WETLANDS, Vol. 29, No. 1, March 2009, pp. 277–287

EFFECTS OF SALINITY AND NUTRIENTS ON MICROBIAL ASSEMBLAGES IN LOUISIANA WETLAND SEDIMENTS

Colin R. Jackson[1] and Scarlett C. Vallaire[2]

[1]*Department of Biology, Shoemaker Hall, The University of Mississippi, University, Mississippi, USA, 38677. E-mail: cjackson@olemiss.edu*

[2]*Department of Biology, Utah State University, Logan, Utah, USA, 84322*

Abstract: To evaluate the effects of saltwater intrusion and nutrient enrichment on wetland microbial communities, we measured changes in sediment microbial structure and function in response to increased salinity and nutrients. Sediments were collected from a cypress-tupelo swamp near Lake Pontchartrain, Louisiana, USA, and maintained in microcosms treated with elevated salinity, nitrogen (N), or phosphorus (P). Impacts on bacterial community diversity and composition were determined via molecular techniques, while effects on function were assessed through measurement of extracellular enzyme activity. Salinity increased bacterial diversity, P had no effect, while N reduced diversity. Deltaproteobacteria dominated all treatments, although their representation, along with that of the Alphaproteobacteria and Planctomycetes, was reduced following N addition. P addition reduced the proportion of Alphaproteobacteria, while salinity increased the proportion of Betaproteobacteria. Exposure to elevated salinity also decreased phosphatase and N-acetlyglucosaminidase activity by almost 20%, with less effect on ß-glucosidase. P addition had no impact on extracellular enzyme activity. Overall, exposure to elevated salinity depresses microbial function and changes the sediment microbial assemblage. These wetlands are likely N-limited, and while N additions may regenerate plant communities, they also change the structure of the sediment microbial community, decreasing diversity and impacting the mineralization of other nutrients.

Key Words: bacterial community structure, enzyme activity, nitrogen, phosphorus

INTRODUCTION

Microorganisms are vital components of wetlands, responsible for key processes in organic matter decomposition and nutrient cycling (Brinson et al. 1981, Wetzel 1992). Despite their importance, few studies have examined the composition or activity of wetland bacterial communities, largely because of methodological constraints. Until the emergence of molecular techniques, accurate assessment of the structure of bacterial communities in natural environments was limited (Pace 1997). By extension, the impacts of changing environmental conditions on bacterial communities have also been difficult to assess. Despite these difficulties, the high surface area to volume ratio of microorganisms means that they are sensitive indicators of environmental change (McArthur 2001) and patterns in microbial community structure could prove useful as bioindicators of degradation in wetlands (Merkley et al. 2004). Functional aspects of microbial assemblages, such as extracellular enzyme activity, can also serve as indicators of ecosystem health (Hinojosa et al. 2004, Hill et al. 2006), and enzyme activities have been measured in various wetland sediments (e.g., Jackson et al. 1995, Wright and Reddy 2001, Corstanje et al. 2007, Jackson and Vallaire 2007).

Little is known of the microbial communities in Louisiana coastal wetlands, or how microorganisms might respond to changes that these wetlands are undergoing. One of the largest areas of freshwater wetlands along the U.S. Gulf Coast occurs around Lake Pontchartrain and Lake Maurepas, Louisiana (Keddy et al. 2007). These wetlands comprise the southeastern extension of the Mississippi alluvial plain, one of the world's largest wetland systems (Shaffer et al. 2005). The area includes almost 1,500 km^2 of wetlands, largely baldcypress (*Taxodium distichum* (L.)) and water tupelo (*Nyssa aquatica* (L.)) swamp. Historically, these wetlands received annual inputs of nutrients and sediment from the Mississippi River. However, channelization and levee construction now prevent this from occurring, and these forested swamps are steadily being converted to macrophyte-dominated marsh and open water (Barras et al. 1994, Keddy et al. 2007).

The leading causes of wetlands loss in coastal Louisiana are saltwater intrusion events during tropical storms and hurricanes (Keddy et al. 2007). Even modest increases in salinity and flooding adversely affect the physiology of plant species which can lead to changes in ecosystem structure (Pezeshki et al. 1989, McLeod et al. 1996). Coupled with long-term vegetation changes associated with sea-level rise (Shirley and Battaglia 2006), it is becoming critically important to assess the impacts of salinity on different components of these wetlands. These systems are also stressed by nutrient limitation (a consequence of reduced inputs from the Mississippi River), and field-scale experimental fertilizer and sediment additions have resulted in significant increases in macrophyte biomass (Keddy et al. 2007). Restoration efforts for the Lake Pontchartrain-Lake Maurepas wetlands focus on freshwater diversions from the Mississippi River and the addition of treated wastewater to address the issues of nutrient limitation and increasing salinity (Keddy et al. 2007). However these efforts are based on studies of wetland vegetation and little is known of how either salinity or nutrient status impact benthic processes or sediment bacterial communities in these wetlands.

