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ORIGINAL ARTICLE

Salt marsh sediment bacteria: their distribution and response to external nutrient inputs

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A primary focus among microbial ecologists in recent years has been to understand controls on the distribution of microorganisms in various habitats. Much less attention has been paid to the way that environmental disturbance interacts with processes that regulate bacterial community composition. We determined how human disturbance affected the distribution and community structure of salt marsh sediment bacteria by using denaturing gradient gel electrophoresis of 16S rRNA in five different habitats in each of four salt marshes located in northeastern Massachusetts, USA. Two of the four marsh creeks were experimentally enriched 15× above background by the addition of nitrogen and phosphorus fertilizers for two or more growing seasons. Our results indicate that extrinsic factors acting at broad scales do not influence the distribution of salt marsh sediment bacteria. Intrinsic factors, controlled by local-scale environmental heterogeneity, do play a role in structuring these sediment microbial communities, although nutrient enrichment did not have a consequential effect on the microbial community in most marsh habitats. Only in one habitat, a region of the marsh creek wall that is heavily colonized by filamentous algae, did we see any effect of fertilization on the microbial community structure. When similar habitats were compared among marshes, there was considerable convergence in the microbial community composition during the growing season. Environmental factors that correlated best with microbial community composition varied with habitat, suggesting that habitat-specific intrinsic forces are primarily responsible for maintaining microbial diversity in salt marsh sediments.
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Introduction

The development of molecular techniques in recent years allows for the examination of microbial community composition without biases introduced by culture-dependant methods. The result has been a flurry of research on whether the concepts of community ecology can be applied at a microbial scale (Horner-Devine *et al.*, 2004; Green and Bohannan, 2006; Hughes Martiny *et al.*, 2006; Woodcock *et al.*, 2006; Crump *et al.*, 2007). In particular, the debate over what structures microbial diversity at local and global scales has received considerable attention. The long-held paradigm that 'everything is everywhere, but, the environment selects' (Bass-Becking, 1934) suggests that microbial taxa have a cosmopolitan distribution (Green and Bohannan, 2006) but that intrinsic environmental conditions acting at local scales dictate actual community composition (Hughes Martiny *et al.*, 2006). The considerable evidence for synchronous shifts in microbial community composition (Stepanauskas *et al.*, 2003; Crump and Hobbie, 2005; Fuhrman *et al.*, 2006; Kent *et al.*, 2007), however, suggests that regional-scale extrinsic factors may also structure the diversity of microorganisms.

The extent to which extrinsic or intrinsic drivers structure the biodiversity of microbial systems is likely to vary with time and habitat. In salt marsh sediments, extrinsic factors such as those that dictate climatic patterns, freshwater delivery and tidal exchange could influence microbial community structure, as has been shown for riverine systems (Crump and Hobbie, 2005). At local scales, the dominant marsh vegetation has been shown to exert strong controls on salt marsh sediment community composition (Blum *et al.*, 2004). Studies

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have examined the role that heavy metals and organic pollution play in altering microbial community structure (Córdova-Kreylos *et al.*, 2006), but less is known about the impact of increasing nutrient supply on microbial community composition, particularly in marine sediments. Urakawa *et al.* (2006) examined the response of ammonia-oxidizing bacteria to wastewater effluent and determined that the diversity of ammonia-oxidizing bacteria decreased with proximity to the wastewater source. Denitrification rates increased as a result of NO_3 addition in sandy sediments but no effect was seen in rocky biofilms (Magalhaes *et al.*, 2005). An examination of bacterial production and the enzymatic breakdown of organic matter showed that all measured microbial processes correlated with the degree of eutrophication in lakes, although the community composition of bacteria was not explicitly examined (Chrost and Siuda, 2006). These results suggest that bacteria may respond to nutrient inputs, but as yet there have been no studies that examine the response of the bulk microbial community to inputs of fertilizer.

Although not much is known about how salt marsh sediment bacteria respond to fertilizer inputs, there is considerable literature on the effects of nutrient enrichment on the ecology of salt marshes, in particular nitrogen (Valiela and Teal, 1974). Nitrogen additions have been shown to shift community composition of dominant macrophytes (Valiela *et al.*, 1985) and increase above-ground and below-ground production of marsh vegetation (Valiela *et al.*, 1982). Furthermore, fertilization has led to increases in the standing stock of benthic microalgae (Van Raalte *et al.*, 1976; Sullivan and Currin, 2000; Deegan *et al.*, 2007), decreases in the rate of nitrogen fixation (Van Raalte *et al.*, 1974), and increases in the density and production of oligochaete and annelid worms (Sarda *et al.*, 1996; Johnson and Fleeger, 2009). Thus, there is considerable evidence that nutrient enrichment will alter the flow of carbon and nitrogen in salt marshes, and this alteration could have a concomitant effect on microbial diversity.

In this study, we examine how coastal eutrophication affects the community structure of salt marsh sediment microbes. We performed a multi-year fertilization experiment in which entire salt marsh habitats were enriched 15-fold above background concentrations with dissolved nitrogen and phosphorus (Deegan *et al.*, 2007). This fertilization did not appreciably change the bacterial production rates except in habitats where benthic algal response was high (Bowen *et al.*, 2009). To investigate community response, bulk sediment DNA was collected monthly from five habitats in fertilized and unfertilized marshes through the 2005 growing season. The goals for this research were to (1) determine if salt marsh sediment bacterial communities show extrinsically controlled synchronous patterns that exist across all salt marsh habitats or if community composition is controlled by local-scale intrinsic environmental variability, (2) determine if the addition of nutrients to the system caused detectable shifts in microbial community composition and (3) identify differences in microbial communities among different salt marsh habitats and determine what factors might be controlling among-habitat variability.

