oligonucleotide probes EUB338, EUK516, and ARCH915, targeting members of the domains *Bacteria*, *Eukarya*, and *Archaea*, respectively, were performed in 9-μl portions of hybridization buffer (0.9 M NaCl, 20 mM Tris-HCl, 5 mM EDTA, 0.01% sodium dodecyl sulfate; pH 7.2) in the presence of formamide (ARCH915 and EUK516, 20% formamide; EUB338, 30% formamide), 1 μl of the probe (25 ng μl^{-1}), and 1 μl of a solution containing the DNA intercalating dye 4′,6-diamidino-2-phenylindole (DAPI) (200 ng μl^{-1}; Sigma) at 42°C for 2 h (57). The analysis was later expanded to other groups in the domain *Bacteria*, including the α-subdivision of the *Proteobacteria* (probe ALF1b; 10% formamide), the β-subdivision of the *Proteobacteria* (probe BET42a; 30% formamide), the γ-subdivision of the *Proteobacteria* (probe GAM42a; 30% formamide), the δ-subdivision of the *Proteobacteria* (probes SRB385 and SRB385Db; 20% formamide), the high-G+C-content gram-positive bacteria (probe HGC69a; 20% formamide), the low-G+C-content gram-positive bacteria (probe LGCb; 20% formamide), the *Cytophaga-Flavobacterium* cluster (probe CF319a; 35% formamide), and the planctomycetes (probe PLA5a; 30% formamide), as outlined by Zarda et al. (57). The preparations were examined with a Nikon high-resolution inverted microscope (Eclipse TE200) fitted for epifluorescence with a high-pressure mercury bulb (100 W) and filter sets UV-2B (EX330-380, DM400, and BA435 for DAPI) and HQ-Cy3 (G535/50, FT565, and BP610/75 for Cy3) at a magnification of ×1,000 (Nikon CFI Plan-Apo 100×/1.30 oil lens).

Microorganisms were counted in 20 0.01-mm^2 fields selected at random from each sample distributed over a circular 53-mm^2 area. Biomass analysis was performed with images captured with a cooled charge-coupled device three-chip color video camera (DEI-750TD; Optronics) and analyzed by an image analysis procedure based on the software Image-Pro Plus program. Ten images were captured for each sample and were analyzed by using the procedure described by Schönholzer et al. (47). After determination of a mean cell biovolume based on about 100 cells, the total biovolume for microbial populations could be determined by using cell numbers obtained by visual counting. This biovolume was converted into biomass by using the conversion factor 310 fg of C μm^{-3} (13).

RFLP analysis of nitrogen-fixing microbial populations. Three soil cores obtained during each sampling period served as replicates of the soil microbial population in a plant community and were used for DNA extraction. Soil samples were processed as described above for in situ hybridization, and the method used subsequently for DNA extraction was based on a bead beating protocol (23). Released nucleic acids were purified by phenol-chloroform extraction followed by Sephadex 200 column chromatography (8). Purified nucleic acids were divided into two portions. The DNA in one portion (1 μl containing the equivalent of the DNA from 5 mg [dry weight] of soil) was used without further modification as the template in a nested PCR targeting *nifH* performed by using the primers and amplification conditions described by Widmer et al. (55). The second portion (containing the equivalent of the DNA from 250 mg [dry weight] of soil) was treated with 2 μl of DNase I (1 U μl^{-1}; Promega, Madison, Wis.) at 37°C for 45 min and then extracted with phenol-chloroform and precipitated with ethanol, and the pellet was air dried. After resuspension in 24 μl of distilled water, cDNA was transcribed from mRNA from half of the portion by using forward primer nifA and Superscript II reverse transcriptase (Life Technologies, Rockville, Md.) according to the manufacturer's instructions. The reverse primer nifrev was used as a negative control with mRNA from the second half of the portion. Ten percent (2 μl) of each cDNA preparation was subsequently used as a template for nested PCR performed with DNA as the template.