Assessing the direct impacts of salinity and nutrient levels on the sediment microbial community is difficult in the field, as either factor would also impact other components of the system (primarily vegetation), and indirectly influence microbial processes. In this study we used a microcosm approach to specifically examine the effects of increased salinity or nutrient levels (N, P) on microbial enzyme activities and benthic bacterial community structure in wetland sediments collected from a cypress-tupelo swamp in the Lake Pontchartrain Basin. As well as examining the effects of each treatment individually, we also examined how a combination of increases in all three factors (salinity, N, and P) might change wetland microbial assemblages. While such conditions (elevated nutrients and salinity) are unlikely to occur in these systems under current conditions, they could potentially occur under future restoration efforts.

METHODS

Experimental Setup

Sediment was collected on 15 August 2005 from the Joyce Wildlife Management Area, an area of cypress-tupelo swamp 10 km north of Lake Pontchartrain, southeastern Louisiana, USA, in which we have previously linked microbial activity to rates of organic matter decomposition and conducted initial surveys of sediment bacterial communities (Jackson and Vallaire 2007, Smart et al. 2008). The area consists largely of secondary baldcypress (*T. distichum*) that has regrown in the area following extensive logging until the early 1900's (Jackson and Vallaire 2007). The system is freshwater (salinity typically 0.3–0.4 ppt), but is hydrologically connected to Lake Pontchartrain and experiences salinity increases from saltwater intrusion during tropical storms and hurricanes. Water depth at time of collection was 2–3 cm, with pH 5.7. Dissolved inorganic nutrient levels in overlying water were < 0.1 mg L^{-1} $N\text{-}NO_3^-$, < 0.05 mg L^{-1} $N\text{-}NO_2^-$, < 0.1 mg L^{-1} $N\text{-}NH_4^+$, and 0.2 mg L^{-1} PO_4^{3-}. Sediment was collected by scooping loose surface material (approximately the top 5 cm) and overlying water into an insulated plastic chest, and immediately transported to the laboratory.

Sediment was mixed and 400 ml dispensed into each of 54 glass beakers (''microcosms''). Treatment conditions were applied following a $2 \times 3 \times 3$ factorial design with variables being salinity, phosphorous, and nitrogen. Salinity was applied as an addition of 40 ml of a sterile artificial seawater solution (27.35 g NaCl, 3.13 g $MgSO_4$, 1.47 g $CaCl_2$, 4.07 g $MgCl_2$, 0.75 g KCl, 1 L H_2O) to bring salinity to 3.5 ppt. This level is typical of increases that can occur in this system during both saltwater intrusion events and periods of drought (Keddy et al. 2007). Microcosms at ambient salinity received 40 ml of water collected on site and subsequently sterilized. Phosphorous was added as KH_2PO_4 to final dissolved inorganic concentrations of +0.5 mg L^{-1} (low P addition) or +5.0 mg L^{-1} (high P addition) over ambient in the overlying water. Nitrogen was added as NH_4NO_3 to dissolved inorganic concentrations of +5.0 mg L^{-1} (low N addition) or +50 mg L^{-1} (high N addition) over ambient in the overlying water. These levels are high for natural systems but span the range of soluble N and P concentrations found in municipal wastewater (Sedlak 1991). Both nutrients were added from concentrated stock solutions so that additions were 2 ml or less, and untreated microcosms served as controls at ambient nutrient levels. Thus, our experimental treatments consisted of microcosms under all possible combinations of two levels of salinity (ambient, elevated), three levels of phosphorous (ambient, low additional P, high additional P), and three levels of nitrogen (ambient, low additional N, high additional N), with three replicate microcosms per treatment. Microcosms were loosely covered to reduce evaporation but still allow air circulation, and maintained at 22°C for 55 days.

Molecular Analyses of Bacterial Community Structure

After 55 days, sediment (5 ml) was collected from each microcosm for molecular analyses of bacterial community structure. Given the number of treatment combinations and replicates, and the results of the enzyme analyses (see below), we focused the molecular analyses on the controls, each treatment (salinity, N, or P) alone, and the combination of all three treatments together (i.e., we excluded the pair wise combinations). DNA was extracted from each sediment sample using a modified version of a protocol originally designed for soil (Zhou et al. 1996) but also used for aquatic sediments (Jackson et al. 2001). Residual humic contamination was removed from extractions using spin columns (Jackson et al. 1997).

DNA from each sample was used as a template in two sets of PCR amplifications using primers specific for Bacteria. One set of amplifications used Bac1070f and Univ1392r primers (Ferris et al. 1996, Jackson et al. 2001) to amplify a 323 base long region of the 16S rRNA gene. Amplification reactions and conditions were as previously described (Jackson et al. 2001) and the Univ1392r primer includes a GC-clamp to facilitate the analysis of amplification products by denaturing gradient gel electrophoresis (DGGE). PCR products were analyzed using DGGE along 40–70% urea-formamide gradients in 8% acrylamide gels. DGGE gels were run at 70 V and 60°C for 18 hours, stained, and community profiles examined using a gel imaging system.