Materials and methods

Study sites

The marsh fertilization experiment took place at the Plum Island Ecosystem Long Term Ecological Research site in northeastern Massachusetts. Details of the enrichment experiment have been described elsewhere (Deegan *et al.*, 2007; Drake *et al.*, 2008; Bowen *et al.*, 2009). Briefly, two pairs of marshes from the Rowley River marsh complex were selected based on similarities in hydrology, nutrient dynamics, benthic microalgae standing stock and vascular plant community. Incoming tidal water of one stream in each pair (Sweeney Creek in the Sweeney Creek/West Creek pair and Clubhead Creek in the Clubhead Creek/Nelson Creek pair) was fertilized to a concentration of 70 μM NO_3^- and 4 μM PO_4^{-3} (equivalent to a loading rate of 300 kg N per ha per year and 10 kg P per ha per year). These concentrations were selected because they reflect the concentrations at which the EPA (2002) designates an estuary to be ‘moderately to severely eutrophied’. Dissolved nutrients were delivered to flooding waters in the two fertilized marshes through a water-flux-calibrated pump system (Deegan *et al.*, 2007). We sampled sediments from five habitats within the marsh, including the marsh creek mudflat (MF), the creek bank wall covered with filamentous algae (FA), the tall form of *Spartina alterniflora* (TSA) habitat, the *Spartina patens* habitat that forms the majority of the marsh platform (SP) and the short form of *S. alterniflora* (SSA) habitat that is found in low-elevation regions on the marsh platform.

Sampling procedure

Sediments were collected from each habitat in all four marshes monthly during the growing season of 2005 (May to October). The top 1-cm of sediment was collected with a sterile 15-mm diameter core tube. At each site, we collected and homogenized 10 sediment cores in a sterile scintillation vial. A portion of the homogenized sediments was removed to a sterile 2-ml centrifuge tube for later DNA extraction. The tubes were stored on ice until frozen at −80 °C. Remaining sediments were used to measure bacterial production by leucine incorporation (Buesing and Gessner, 2003) and the carbon and nitrogen content of the sediments using standard

methods (Bowen *et al.*, 2009). Biomass of benthic microalgae was also determined monthly in four of the five habitats (short-form *S. alterniflora* was not sampled) by measuring total chlorophyll *a* (mg chl *a* per cm^2) following the method of Lorenzen (1967).

Denaturing gradient gel electrophoresis
DNA was extracted from bulk sediment using a PowerSoil Soil DNA Isolation kit (Mo Bio Laboratories, Carlsbad, CA, USA) following the manufacturer's instructions. Extracted DNA was amplified using PCR with primers for the bacterial 16S rRNA