PCR products (371 bp) were used to generate RFLP patterns with restriction endonuclease *Hae*III (55). After gel electrophoretic separation of the different fragments on nondenaturing 7.5% polyacrylamide gels, the gels were stained with silver nitrate by using the procedure described by Bassam et al. (5). Stained gels were scanned with a ScanJet 4200C (Hewlett-Packard, Palo Alto, Calif.). Fragments between 105 and 258 bp long in the images obtained were analyzed by using the GelManager 1.5 program (Biosystematica). GelManager 1.5 em-

ploys the Pearson product moment coefficient (r) to generate a band similarity matrix by using information about the apparent molecular weights of the bands and band spacing. The results were then used to construct unweighted pair group method with arithmetic average (UPGMA) dendrograms.

A comparative analysis could be performed only when DNA was used as the template in a nested PCR. Reverse transcription-PCR (RT-PCR) performed with samples from the *Phragmites* site did not result in amplification products, although the method worked well with samples from the *Spartina* site. Three PCR amplifications were performed for each soil core, and the products of the three amplifications for each sample were analyzed separately to assess within-replicate variability and subsequently combined. RFLP analyses performed with *Hae*III and replicate samples (i.e., amplicons generated by using DNA isolated from replicate cores [$n = 3$] from the same season as the template) always resulted in band pattern similarity values greater than 0.8, and duplicate samples (i.e., amplicons generated by using DNA from the same extraction as the template) always resulted in band pattern similarity values greater than 0.9 (data not shown), indicating that the reproducibility of the results was very high.

Statistical analysis. Two-way analysis of variance (ANOVA) (JMP statistical software; SAS Institute Inc., Cary, N.C.) was used to determine whether there were differences in the soil microbial populations or environmental conditions for the two plant species at four key periods during the growing season (dormancy, vegetative growth, reproduction, and senescence). One-way ANOVA (JMP statistical software) was used to determine differences within plant species during the growing season. Post hoc means comparisons were made by using the least-significant difference, and general linear contrasts (t test) were used to test the significance of specific differences that underlay any higher-order interaction. Linear regressions (JMP statistical software) were used to determine the relationship between microbial populations and SO_4^{2-}, NH_4^+, redox potential, pH, conductivity, temperature, root biomass, macroorganic matter, or AMF colonization. To eliminate the possibility that bacterial populations could influence one another, we also performed linear regressions in which bacterial populations were the independent variable by using the same procedure. Due to the heterogeneous nature of the below-ground landscape, an α of 0.10 was considered significant for linear regression analyses. A statistical comparison of N-fixing populations was performed by using Pearson product moment coefficients calculated within the GelManager 1.5 program. Band similarities greater than 0.7 indicated that there were not significant differences between adjacent patterns, which were assumed to be products of the same population.

RESULTS

Analysis of environmental characteristics. Only small differences in environmental conditions were found for adjacent sites covered by either *S. patens* or *P. australis* during the growing season. While water contents (77 to 86%) and pH values (pH 6 to 7) remained relatively constant over the growing season, other physicochemical characteristics (soil temperature, redox potential, conductivity, pore water concentrations of sulfate and ammonium) displayed seasonal patterns. Peak temperatures of around 30°C were obtained at both sites during reproduction; for the *S. patens* and *P. australis* sites the temperatures were about 11 and 7°C, respectively, during dormancy and 19 and 16°C, respectively, during senescence. A similar profile was obtained for redox potentials, which were always negative. At the *S. patens* site the lowest values were obtained during reproduction (-285 ± 31 mV), and the highest values were obtained during dormancy (-130 ± 9 mV). The values were slightly lower at the *Phragmites* site (-293 ± 4 and -185 ± 4 mV, respectively). At both sites, soil conductivity increased continuously during the growing season from 2.6 ± 0.1 to 11.4 ± 0.6 mS. The pore water concentrations of sulfate increased significantly during the season, from 96 ± 15 to 655 ± 177 mg liter^{-1} following seasonal changes in conductivity. Differences between the sites, however, were not apparent. The ammonium levels declined from about 9.5 ± 2.3 to 2.0 ± 0.5 mg liter^{-1} at both sites during the season.