The second set of amplifications used Bac8f and Univ1492r primers (Jackson et al. 2001) to amplify a larger portion of the 16S rRNA gene. PCR products from replicate microcosms under the same treatment were pooled, cloned into artificial plasmid vectors, and a clone library established (five libraries total, representing control, salinity, high N, high P, and the combination salinity + high N + high P treatments). 16S rRNA gene inserts from 96 clones in each library were amplified and separated into ribotypes using restriction enzyme digests (*Eco*RI, *Rsa*I, and *Hae*II; Jackson et al. 2006). The frequency of ribotypes in each clone library were used to estimate overall bacterial diversity in each sediment sample as the non-parametric diversity index S_{Chao1} (Chao 1987) using a web-based application (Kemp and Aller 2004a).

The first 600–700 base pairs of selected ribotypes (primarily those represented by multiple clones, representing the most abundant bacterial populations in each community) were sequenced and sequences entered into ARB software (Ludwig et al. 2004). Sequences were aligned and phylogenetic relationships determined using the same methods as Jackson et al. (2006). A phylogenetic tree showing relationships between the sequenced ribotypes was exported from ARB into UniFrac (Lozupone and Knight 2005, Lozupone et al. 2006), a web application that tests for differences among clone libraries from multiple bacterial communities based on 16S rRNA phylogenetic relationships. Within the UniFrac application we performed a number of tests to determine if either nutrient or salinity treatments had resulted in shifts in the dominant wetland sediment bacterial populations. Broad differences between communities were tested using the UniFrac metric, which tests if differences between communities are due to chance or reflect evolutionary adaptation to different conditions (Lozupone and Knight 2005). Values obtained were used to generate an environmental distance matrix reflecting each pairwise combination of treatments, which was used in principle components analysis (PCA) to explore potential patterns in the response of the sediment bacterial community to elevated salinity and nutrient levels. A more thorough description of the multivariate approaches used by UniFrac in the analysis of bacterial communities is given by Lozupone et al. (2006).

Microbial Extracellular Enzyme Activity

Sediment microbial enzyme activities in each microcosm were assayed using procedures that have been commonly used in the analysis of soils and sediments (e.g., Jackson et al. 1995, Alvarez and Guerrero 2000, Jackson and Vallaire 2007). Surface sediment (5 ml) was collected from each microcosm and brought to a total of 15 ml in 50 mM pH 5.5 acetate buffer. This suspension was homogenized and four 150 μl replicates were mixed with 150 μl of substrate solution in deep-well microtiter plates. *p*NP-β-D-glucopyranoside, *p*NP-N-acetyl-β-D-glucosaminide, *p*NP-phosphate were used at 5 mM to assay for the activity of β-1,4-glucosidase, β-N-acetylglucosaminidase (NAGase), and acid phosphatase, respectively. Duplicate analytical controls consisted of 150 μl of sample suspension with 150 μl of acetate buffer. Following incubation (25°C, 2–4 h) plates were centrifuged (5 min, 1,000 g) to pellet particles and 150 μl of the supernatant transferred to a microplate and mixed with 150 μl 0.067 M NaOH. Absorbance of the colored end product (*p*NP) was determined using a microplate reader and adjusted according to controls. Enzyme activity was calculated by dividing the adjusted absorbance by 16.9 the absorbance of 1 μmole *p*NP under these specific

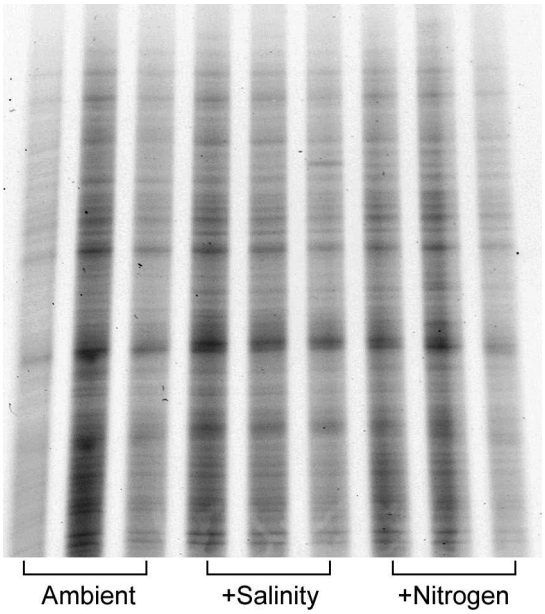

Figure 1. Denaturing gradient gel electrophoresis (DGGE) analysis of bacterial communities in wetland sediments after 55 days in microcosms under different treatments: ambient, elevated salinity (to 3.5 ppt), or elevated nitrogen (+50 mg L^{-1}). Each vertical lane represents the bacterial community present in one replicate microcosm (3 replicates per treatment), and each band represents one distinct genetic type. Because the banding patterns are complex, all nine samples appear to have similar, diverse bacterial communities when analyzed by DGGE, as did other treatment combinations (not shown).

assay conditions; Jackson et al. 2006). An additional 5 ml sample of sediment was collected from each microcosm on each date and weighed, dried (65°C, 48 h), and ashed 500°C, 2 h) so that enzyme activity could be expressed per g OM (i.e. μmole substrate consumed h^{-1} g OM^{-1}). Statistical comparisons of enzyme activity under different treatments on each date were analyzed by three-way factorial analysis of variance (ANOVA) and post-hoc tests (Tukey's HSD) using SPSS 15.0 software.