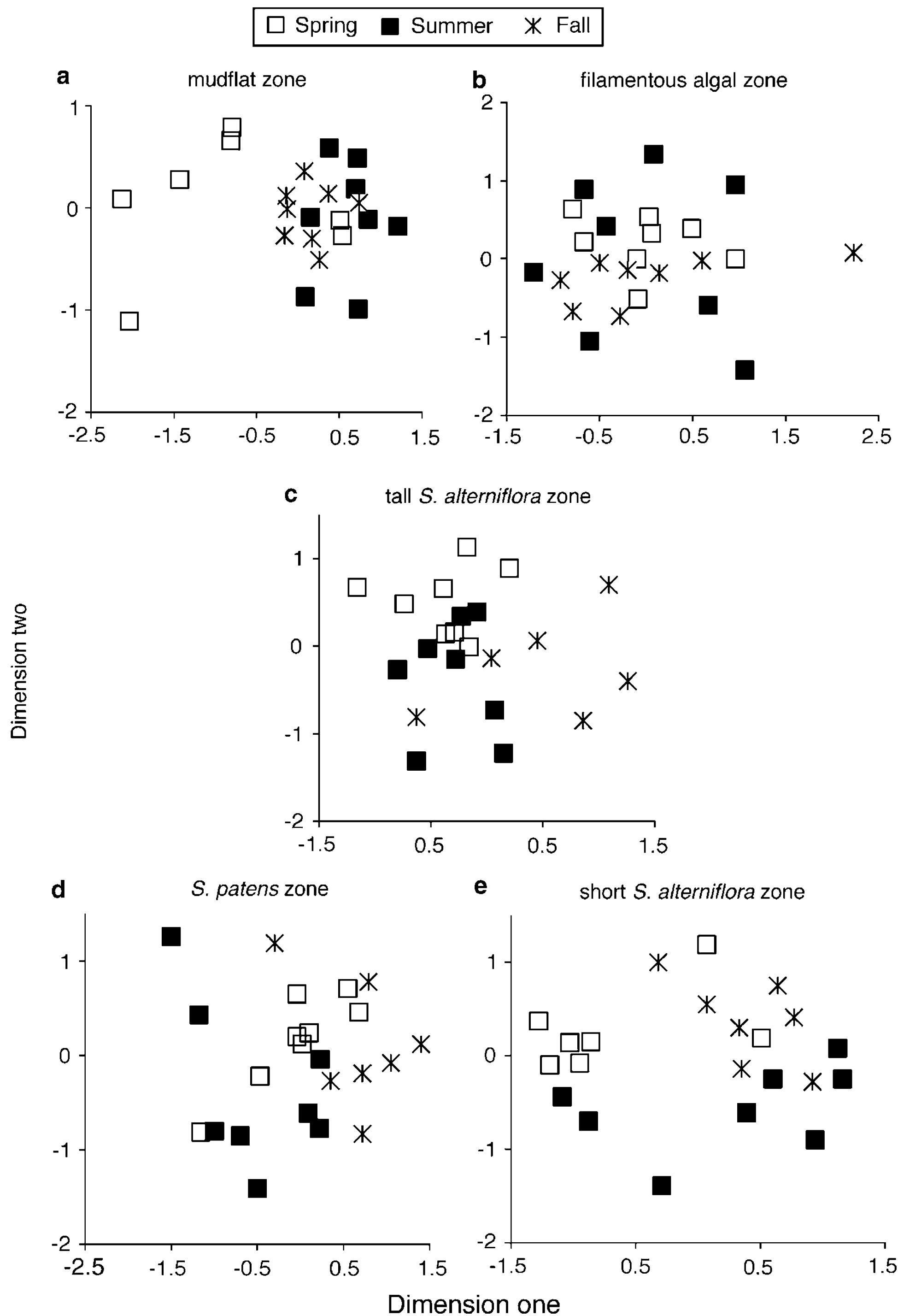

Figure 1 Temporal patterns in bacterial community composition within each habitat. Non-metric multidimensional scaling plots of bacterial community composition in five salt marsh habitats. Replicates are from four adjacent marshes sampled in spring (May and June), summer (July and August) and fall (September and October) of 2005 in the five habitats: mudflat zone (**a**, stress = 0.17), filamentous algal zone (**b**, stress = 0.20), tall-form *S. alterniflora* habitat (**c**, stress = 0.14), *S. patens* habitat (**d**, stress = 0.19) and short-form *S. alterniflora* habitat (**e**, stress = 0.17).

gene (357F: TACGGGAGGCAGCAG, 519R: ACCGCG GCTGCTGGCAC). An additional GC clamp was appended to the 5′-end of the forward primer (CGCC CGCCGCGCCCCGCGCCCGGCCCGCCGCCCCCGCCC CCC). Amplification conditions followed the method of Crump and Hobbie (2005) except that the final extension step was increased from 5 min to 1 h at 72 °C. The denaturing gradient gel electrophoresis (DGGE) conditions of Crump and Hobbie (2005) were used although gels in this study were allowed to run for 24 h to eliminate ghost bands. Gels were run on a CBS Scientific (Del Mar, CA, USA) DGGE system. Bacterial operational taxonomic units were defined from high-resolution photographs of the DGGE gels taken with the Chemi-Doc gel documentation system (BioRad, Hercules, CA, USA).

Statistical analyses

The banding patterns in each sample were aligned and their relative band height quantified using GelComparII software package (Applied Maths, Austin, TX, USA). We square-root transformed the relative band height data to minimize the impact of highly dominant operational taxonomic units (Clarke and Warwick, 2001) and then calculated similarity matrices of each sample within a gel using the Bray–Curtis similarity index calculation (Legendre and Legendre, 1988; Rees *et al.*, 2004) with Primer V5 (Primer-E Ltd, Plymouth, UK). Similarity matrices were then ordinated using non-metric multidimensional scaling (NMDS) in Primer V5. NMDS plots are iteratively derived spatial representations of community similarity in which each sample is defined by its proximity to the most closely related samples. Goodness of fit of the NMDS plots was determined based on the calculated stress of the plot, with a stress value of 0.2 or below considered acceptable (Clarke, 1993).

We examined the statistical significance of the differences among communities using the Analysis of Similarity feature of Primer V5. Analysis of Similarity clusters samples in the similarity matrix by predetermined factors (habitat, season and so on) and determines the rank similarities of each factor in the matrix. It calculates a global test statistic, R, the value of which can range between $+1$ and -1 (Clarke and Warwick, 2001). A value close to $+1$ indicates a nearly complete separation of the communities among the given factors (Ramette, 2007). For example, a significant R-value, with habitat as a factor, would indicate that there are significant differences among all the habitats defined in the similarity matrix. Values close to 0 indicate highly similar communities that do not separate by the predetermined factor. R-values that are negative indicate that the variability within a given factor is greater than the variability among factors (Clarke and Warwick, 2001).

Measures of DGGE band richness and Shannon's Diversity Index were calculated using the DIVERSE feature of Primer V5. To determine correlations between both band richness and Shannon's Diversity Index with concentrations of benthic chl *a,* we first performed a Shapiro–Wilkes test in SPSS (SPSS 11.0.2.; SPSS Inc., Chicago, IL, USA) to test for deviations from normality. We then calculated a Pearson product–moment coefficient in SPSS to assess the degree of correlation between the richness/diversity and chl *a* concentrations.

The BIO-ENV program in Primer V5 was used to determine which measured environmental factors were influencing bacterial community composition. Environmental parameters (sampling month, habitat, fertilization status, C:N ratio, benthic chl *a* concentrations and bacterial production rates) were log transformed as needed and Euclidean distance was used to construct a similarity matrix for each community that was parallel to the Bray–Curtis similarity matrix calculated above. BIO-ENV uses a rank correlation (Spearman's) method to assess the degree of association between two similarity matrices. BIO-ENV calculates a Spearman's correlation coefficient (ρ) that can range in value from -1 (complete opposition) to 1 (complete concordance).

Table 1 ANOSIM results comparing bacterial community composition within each salt marsh habitat during three seasons of 2005

Interaction	R-*value*		
	Spring: summer	*Spring: fall*	*Summer: fall*
Mudflat	0.271**	0.281**	NS
Filamentous	0.156*	0.244**	NS
Tall *Spartina alterniflora*	0.750**	0.421**	NS
Spartina patens	0.214*	0.334**	0.263**
Short *S. alterniflora*	0.434*	0.545**	0.248**

Abbreviations: ANOSIM, Analysis of Similarity; NS, not significant.
$^{*}P<0.05$; $^{**}P<0.01$.