At a soil depth of 2.5 cm, the root biomass of *S. patens* ranged from 311 ± 52 mg (dry weight) g of soil^{-1} during vegetative growth to 499 ± 67 mg (dry weight) g of soil^{-1} during senescence. No significant seasonal increases were observed for root biomass of *P. australis* due to high within-sample variability (range, 96 ± 20 mg [dry weight] g of soil^{-1} during dormancy to 421 ± 202 mg [dry weight] g of soil^{-1} during senescence). The macroorganic matter content increased from 142 ± 15 to 178 ± 40 mg (dry weight) g of soil^{-1} at the *Spartina* site and then decreased to 137 ± 19 mg (dry weight) g of soil^{-1}. At the *Phragmites* site, slightly greater fluctuations were noted; the values for the dormancy, reproduction, and senescence stages were 76 ± 19, 214 ± 81, and 156 ± 15 mg (dry weight) g of soil $^{-1}$, respectively.

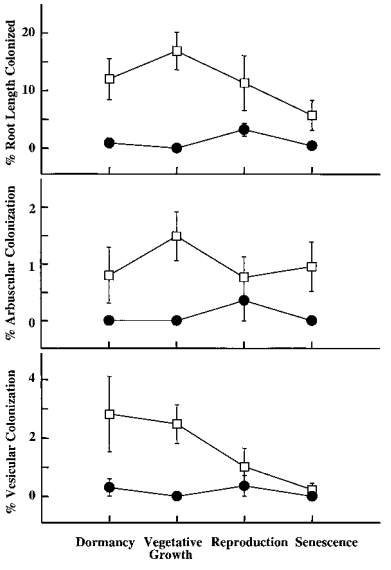

FIG. 1. Seasonal AMF colonization of *S. patens* (□) and *P. australis* (●).

Analysis of AMF. Significantly greater colonization was found on *S. patens* than on *P. australis* when we examined the root length colonized (two-way ANOVA, $F = 16.6$, $P < 0.001$), arbuscular colonization ($F = 12.8$, $P < 0.005$), and vesicular colonization ($F = 10.8$, $P < 0.005$) (Fig. 1). *P. australis* generally did not form AMF arbuscules and vesicles during the season (Fig. 1), and low levels of colonization were observed only at the time of reproduction ($3.2\% \pm 1.1\%$ of the root length was colonized), while *S. patens* formed AMF arbuscules and vesicles during the whole growing season, and both arbuscules and vesicles were encountered in root samples (Fig. 1). Seasonal increases in the root length colonized and arbuscules were noted but were generally not significant. Specific analysis of seasonal differences using t tests revealed that there were significant decreases in the percentage of root length colonized between the time of vegetative growth ($16.9\% \pm 3.3\%$) and the time of senescence ($5.7\% \pm 2.6\%$) (t-stat = 2.7, $P < 0.05$), as well as significant decreases in vesicular colonization during the same time frame (t-stat = 3.2, $P < 0.02$). Vesicular colonization was greatest during plant dormancy ($2.8\% \pm 1.3\%$) and vegetative growth ($2.5\% \pm 0.7\%$) and lowest during senescence ($0.3\% \pm 0.3\%$).