RESULTS

Molecular Analysis of Bacterial Community Structure

DGGE analysis of the bacterial community present in wetland microcosms showed no clear impact of any treatment. DGGE profiles were complex, contained at least 35–40 visible bands, and repeatedly showed the same banding patterns regardless of treatment (Figure 1). While replicate microcosms for each treatment had the same banding patterns, the large number of bands suggested that this method would not reliably detect changes in community structure for these samples.

Larger (bases 8–1492) 16S rRNA fragments were amplified from representative microcosms for each of the control, salinity, high N, high P, and salinity + high N + high P treatments. Analysis of clone libraries generated from each microcosm showed appreciable bacterial diversity, with S_{Chao1} richness estimates suggesting 100–200 distinct phylogenetic types (Table 1). Richness was increased under the salinity treatment (S_{Chao1} of 215 compared to 121 under ambient conditions), while the other treatments all reduced S_{Chao1}. This reduction was most pronounced for the high N treatment (S_{Chao1} of 79).

Sequencing of representative clone libraries revealed differences in the composition of sediment bacterial communities under each treatment (Figure 2). Representative of four subdivisions of the Proteobacteria (Alpha-, Beta-, Gamma-, and Delta-) were detected in each of the sampled microcosms, and the Deltaproteobacteria were typically the most abundant lineage, accounting for 25–30% of the sequences in our clone libraries. Exceptions were the microcosms that received N addition (either alone or combined with other treatments), where the proportional abundance of Deltaproteobacteria was 15%. N addition, alone or in combination with other treatments also reduced the proportion of Alphaproteobacteria and Planctomycetes. N also increased the representation of the Nitrospira from barely detectable levels to almost 10% of the 16S rRNA sequences sampled (Figure 2). Elevated P reduced the proportion of Alphaproteobacteria, and P treatments (alone or in combination) also increased the representation of the Verrucomicrobia. The effects of salinity on overall community composition were less clear, with increased salinity alone producing changes that weren't always seen

Table 1. Community characteristics of bacterial assemblages in wetland sediments in microcosms amended with increased salinity (to 3.5 ppt), phosphorous (+5 mg P L^{-1} as KH_2PO_4), nitrogen (+50 mg N L^{-1} as NH_4NO_3), or a combination of all three. Indices are derived from 16S rRNA clone libraries generated from each treatment and represent estimates of species diversity (S_{Chao1}) and the proportion of the community sampled (Coverage).

Treatment	S_{Chao1}	Coverage
Ambient	121	0.41
Salinity	215	0.24
Phosphorous	113	0.39
Nitrogen	79	0.48
Combination	90	0.52