Table 2 The maximum Spearman's correlation coefficient that describes the rank correlation between measured environmental variables and microbial community composition within each habitat

Habitat	*Contributing factors*	*Spearman's coefficient*
Mudflat	BP [0.206]+month [0.101]	0.261
Filamentous	Month [0.312]+C:N [0.298]+chl *a* [0.176]	0.399
Tall *S. alterniflora*	Month [0.480]	0.480
S. patens	BP [0.283]+month [0.247]	0.368
Short *S. alterniflora*	Month [0.229]	0.229

Environmental factors that contributed to the highest Spearman's coefficient listed here include bacterial production (BP), sampling month, C:N ratio and chl *a* concentrations. Bracketed numbers indicate the correlation of each individual contributing factor if it were the only factor describing the community composition.

Results

Extrinsic controls on bacterial community composition
If regional-scale extrinsic forces were influential in structuring bacterial communities in salt marsh sediments, there would likely be synchronous changes in microbial composition in each of the marsh habitats. Visual examination of NMDS plots of the Bray–Curtis similarity matrices reveals no clear seasonal shifts in microbial community composition within each of the salt marsh habitats (Figure 1). Analysis of Similarity calculations detected some significant differences among the microbial communities within each habitat, but

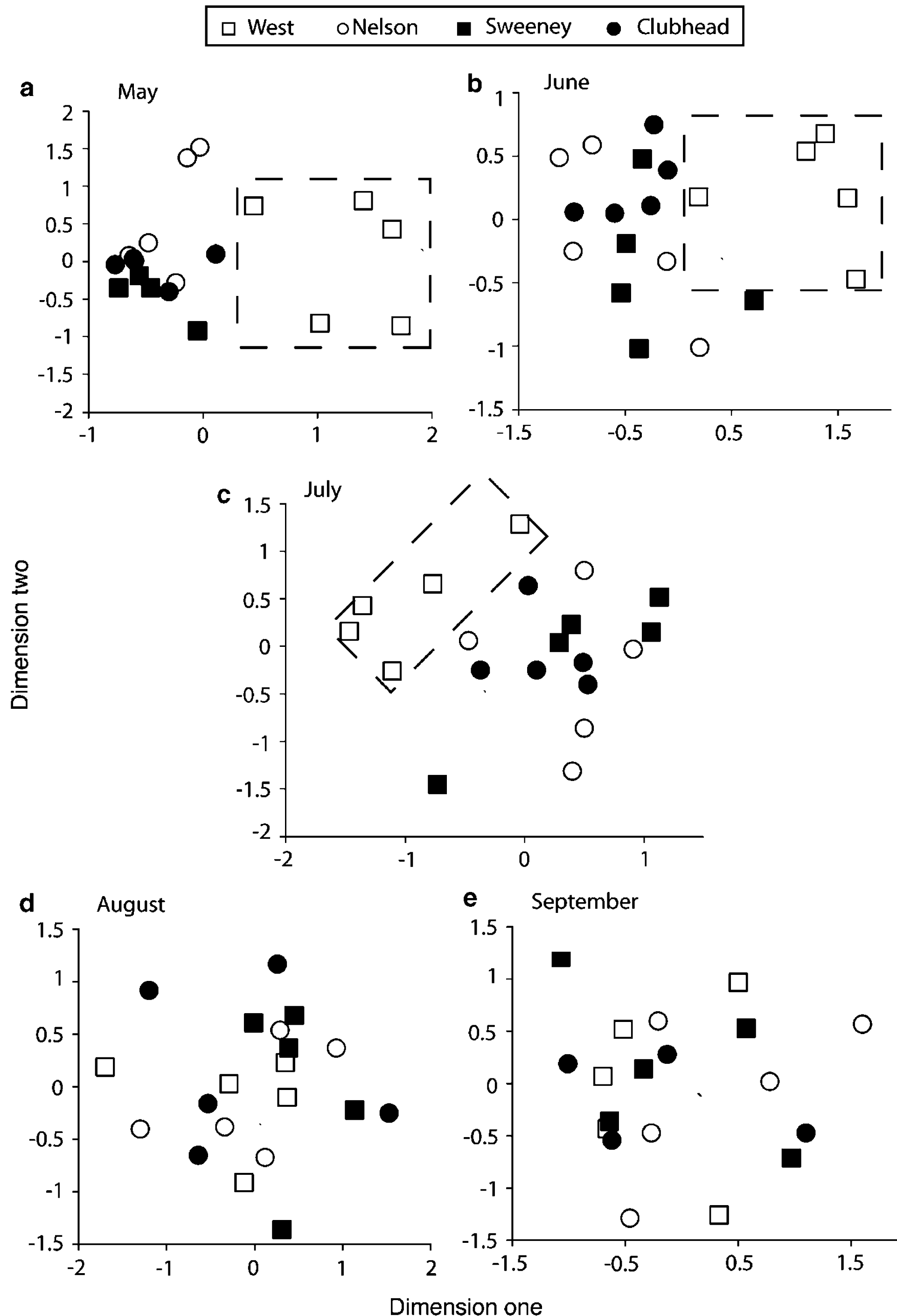

Figure 2 Non-metric multidimensional scaling plot of bacterial community composition in each month of the 2005 growing season. The different habitats are used as replicates to examine differences among the four streams included in this study. Open symbols are reference marshes and closed symbols are marshes that received fertilizer. Stress values of the plots are as follows: (**a**) May = 0.13, (**b**) June = 0.16, (**c**) July = 0.20, (**d**) August = 0.18 and (**e**) September = 0.19.

these differences were not synchronous and the degree of separation, in most cases, was small (Table 1). In all but two of the 15 possible pair-wise comparisons among seasons within each habitat, the R-value was lower than 0.5 ($R = 1.0$: completely different communities; $R = 0.0$: completely indistinguishable communities).