TABLE 1. Numbers of cells for microorganisms in soil covered by *S. patens* or *P. australis* at key stages of the growing season

Analysis	10^7 cells g (dry wt) of soil^{-1}							
	S. patens site				*P. australis* site			
	Dormancy	Vegetative growth	Reproduction	Senescence	Dormancy	Vegetative growth	Reproduction	Senescence
DAPI staining targeting all organisms	3,790 (640)[a]	3,860 (550)	4,980 (440)	3,550 (830)	1,400 (120)	1,770 (90)	2,800 (940)	1,680 (240)
Probes targeting organisms on the domain level								
ARCH915	3 (2)	14 (10)	17 (5)	22 (4)	19 (4)	12 (3)	23 (9)	8 (4)
EUK516	12 (5)	9 (4)	20 (3)	17 (11)	1 (1)	5 (1)	10 (9)	3 (2)
EUB338	1,330 (210)	1,380 (210)	1,370 (80)	1,220 (320)	370 (60)	470 (20)	720 (20)	320 (10)
Probes targeting specific groups of the domain *Bacteria*								
ALF1b	450 (60)	750 (70)	1,030 (50)	660 (20)	170 (40)	140 (10)	360 (120)	70 (40)
BET42a	49 (3)	18 (3)	24 (11)	49 (36)	2 (1)	6 (2)	9 (5)	4 (2)
GAM42a	120 (20)	130 (40)	50 (20)	57 (19)	12 (4)	19 (8)	39 (23)	51 (44)
SRB385	170 (30)	100 (10)	210 (50)	360 (80)	32 (9)	56 (9)	69 (21)	57 (28)
SRB385Db	190 (30)	67 (20)	120 (40)	160 (50)	55 (8)	96 (12)	130 (62)	59 (23)
CFB319a	25 (12)	22 (5)	14 (3)	23 (21)	5 (2)	4 (2)	2 (1)	6 (5)

[a] The values are averages (standard errors).

In situ analysis of microbial communities. The numbers of DAPI-stained cells at the two sites differed significantly (two-way ANOVA, $F = 28.2$, $P < 0.001$); the number of cells in samples from the *Spartina* site was about twice the number of cells in samples from the *Phragmites* site. Seasonal differences were not observed for samples from either plant species (Table 1), although the highest numbers were obtained for samples from both species during reproduction. Similar results were obtained by in situ hybridization with probe EUB338, targeting members of the domain *Bacteria* (Table 1). This probe detected 28 to 35% of the DAPI-stained cells in samples from the *Spartina* site and 18 to 26% of the DAPI-stained cells in samples from the *Phragmites* site. Cells that hybridized with probe EUK516, targeting members of the domain *Eukarya*, and with probe ARCH915, targeting members of the domain *Archaea*, generally accounted for less than 1% of the DAPI-stained cells, and there were no differences between sites (Table 1). Fungal hyphae were detected occasionally with probe EUK516; however, no significant differences between sites were found.

Specific analyses of groups within the domain *Bacteria* generally resulted in detection of higher numbers of cells in samples from the *Spartina* site than in samples from the *Phragmites* site (Table 1). Cells hybridizing to probe ALF1b, targeting members of the α-subdivision of the *Proteobacteria* formed the largest group in samples from both the *Spartina* site and the *Phragmites* site, and there were significant seasonal increases; the numbers of cells peaked during reproduction, and were 75 and 50%, respectively, of the cells detected by EUB338 (Table 1). The numbers of cells detected with probe BET42a, targeting members of the β-subdivision of the *Proteobacteria* were small (1 to 4% of the cells detected by EUB338) in samples from both sites, and there was no seasonal variation (Table 1). Even lower numbers of cells were detected with probes PLA5a, HGC69a, and LGCb; these cells consistently accounted for less than 1% of the cells detected by EUB338. No significant seasonal variation was observed with probe CFB319a, targeting members of the *Cytophaga-Flavobacterium* cluster, and with GAM42a, targeting members of the γ-subdivision of the *Proteobacteria*. When the latter probe was used, however, there was a slight decline in the numbers of cells from the vegetative growth stage to the senescence stage; the numbers of cells detected with this probe decreased from 9 to 4% of the cells detected by EUB338 in samples from the *Spartina* site (Table 1).