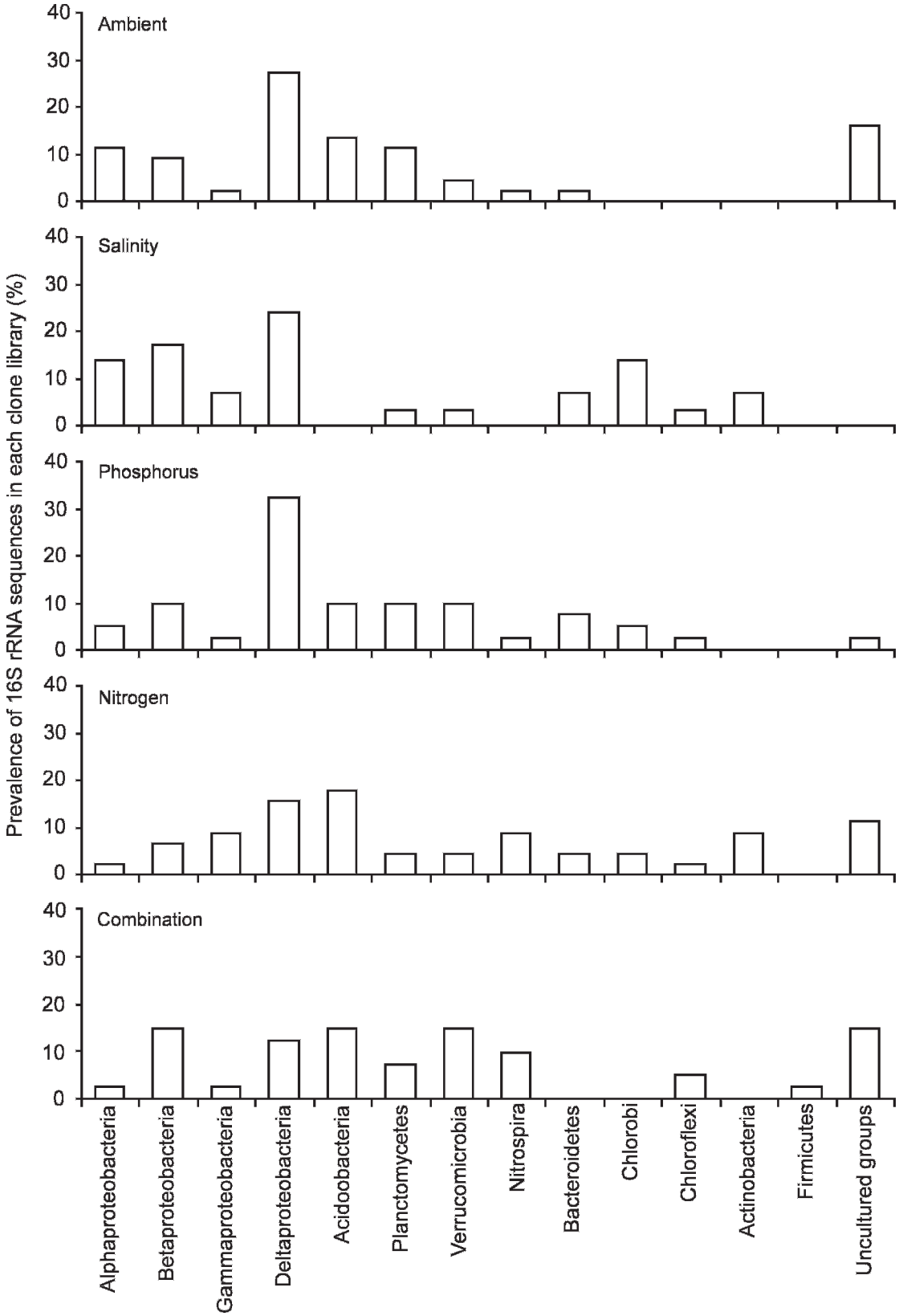

Figure 2. Representation of major bacterial lineages in 16S rRNA clone libraries generated from PCR-amplified DNA extracted from wetland sediment microcosms under ambient conditions (Ambient) or amended with salinity to 3.5 ppt (Salinity), +5 mg P L^{-1} as KH_2PO_4 (Phosphorus), +50 mg N L^{-1} as NH_4NO_3 (Nitrogen), or a combination of salinity, nitrogen, and phosphorus (Combination). Values are % of 16S rRNA gene sequences affiliated with that lineage in a particular clone library.

under the combination treatment. No 16S rRNA sequences affiliated with the Acidobacteria were detected in the salinity alone treatment, compared to that lineage accounting for 14% of the clone library under ambient conditions. However, they accounted for the same proportion of the clone library when salinity was increased in combination with N and P (Figure 2). Conversely, the Chlorobi were not detected in either ambient or combination treatment microcosms, but accounted for 14% of sequences sampled in the salinity alone treatment. Exposure to elevated salinity increased the proportion of Betaproteobacteria in the 16S rRNA sequences sampled, and this increase also occurred when salinity was raised in combination with N and P (Figure 2).

UniFrac analysis of differences between the bacterial 16S rRNA gene sequences obtained from each microcosm community showed no statistically significant differences (UniFrac metric, $p > 0.1$) between any pair of communities. Distance matrices between the different microcosms suggested some patterns in the distribution of 16S rRNA sequences between them, and PCA separated the five clone libraries (Figure 3). The first component (horizontal axis) accounted for 32.5% of the variation and separated the N-amended and combination (salinity + N + P) microcosm communities from the others. The second component (vertical axis) accounted for 26% of the variation and distinguished the salinity and combination treatments from the ambient and nutrient only microcosms (Figure 3).

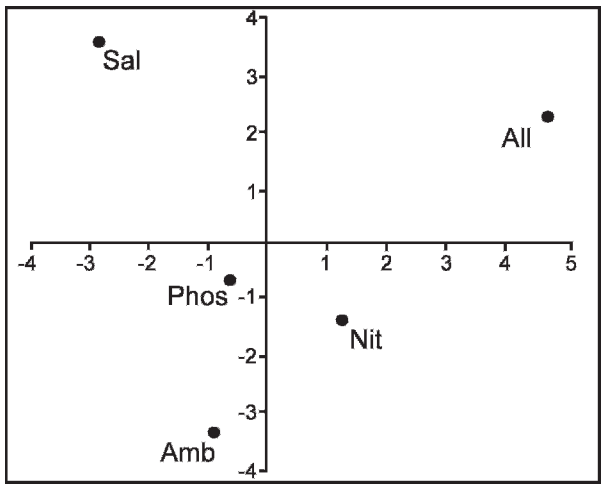

Figure 3. Principle components analysis (PCA) of bacterial community structure under different treatments: ambient conditions (Amb), elevated salinity (Sal), elevated nitrogen (Nit), elevated phosphorus (Pho), or a combination of salinity, nitrogen, and phosphorus (All). The results of PCA are displayed along the horizontal (principle component 1, 32.0% of variation) and vertical (principle component 2, 26.5% of variation) axis. Differences in community structure were evaluated using UniFrac software to examine phylogenetic relationships between dominant 16S rRNA gene sequence types present under each treatment.

Microbial Extracellular Enzyme Activity

Three-factorial ANOVA revealed no interactions among the three treatments ($p > 0.05$ for all) on the activity of any microbial enzyme, although there were some individual effects of each treatment on microbial enzyme activity. Salinity decreased the activities of NAGase and acid phosphatase by almost 20% (Figure 4; $F = 15.81$ and 26.69, respectively, d.f. = 1, 53, $p < 0.001$ for each). Salinity also appeared to decrease β-glucosidase activity (Figure 4) but this was just outside of our significance criteria ($p = 0.09$). P amendment had no affect on the activity of any of the three enzymes, while the high N treatment increased acid phosphatase activity by 20% (Figure 4; $F = 10.95$, d.f. = 2, 53, $p < 0.001$).