Despite the lack of any clear synchronous patterns in the data, when we examined a suite of environmental variables that could explain the bacterial community composition within each marsh habitat, the sampling month explained the greatest amount of the rank correlation between environmental variables and community structure in three of the five habitats (Table 2). In the other two habitats, bacterial production explained the rank correlation best, followed by the month of sampling. With the environmental factors that we measured (month of sampling, bacterial production, C:N ratio, %C, %N, benthic chl *a* and whether or not the community received fertilization) we were able to explain between 23% and 48% of the rank correlation between our measured environmental variables and the microbial community composition (Table 2).

Intrinsic controls on bacterial community composition

If microbial community structure is dictated by local intrinsic conditions instead of regional-scale extrinsic conditions, we would likely see differences in community composition with increasing environmental heterogeneity. The increase in heterogeneity may occur from differences among our four marshes, differences among habitats within each of the marshes or, for the marshes that received fertilizers, distance from the point of perturbation. NMDS plots of similarity matrices indicate tremendous community similarity among three of the four marshes throughout the growing season (Figure 2). One of the four marshes, West Marsh, had a very different microbial community composition ($R = 0.692$–0.738) than the rest of the marshes (Table 3, left two columns) during the early part of the growing season. Over the course of the growing season, however, the community in this divergent marsh became more similar to that in the other marshes such that by August and September there were no differences among any of the marshes in our study (Figure 3).

As there were largely no differences among the four marshes, we used each marsh as a replicate and examined differences among habitats for each month of the 2005 growing season (Figure 4, Table 3, right two columns). At the beginning of the growing season, there were no significant differences in bacterial community composition among the various habitats (Figure 4, top two panels). As the growing season progressed, however, the microbial communities within each habitat converged (Figure 3) so that by September microbial community composition within each habitat was highly similar and different from adjacent habitats ($R = 0.521$–1.00; Table 3).

Table 3 ANOSIM results comparing bacterial community composition among marshes and within habitats for each month of the 2005 growing season. Only significantly different interactions are listed here

Among marshes		*Among habitats*	
Interaction	R-*value*	*Interaction*	R-*value*
May		July	
West–Sweeney	0.738	MF–SSA	0.417
West–Clubhead	0.716	TSA–SSA	0.615
West–Nelson	0.692	August	
June		FA–SP	0.458
West–Sweeney	0.604	FA–SSA	0.656
West–Clubhead	0.680	MF–SSA	0.531
West–Nelson	0.700	SP–TSA	0.708
Sweeney–Clubhead	0.396	SSA–TSA	0.781
Sweeney–Nelson	0.328	September	
July		MF–TSA	0.698
West–Sweeney	0.540	MF–SSA	0.958
West–Clubhead	0.728	FA–TSA	0.635
West–Nelson	0.404	FA–SP	0.698
		FA–SSA	0.594
		TSA–SP	0.521
		TSA–SSA	1.00

Abbreviations: ANOSIM, Analysis of Similarity; FA, filamentous algal zone; MF, mudflat; SP, *S. patens*; SSA, short form *S. alterniflora*; TSA, tall form *S. alterniflora*.
All R-values were significant at $P < 0.05$.

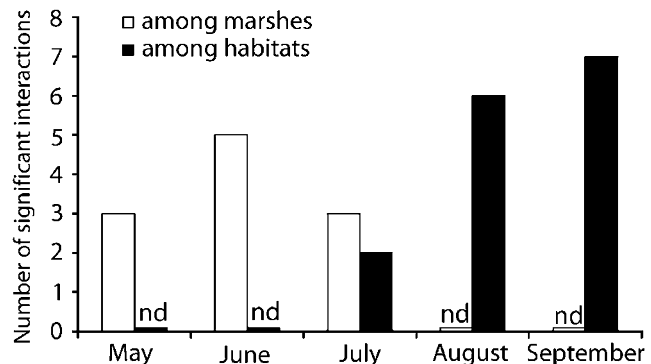

Figure 3 Spatial patterns in bacterial community composition among marshes. The number of significant differences within the bacterial community compositions among marshes and among habitats during each month of the growing season as calculated by Analysis of Similarity. ND indicates that there were no significant differences within that month.

We examined Spearman's correlation coefficients between bacterial community composition and measured environmental variables for each month (Table 4). This has the effect of removing any temporal variability and allowing us to examine the environmental factors that may be influencing bacterial community composition. The environmental factors that best explained the ranked microbial community composition varied on a month-to-month basis. Bacterial production and whether or not the system was fertilized were important in the

earlier part of the growing season, and the C:N ratio of the sediments and the specific habitat type were more important in the later part of the growing season.

Effects of fertilization

As fertilization seemed to play a role in structuring the microbial communities in some months (Table 4), we also wanted to determine if there were specific effects of fertilization in each of the marsh habitats. By comparing microbial community composition between fertilized and reference marshes in each marsh habitat, we detected a positive response to fertilization only in the bacterial community composition of the filamentous algal zone (Figure 5) although this response could be explained by a fertilization-induced increase in microalgal biomass that was detected by our primers selecting for chloroplast DNA.