Differences between sites were observed when the numbers of cells were determined with probes SRB385 and SRB385Db, targeting members of the δ-subdivision of the *Proteobacteria* (including sulfate-reducing bacteria). The numbers of cells detected with probe SRB385 were lowest in samples from the *Spartina* site during vegetative growth (7% of the cells detected by EUB338) and highest during senescence (30% of the cells detected by EUB338) (Table 1). In samples from the *Phragmites* site, the numbers of these cells remained fairly constant (Table 1). The numbers of cells hybridizing with SRB385Db specifically targeting members of the family *Desulfobacteriaceae* varied much less seasonally (Table 1); the highest numbers of such cells were observed with samples from the *Spartina* site collected during dormancy (14% of the cells detected by EUB338), and the lowest numbers were detected during vegetative growth (5% of the cells detected by EUB33) (t-stat = 3.7, $P < 0.02$). In samples from the *Phragmites* site, the numbers of these cells increased from a low value during dormancy (15% of the cells detected by EUB338) and peaked during reproduction (18% of the cells detected by EUB338), although the increase was not statistically significant (Table 1).

The mean biovolumes of DAPI-stained cells ranged from 0.10 ± 0.03 to 0.13 ± 0.01 μm^3 in samples from the *Spartina* site and from 0.05 ± 0.01 to 0.13 ± 0.06 μm^3 in samples from the *Phragmites* site. The cell biovolume estimates for specific bacterial populations detected by in situ hybridization with probes ALF1b (average biovolume, 0.18 ± 0.03 μm^3), GAM42a (0.30 ± 0.04 μm^3), SRB385 (0.28 ± 0.05 μm^3), and SRB385Db (0.28 ± 0.06 μm^3) were larger than the estimates for DAPI-stained cells, but significant differences between sites and between seasons were not observed. Biomass calculations that were based on cell numbers and average biovolumes, therefore, resulted in patterns similar to those described above for cell numbers.

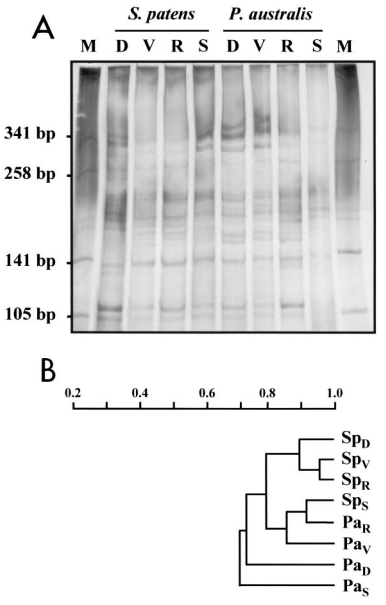

FIG. 2. (A) RFLP analysis of *Hae*III-restricted *nifH* amplicons from DNA extracted from composite bulk and rhizosphere soil obtained at the *S. patens* and *P. australis* sites at a depth of 2.5 cm during major plant phenological stages, including dormancy (lanes D), vegetative growth (lanes V), reproduction (lanes R), and senescence (lanes S). The size marker (lanes M) was pUC19 cleaved with *Sau*3A. (B) Corresponding UPGMA dendrogram based on analysis of fragments that were between 105 and 258 bp long. If the level of similarity between patterns was greater than 0.7, the patterns were not considered different. Sp, *S. patens*; Pa, *P. australis*; D, dormancy; V, vegetative growth; R, reproduction; S, senescence.