DISCUSSION

Increasing salinisation is a major concern to aquatic environments worldwide and can arise from short-term saltwater intrusion events, mid-term periods of drought and low water, and long term changes associated with global climate change and sea level rise (Williams 1999, Nielsen et al. 2003, Keddy et al. 2007). Coastal wetlands in Louisiana are exposed to salinity through all of these mechanisms, and while we examined changes that are typical of saltwater intrusion events (raising salinity to 3.5 ppt), the duration of the study (55 days) is also representative of droughts that can raise salinity further (Keddy et al. 2007). Increased salinity lowered phosphatase and NAGase activity by almost 20% compared to controls. Activities of these enzymes are closely tied to rates of P and N mineralization (McGill and Cole 1981, Olander and Vitousek 2000, Kang et al. 2005), and changes in enzyme activity likely resulted in changes in nutrient cycling. Similarly, β-glucosidase activity has been linked to rates of organic matter decomposition in these and other wetlands (Jackson et al. 1995, Jackson and Vallaire 2007), and while ß-glucosidase activity was not statisticall different between salinity treatments and controls ($p = 0.09$), there was the suggestion that salinity slightly reduced activity. Wetlands exposed to elevated salinity would already be experiencing the effects of saltwater stress on vegetation (Pezeshki et al. 1989, Baldwin and