If this response to fertilization resulted from microalgal chloroplasts detected by the primers, then we would expect there to be a correlation between DGGE band richness and the concentration of chl *a* (as a proxy for benthic microalgal biomass). We do not intend the use of DGGE band richness/

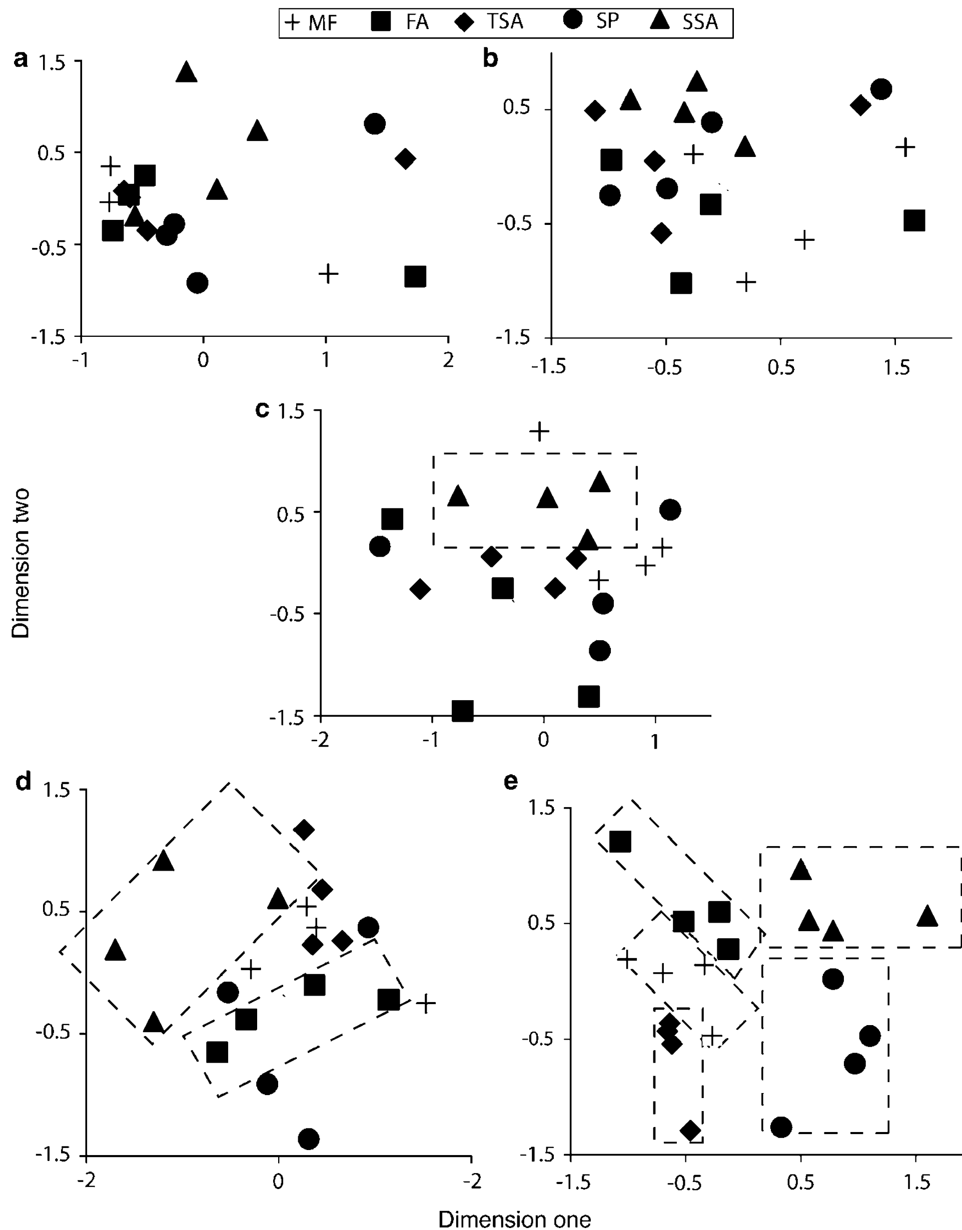

Figure 4 Spatial patterns in bacterial community composition among habitats. Non-metric multidimensional scaling plots of bacterial community composition in each month of the 2005 growing season. Here the different streams are treated as replicated to examine difference among habitats. Stress for each plot is the same as in Figure 2. MF = mudflat zone, FA = filamentous algal zone, TSA = tall-form *S. alterniflora* habitat, SP = *S. patens* habitat and SSA = short-form *S. alterniflora* habitat. (**a**) May, (**b**) June, (**c**) July, (**d**) August and (**e**) September.

Table 4 The maximum Spearman's correlation coefficient that describes the rank correlation between measured environmental variables and microbial community composition for all marsh habitats in each month of the 2005 growing season

Habitat	*Contributing factors*	*Spearman's coefficient*
May	BP [0.309]	0.309
June	fert [0.176]+BP [0.075]	0.109
July	fert [0.466]+chl *a* [0.391]+habitat [0.086]	0.695
August	C:N [0.343]+habitat [0.337]	0.396
September	C:N [0.593]+habitat [0.437]	0.603

Environmental factors that contributed to the highest Spearman's coefficient listed here include bacterial production (BP), habitat designation, whether the system received fertilizers or not (fert), C:N ratio and chl a concentrations. Bracketed numbers indicate the correlation of individual contributing factors if they were the only factor describing the community composition.