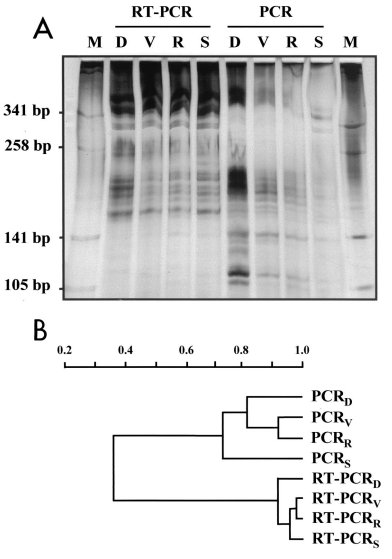

FIG. 3. (A) RFLP analysis of *Hae*III-restricted *nifH* amplicons using mRNA (RT-PCR) or DNA (PCR) extracted from composite bulk and rhizosphere soil obtained at the *S. patens* site at a depth of 2.5 cm during major plant phenological stages, including dormancy (lanes D), vegetative growth (lanes V), reproduction (lanes R), and senescence (lanes S), as the template. The size marker (lanes M) was pUC19 cleaved with *Sau*3A. (B) Corresponding UPGMA dendrogram based on analysis of fragments that were between 105 and 258 bp long. If the level of similarity between patterns was greater than 0.7, the patterns were not considered different. D, dormancy; V, vegetative growth; R, reproduction; S, senescence.

RFLP analysis of nitrogen-fixing microbial populations. RFLP analysis of *nifH* when DNA was used as the template for PCR amplification revealed no significant differences in the RFLP patterns between samples from the two sites (band similarity for all patterns, >0.7) at any point during the growing season (Fig. 2). Samples from the *Spartina* site exhibited similarity values of 0.8 or more at all stages, and the values were within the range of values for replicate samples. Similar results were obtained for samples from the *Phragmites* site, which had similarity values of 0.7 or more (Fig. 2).

RFLP patterns generated after RT-PCR performed with *nifH* mRNA from the *Spartina* site as the template resulted in even less variability between seasons (Fig. 3). Similarity values of more than 0.9 for all samples indicated that the values were not different. Comparative analysis of patterns generated after PCR and RT-PCR showed that the band patterns were similar; however, large differences in the intensities of certain fragments resulted in significant differences, and the levels of similarity between patterns generated after PCR or RT-PCR were less than 0.4 (Fig. 3).

Analysis of interrelationships. Linear regression analysis revealed that there was a significant positive association between the root length colonized by AMF at the *S. patens* site and redox potential ($R^2 = 0.35, P < 0.05$), and more positive redox potentials were associated with greater root length colonized. In addition, a significant positive relationship was observed between root length colonized and vesicular colonization for *Spartina* root samples ($R^2 = 0.76, P < 0.001$). In samples from the *Spartina* site a weakly significant negative relationship was observed for root length colonized by AMF and number of cells hybridizing with probe ALF1b ($R^2 = 0.27, P = 0.08$), but AMF had a weakly significant positive relationship with cells hybridizing with probe GAM42a ($R^2 = 0.26, P = 0.09$) (Fig. 4). In contrast, when samples from the *Phragmites* site were ex-

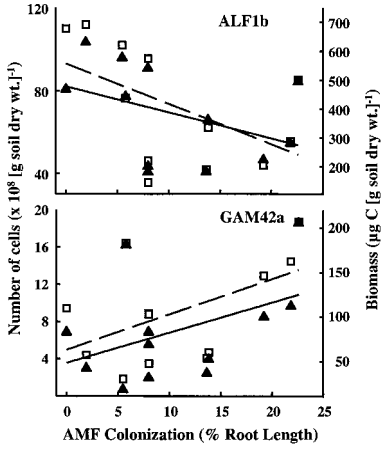

FIG. 4. Linear regression analysis for numbers of cells obtained after in situ hybridization with probes ALF1b and GAM42a for samples associated with *S. patens.* Numbers of cells are indicated by squares and dashed lines, and estimated biomasses are indicated by triangles and solid lines.