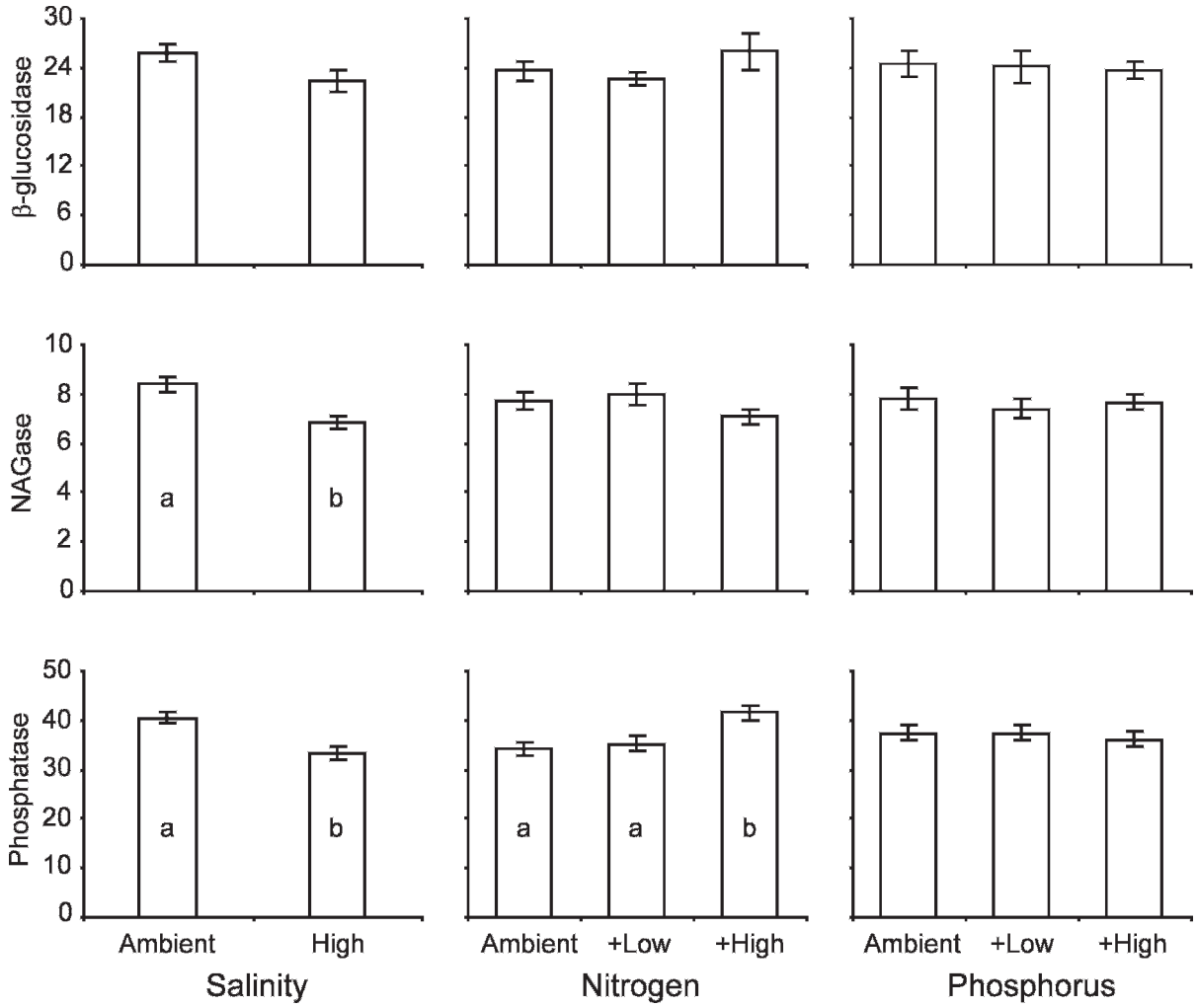

Figure 4. Effects of 55 day exposure to salinity or nutrient addition on the activities of three extracellular enzymes (ß-glucosidase, N-acetylglucosaminidase, acid phosphatase) In wetlands setiments. Activities are in μ mol h^{-1} gOM^{-1}, and reflect mean (± standard error) of replicate microcosms (n varies from 18–27 depending on treatment). Treatments are to 3.5 ppt (high) salinity, +5.0 mg L^{-1} (+low) or +50 mg L^{-1} (+high) nitrogen, and +0.5 mg L^{-1} (+low) or +5.0 mg L^{-1} (+high) phosphorus compared to untreated (ambient) concentrations. Small letters within each plot indicate treatments showing significant differences in enzyme activity ($p < 0.05$).

Mendelssohn 1998, Willis and Hester 2004) so that even 15–20% reductions in rates of C, N, or P mineralization could be ecologically significant. Given the low levels of nutrients in these wetlands, such reductions in mineralization rates would potentially impact nutrient availability to wetland macrophytes, adding an additional stressor in combination with already increased salinity levels. These findings also highlight potential indirect effects of salinity on wetland vegetation (by depressing rates of nutrient cycling) that are rarely considered.

A likely restoration strategy for wetlands along the Louisiana coast is to increase nutrient levels through river diversions or municipal wastewater application (DeLaune et al. 2005, Keddy et al. 2007). Typically, a negative relationship exists between P concentration and phosphatase activity in wetland sediments (Kang and Freeman 1999, Wright and Reddy 2001), and phosphatase activity can be an indicator of eutrophication (Newman et al. 2003). Surprisingly, P addition did not change the activity of phosphatase or any other enzyme in wetland microcosms, even at high (+5.0 mg L^{-1}) loadings. Phosphatase activity was stimulated by nitrogen, with a 20% increase in the high N microcosms. Increased enzyme activity in N amended microcosms could be a result of increased microbial biomass, however, only phosphatase showed an increase following N addition. The likely explanation is that microbial processes in these wetlands are not P limited, but are N limited. Following N

additions, the demand for P became higher leading to increased phosphatase activity. This has been reported for other systems (Nausch and Nausch 2004) and would explain the lack of response to P treatments. Coastal wetlands around the Laurentian Great Lakes and other North American marshes are thought to be N limited (Bedford et al. 1999, Hill et al. 2006), and Lake Pontchartrain wetlands may show the same trend. Phytoplankton communities in the Lake Pontchartrain estuary itself are N limited (Turner et al. 2002) so N limitation of adjacent wetlands is not necessarily surprising.

Initially we used DGGE to examine structural changes in the sediment bacterial community under various treatments, as this method can quickly assess community profiles in a large number of samples (Jackson et al. 2001, Crump and Hobbie 2005, Lyautey et al. 2005). Approximately 40 distinct bands were visible in DGGE profiles, a typical number for sediment communities, but towards the upper limit of resolution of DGGE analysis (Jackson and Churchill 1999). All microcosms examined showed the same complex patterns, and we were unable to resolved differences in bacterial assemblages using this method. DGGE is a fairly crude measurement of community structure and underestimates diversity for complex communities (Woodcock et al. 2006). Given our DGGE results we focused our effort on the more qualitative approach of 16S rRNA cloning and sequencing of selected microcosms, with UniFrac analysis.

The UniFrac metric revealed no significant differences between the communities in the different microcosms, suggesting that neither salinity nor nutrient amendment resulted in a bacterial community that showed specific evolutionary adaptations to those particular environmental conditions. This finding is not necessarily surprising as while each community examined came from different treatments, these treatments all received the same initial sediment (and hence the same initial inoculum of bacterial populations). The UniFrac metric may be better suited to analyses of bacterial communities that show clearer spatial separation (e.g., Jackson and Weeks 2008) rather than examining how a community responds to changing environmental conditions. PCA suggested subtle differences in the bacterial communities under each treatment and supported the patterns seen in microbial extracellular enzyme activity. One axis separated the bacterial assemblages based on nitrogen status, while the second axis suggested an effect of salinity. Thus, as with enzymatic activity, there was a suggestion that both salinity and N additions may have resulted in changes in the sediment bacterial community.

Through DNA sequencing we identified dominant groups of bacteria under each treatment. The Proteobacteria are typically the most abundant lineages of bacteria found in freshwater sediments (Nold and Zwart 1998), and these groups dominated the wetland microcosms in this study. P enrichment had only a moderate effect on community composition and led to just an increase in the Verrucomicrobia and a decline in the Alphaproteobacteria. In contrast, N enrichment substantially reduced the proportions of Alphaproteobacteria, Deltaproteobacteria, and Planctomycetes, and slightly reduced the proportion of Betaproteobacteria. The Alphaproteobacteria and Betaproteobacteria isolated from wetlands are typically oligotrophic (Mitsui et al. 1997) which would explain a reduction in these groups following nutrient amendment. The Betaproteobacteria showed the most consistent response to salinity, increasing under both salinity alone and the combination treatment. However, this lineage is ecologically diverse making their response to salinity difficult to explain.