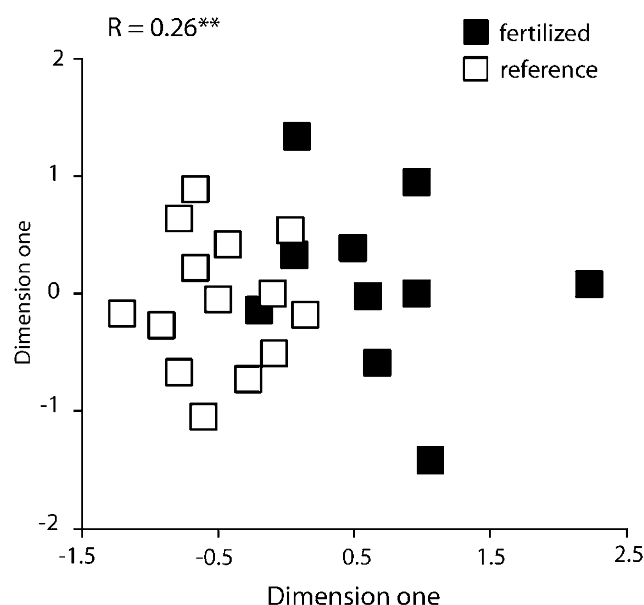

Figure 5 Non-metric multidimensional scaling plots of bacterial community composition in fertilized and reference marshes within the filamentous algal marsh zone (stress = 0.20). **Significant at $P < 0.01$.

diversity as a measure of total microbial richness/diversity in marsh sediments but instead use these numbers as a minimum relative richness/diversity within each habitat. In the filamentous algal habitat, the only habitat where there was a positive effect of fertilization (Figure 5), there was no correlation between DGGE band richness (or Shannon's Diversity Index) and chl *a* concentrations (Figure 6, top two graphs). We did, however, find a significant positive correlation between DGGE band richness and diversity and chl *a* concentrations in the MF habitat and significant negative correlations in the tall *S. alterniflora* habitat (Figure 6).

Discussion

Two mechanisms are commonly proposed to explain bacterial community composition: historical contingencies, such as those that are defined by ancestral interactions with the environment and other organisms, and contemporary disturbances (Hughes Martiny *et al.*, 2006). If historical contingencies dictate microbial community composition then we would expect to see patterns in composition that play out on regional scales. Examples of these regional-scale drivers include climate-driven seasonal shifts or community compositions that can be predicted based on landscape position or some other geographical distribution. Both of these patterns have been described for bacterioplankton, with evidence for seasonal synchrony in rivers (Crump and Hobbie, 2005), lakes (Crump *et al.*, 2003; Kent *et al.*, 2007), estuaries (Kan *et al.*, 2007) and the open ocean (Fuhrman *et al.*, 2006).

By contrast, controls on community composition that are induced by intrinsic environmental variables are expressed at local scales and can often be correlated with biogeochemical or other environmental factors. Franklin *et al.* (2002) indicated that although bacterial abundance did show significant spatial autocorrelation in salt marsh sediments, bacterial community composition did not share this feature. They suggested that this may reflect variability arising from environmental heterogeneity. Horner-Devine *et al.* (2004) showed that salt marsh sediment microbes displayed a significant taxa area relationship that was largely explained by environmental variables. Hewson *et al.* (2007) showed that environmental factors were responsible for the differences in sediment microbial communities found along a 35-km transect off the coast of Southern California. Microbial community composition in fine benthic organic matter in the streams of the Hubbard Brook Forest appeared to be largely structured by stream pH (Fierer *et al.*, 2007), and in a comparison of soil microbial communities from 98 locations across North and South America, soil pH explained much of the differences among the communities (Fierer and Jackson, 2006). All of these terrestrial examples indicate that local-scale environmental factors predominate over historical contingencies in structuring sediment microbial communities.

As with these terrestrial examples, our DGGE data from salt marsh sediments provide evidence that the community composition of the dominant microbial taxa is structured by local environmental factors, rather than by extrinsic factors that act at regional scales. These marsh communities show no evidence of synchrony, in fact as yet there have been no reports of synchrony in soil or sediment communities, suggesting that there may be fundamental differences in the forces structuring microbial diversity in sediment versus planktonic systems.