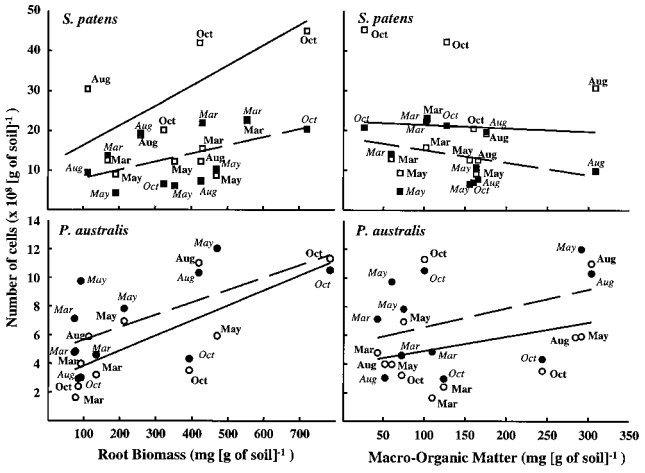

FIG. 5. Linear regression analysis for numbers of cells detected with SRB385 (open symbols, solid lines) and SRB385Db (solid symbols, dashed lines) for samples associated with *S. patens* and *P. australis*.

amined, probes ALF1b and GAM42a did not reveal any significant interrelationships with biotic or abiotic components.

The results were different for cells hybridizing with probes SRB385 and SRB385Db (Fig. 5). In samples from the *Phragmites* site cells hybridizing with probe SRB385 had a significant positive relationship with root biomass ($R^2 = 0.58$, $P < 0.005$), but this was not the case in samples from the *Spartina* site ($R^2 = 0.60$, $P = 0.48$). In samples from the *Spartina* site cells hybridizing with probe SRB385 had a significant positive relationship with the sulfate concentration in pore water ($R^2 = 0.75$, $P < 0.001$). Such a relationship was not evident in samples from the *Phragmites* site (data not shown). Cells detected with probe SRB385Db had significant positive relationships with root biomass for both plant species (for *P. australis*, $R^2 = 0.38$ and $P < 0.05$; for *S. patens*, $R^2 = 0.26$ and $P = 0.09$). No significant relationships were detected between the numbers of cells detected with both probes and soil macroorganic matter content.

DISCUSSION

At Piermont Marsh generally only small differences in environmental conditions (water content, conductivity, pH, temperature, ammonia and sulfate concentrations), as well as in macroorganic matter content and below-ground biomass production, were found at adjacent sites covered by *S. patens* and by *P. australis* during the growing season. Both sites supported microbial populations at levels that compared favorably with estimates obtained by other workers for bacterial population size and biomass at the depth examined (20). The vast majority of cells belonged to the domain *Bacteria*, and only low numbers of *Eukarya* and *Archaea* were detected. Due to the reducing conditions at this depth, protozoans and fungi were not expected to play a significant role numerically, although at the surfaces of estuarine sediments, for example, fungi have been found to account for up to 10% of the microbial biomass (46). The low number of *Archaea* was also anticipated since these organisms are typically outcompeted for energy sources by large populations of sulfate-reducing bacteria in sulfate-rich sediments (20, 25).

Only small differences in microbial community structure were noted between sites when major phylogenetic groups or the functional group of nitrogen-fixing bacteria was analyzed. This lack of variation might reflect relative stability of microbial populations in marsh soils, as demonstrated for diazotrophic bacteria, despite seasonal variation in plant growth or experimental manipulations that reduced C excretion (39–41). However, it might also reflect methodological constraints concerned with the resolving power of the analysis procedures or the sampling strategy used in this study. In our study, the number of cells detected with probe SRB385Db, targeting sulfate-reducing bacteria of the family *Desulfobacteriaceae* (42), showed only weak seasonal variation. A similar result for these sulfate-reducing bacteria was obtained for samples from sediments covered with *S. alterniflora*, while populations in rhizosphere samples showed significant seasonal variation (19), supporting the assumption that populations of sulfate-reducing bacteria rely on plant carbon sources, especially organic acids excreted as root exudates (44, 45). The analysis of composite samples from bulk and rhizosphere soil performed in our study might have masked rhizosphere effects of both plant species, and the results, therefore, more accurately reflect populations of organisms most affected by abiotic factors, such as soil temperature and tidal flooding, rather than populations of organisms affected by plant root characteristics. This assumption is supported by the lack of association between bacterial populations detected with probe SRB385, targeting sulfate-reducing bacteria of the family *Desulfovibrionaceae*, and root biomass in samples from the *Spartina* site, although a positive association between this population and pore water sulfate concentration was noted and accompanied significant seasonal variation. The overall populations detected with probe SRB385 were significantly larger in samples from the *Spartina* site than in samples from the *Phragmites* site, where no seasonal variation was observed for these populations, although the pore water conditions were similar and there was a positive association between the populations detected with probe SRB385 and root biomass.