Little is known of the Verrucomicrobia and Planctomycetes although they are widespread in soils and sediments (Hedlund et al. 1997, Janssen 2006). Sequences from both groups were well-represented in our study and the proportion of Verrucomicrobia increased when sediments were exposed to P, as others have reported (Lindström et al. 2004). Planctomycetes have been recovered from aquatic environments that vary greatly in salinity or nutrient status (Schlesner 1994) so declines in their relative abundance under salinity or N treatments is not easily explained. It may be that other groups (such as the Nitrospira under N, or Betaproteobacteria under salinity) became more competitive.

Linking functional changes (such as in enzyme activity) to changes in community structure is notoriously difficult in microbial ecology. Phosphatase and NAGase are produced by many microbial taxa and patterns in enzyme production vary greatly between closely related species (Rossolini et al. 1998; Dahiya et al. 2006). Changes in enzyme activity may also reflect changes in cell physiology (i.e., differences in enzymatic activity or production under different conditions) as well as changes at the community level (i.e., shifts in the microbial populations responsible for enzyme production). Separating these causal mechanisms is difficult when dealing with communities that are diverse and contain many uncultured organisms of unknown physiology. We did not explicitly try to link function to community structure, but rather sought to investigate how changes in salinity or nutrient status

would affect these different aspects of wetland sediment communities. Our results suggest that salinity and N additions affected community structure and reduced enzyme activities and regardless of the mechanism; increased salinity and/or N are likely to lead to changes in rates of nutrient mineralization in Lake Pontchartrain wetland sediments.

Estimates of overall bacterial diversity under ambient and most treatment conditions were typical of those derived for aquatic environments (Kemp and Aller 2004b). Salinity substantially increased diversity, while diversity was reduced under the high N treatment. That N addition reduced bacterial diversity while P addition did not lends additional support to the suggestion that these wetland sediments are N limited. Salinity has been shown to impact bacterial diversity in estuaries (Bouvier and del Giorgio 2002, Kirchman et al. 2005) and it may be one of the most important global determinants of microbial community structure (Lozupone and Knight 2007). However, most studies have generally focused on short-term tidal changes in salinity or long term salinity effects as seen in isolated saline lakes (Wu et al. 2006). Wetlands in southeastern Louisiana are more likely to be exposed to modest increases in salinity over the moderate time frames tested in this study, and the increase in bacterial diversity may be a result of slight changes in environmental conditions that reduced the dominance of certain populations.

Because we amended microcosms with sterile solutions, any bacterial populations present in the salinity or nutrient treatments must have been present in the initial sediment, albeit at levels below detection. This demonstrates that bacterial diversity estimates are not necessarily accurate; a result of limited sampling of dominant bacterial populations, and true bacterial diversity in most aquatic environments is much higher (Kemp and Aller 2004b). That said, the changes in diversity associated with salinity or N addition, coupled with functional changes in enzyme activity, and shifts in the phylogenetic make-up of the assemblages as suggested by PCA, suggest that the impacts on the sediment microbial community are not just an artifact of sampling. Under real world conditions, saltwater intrusion or nutrient additions through wastewater or river diversions may also bring in additional bacterial populations, potentially increasing the response of the sediment microbial community even more.

We used a microcosm approach to examine the impacts of a moderate salinity increase and two levels of N or P addition on the bacterial community in wetland sediments from a Lake Pontchartrain coastal wetland. While additional treatment levels would be useful, molecular analyses of bacterial communities such as by DGGE or DNA sequencing are complex and expensive to perform (Merkley et al. 2004), which limits experimental design. However, our results do show that a modest increase in salinity depressed microbial enzyme activity suggesting reduced rates of nutrient cycling following saltwater intrusion events. Salinity also increased overall bacterial diversity, and led to shifts in assemblage structure towards a community dominated by Betaproteobacteria, and the already dominant Deltaproteobacteria. High N loads stimulated phosphatase activity, implying that these wetlands are nitrogen limited, and that N additions through wastewater inputs or river diversions will change overall nutrient dynamics. Elevated N inputs also changed the structure of the bacterial assemblage, reducing the proportional abundance of the Betaproteobacteria, Deltaproteobacteria, and Planctomycetes, which resulted in an increase in the number of bacterial groups detected. However, in contrast to increased salinity, N amendment led to an apparent reduction in actual species diversity within these groups. Plant assemblages in Louisiana wetlands are already recognized as being stressed by increasing salinity and nutrient status, and this study shows that microbial communities in these systems are also impacted by these conditions. Given their critical importance to biogeochemical processes, sediment bacterial communities should be considered when evaluating wetland regeneration and restoration efforts.

ACKNOWLEDGMENTS

This work was supported by a U.S. Environmental Protection Agency award to CRJ through the West Lake Pontchartrain Research Program. Haley Echlin helped with much of the molecular work and Hsuanhua Smart helped with sequence analysis. Two anonymous reviewers provided useful comments on an earlier version of this manuscript.

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Manuscript received 31 March 2008; accepted 25 September 2008.