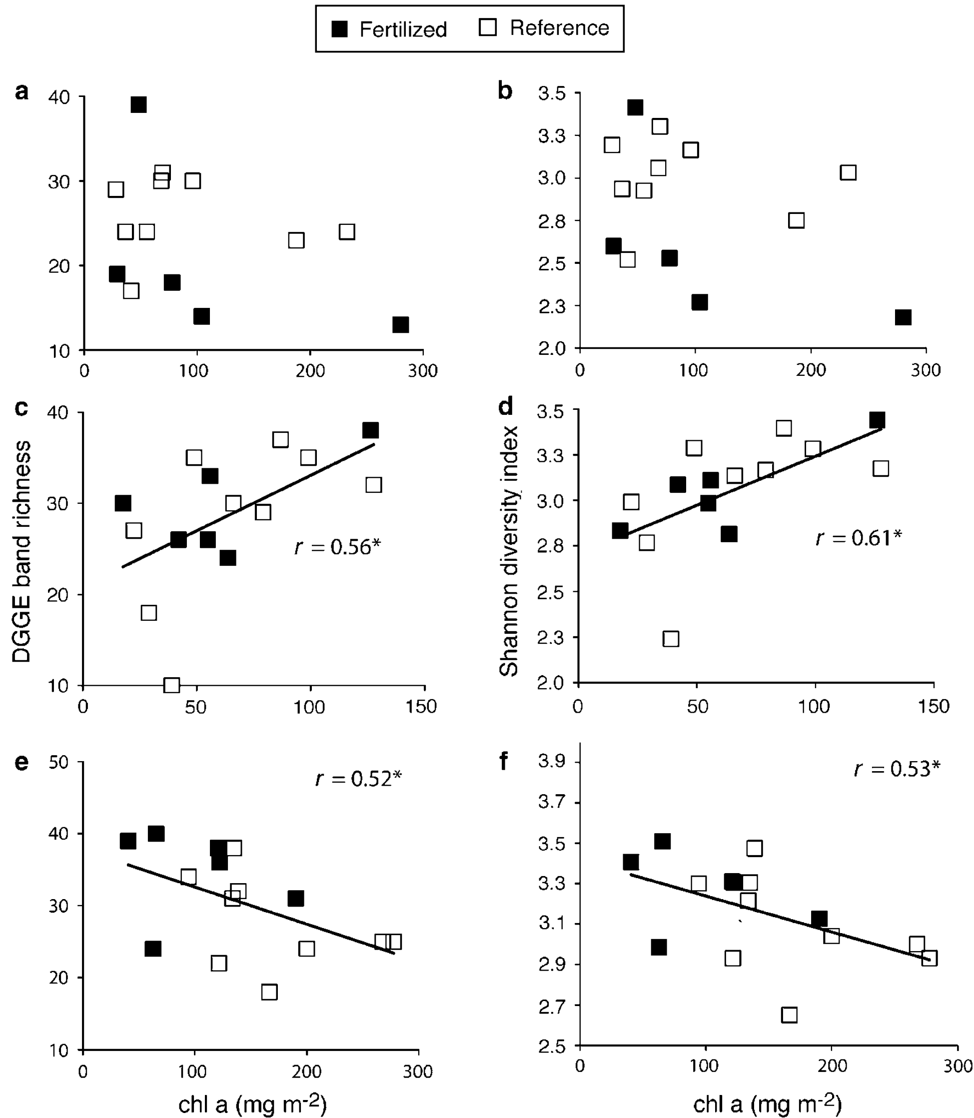

Figure 6 DGGE band richness (panels **a**, **c** and **e**) and Shannon's Diversity Index (panels **b**, **d** and **f**) calculated from DGGE band data plotted as a function of benthic chl *a* concentrations for three marsh habitats: the filamentous algal zone (panels **a** and **b**), the mudflat zone (panels **c** and **d**) and the tall form of *S. alterniflora* zone (TSA). Shapiro–Wilkes tests indicated that none of the data deviated significantly from a normal distribution (panels e and f). Significant Pearson product–moment coefficients are indicated by *($P<0.05$) or **($P<0.01$).

Rather than synchronous changes that act across different habitats in the marsh, these DGGE results indicate that within-habitat environmental conditions exert the strongest control on microbial diversity.

Sediment microbial communities appear to segregate themselves by habitat as the growing season progresses (Figure 4). In the earliest part of the growing season, microbial communities within habitats do not appear to resemble one another. As the growing season proceeds, however, the communities within a given habitat become more and more similar. The result is greater similarities among given habitats of different marshes spanning a region of several kilometers than among different habitats within a marsh that are separated by not more than 50 m. The environmental factors that most strongly correlate with community composition vary with habitat, but C:N ratio and rates of bacterial production explain the greatest proportion of the correlation coefficients in many habitats.

In light of the critical role of the local environment in structuring these sediment communities, we must also consider the role that increasing human disturbance plays in structuring microbial diversity. In the context of eutrophication in coastal environments, our results are particularly meaningful. The extent to which increasing nutrient enrichment restructures salt marsh microbial communities could have important implications for the

biogeochemistry of these critical habitats. Only in one salt marsh habitat of the five studied was there a direct response of the microbial community to the addition of nitrogen and phosphorus fertilizers. This zone, typified by the growth of filamentous algae along marsh creek walls, showed a clear difference in the microbial community composition as a result of the inputs of fertilizer. If the microbial community associated with the filamentous algal habitat responded directly to added nutrients, similar shifts in community composition should occur in other marsh habitats that were exposed to the nutrient treatment. Instead, as the microbial community composition changed only in this habitat, it suggests that a shift in the carbon supply from the filamentous algae could have led to the differentiation in the microbial community.

It should be noted that if the response to fertilization detected in the filamentous algal zone was an artifact of our method resulting from the amplification of chloroplast DNA from microalgae, there would likely be a positive correlation between community richness and chl *a* concentrations. No such correlation is apparent in the filamentous algal habitat; however, there was a significant positive correlation between chl *a* concentrations and band richness in the MF habitat (Figure 6, middle two figures) and a significant negative correlation between the two in the tall *S. alterniflora* habitat. These correlations could result from real differences in microbial diversity that correlate with changes in the benthic microalgal community through, for example, the promotion of a competitively dominant phylotype in the tall *S. alterniflora* habitat. They could also result from changes in the diversity of the microalgal community that is detected as an artifact of the method. As there was no correlation between chl *a* and diversity in the habitat dominated by microalgae, we suspect that any artifact would be a small component of the overall band richness and that these correlations do suggest meaningful differences among habitats. However, more work is needed to explore these relationships.

The methodologies employed in this study provide only a coarse understanding of microbial diversity in this system. Fingerprinting tools such as DGGE of 16S rRNA capture the community dynamics of the dominant taxa, but there is evidence that the choice of molecular tool can skew the interpretation of biogeographical data (Cho and Tiedje, 2000). It has even been argued that the resolution of 16S rRNA may not be sufficient to differentiate among some ecologically relevant genotypes (Ramette and Teidje, 2007). Furthermore, we are extrapolating from very small sample sizes to entire marsh habitats. Such scaling is a necessary feature of culture-independent microbial work as a comprehensive coverage of microbial communities that number on the order of 10^9 individuals per cm^3 of marsh sediment is practically impossible (Ramette and Teidje, 2007). We suggest that the emergence of significant patterns in this study, in spite of these recognized limitations, indicates that as finer scale tools become available we will begin to see much greater structure in microbial community composition (Papke *et al.*, 2003; Horner-Devine *et al.*, 2004; Ramette and Teidje, 2007).

Conflict of interest

The authors declare no conflict of interest.

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