The contradicting results described above suggest that although specific rhizosphere effects may have been masked by our sampling strategy, there are substantial differences between plant species that effect the dynamics and size of the microbial populations. Although the below-ground biomasses were not significantly different at sites covered by *S. patens* and sites covered by *P. australis,* the different responses of the microbial populations at the two sites might reflect differences in root-rhizome production and carbon allocation to below-ground biomass that affect microbial resource availability. Another differentiating factor between the two sites was the fact that AMF effectively colonized *S. patens*, while little AMF colonization was observed for *P. australis* over the course of the season. An absence of colonization or very low levels of colonization have been reported previously for *P. australis* (49), although other studies showed that there was significant AMF colonization (11, 34). The latter studies suggested that AMF colonization of *P. australis* was particularly sensitive to soil water content, with AMF colonizing plants only in drier sediments (34). In our study, soil flooding might have been the

most prominent environmental factor preventing AMF colonization of *P. australis* but not AMF colonization of *S. patens*, which is commonly found in association with AMF (10, 35). The levels of colonization of *S. patens* roots by AMF in our study are consistent with levels of colonization previously described by other authors (10, 22).

AMF are known to alter root architecture (18) and may reduce the production of extracellular mucilage around plant roots (31), which can have an impact on root sheath development in grasses (38). They can also alter both the distribution and the quality of carbon excreted into the environment by plant roots (15, 43). Since C availability is limited in marsh sediments (33, 54) and AMF act as C sinks within the plant-fungus system (21), the presence of AMF could affect C availability in both the rhizosphere and the surrounding bulk soil. Specific rhizosphere microbial populations could be affected by changes in root exudation due to AMF colonization (30), and bulk soil microbial populations could be affected by the reacquisition of excreted substances by the extraradical mycelium (50). The presence of AMF could therefore intensify microbial competition, leading to smaller microbial populations in the rhizosphere. This hypothesis is supported by studies that showed that the presence of certain species of *Glomus* reduced the biomass of N-fixing microorganisms, a result of competition between root symbionts for C-containing plant exudates (16, 36). In our study, however, the microbial populations in samples from the *Spartina* site were at least twice as large in terms of number and biomass as the microbial populations in samples from the *Phragmites* site, indicating that basic differences in plant growth and carbon allocation strategies are responsible for the observed differences in microbial populations. Yet there are indications that bacterium-fungus interactions involving populations of the α-subdivision of the *Proteobacteria* are negatively associated with AMF colonization and that bacterium-fungus interactions involving the γ-subdivision of the *Proteobacteria* are positively associated with AMF colonization.

Thus, although our results indicate that there are specific interactions between plant species and microorganisms in salt marsh environments, additional work with populations specifically separated from bulk and root-rhizosphere soil may reveal larger differences in these populations, the result of the more important effect of plant root activity on the community in the rhizosphere, and help establish spatial preferences. Future studies should also include investigations of fungal community structure, which was neglected in this study but might also change seasonally and have an impact on microbial communities, as well as specific studies to investigate the effects of AMF colonization on microbial populations.

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