

ORIGINAL ARTICLE

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

Jennifer L Bowen^{1,3}, Byron C Crump², Linda A Deegan¹ and John E Hobbie¹¹Marine Biological Laboratory, The Ecosystems Center, Woods Hole, MA, USA and ²Horn Point Laboratory, University of Maryland College of Environmental Science, Cambridge, MD, USA

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.

The ISME Journal (2009) 3, 924–934; doi:10.1038/ismej.2009.44; published online 7 May 2009

Subject Category: microbial population and community ecology

Keywords: salt marsh; bacterial community composition; denaturing gradient gel electrophoresis; eutrophication; wetlands

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

Correspondence: JL Bowen, Department of Geosciences, Princeton University, Guyot Hall, Princeton, NJ 8544, USA.

E-mail: jlbowen@princeton.edu

³Current address: Department of Geosciences, Princeton University, Princeton, NJ 08540, USA

Received 11 December 2008; revised 24 March 2009; accepted 30 March 2009; published online 7 May 2009

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 $70 \mu\text{M NO}_3^-$ and $4 \mu\text{M PO}_4^{3-}$ (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 cm²) 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

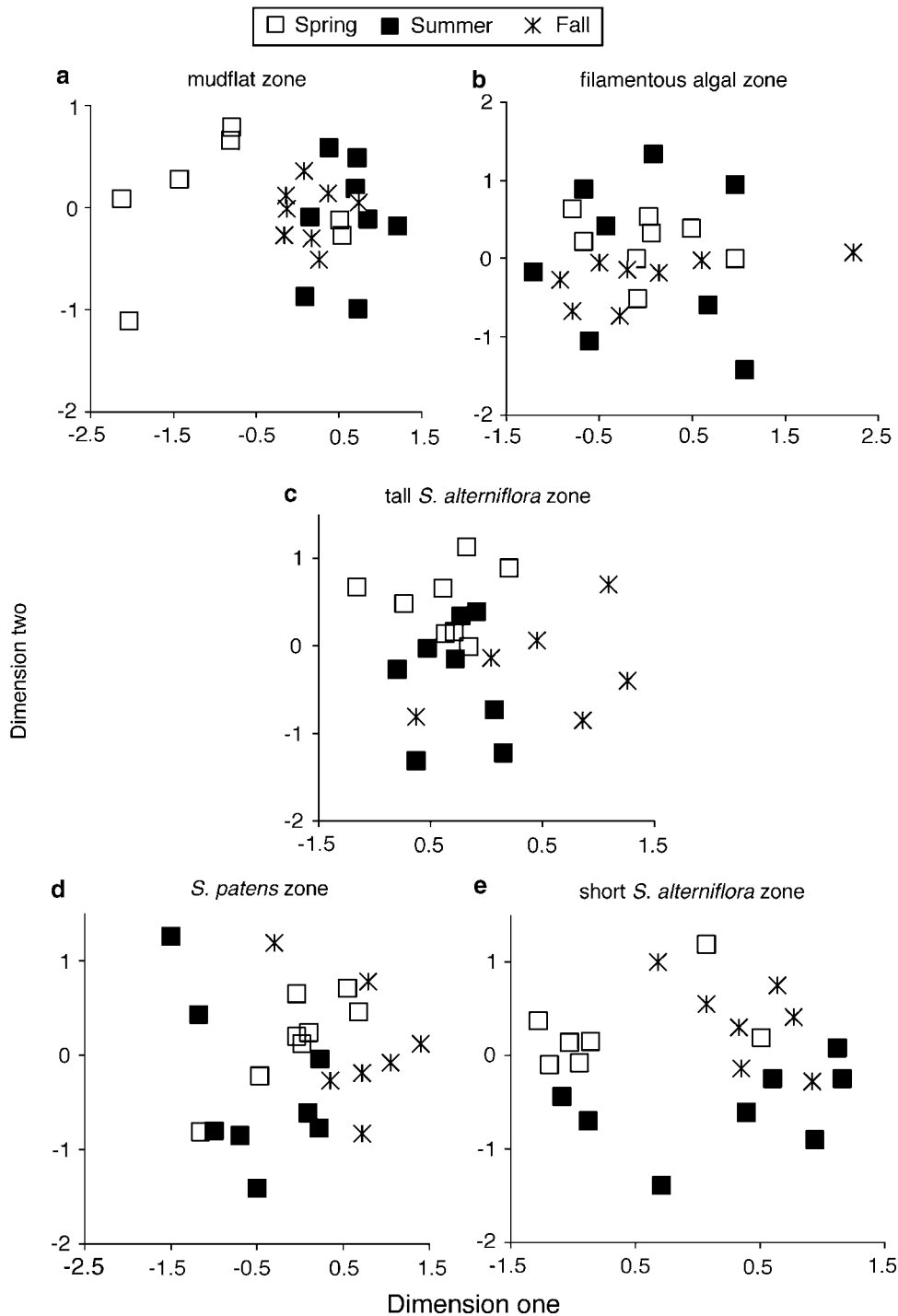


Figure 1 Temporal patterns in bacterial community composition within each habitat. Non-metric multidimensional scaling plots of bacterial community composition in five salt marsh habitats. Replicates are from four adjacent marshes sampled in spring (May and June), summer (July and August) and fall (September 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 CGCCGCGCCCCGCGCCCCGCCCCGCCCCGCCCCGCCCC 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 <i>Spartina alterniflora</i>	0.750**	0.421**	NS
<i>Spartina patens</i>	0.214*	0.334**	0.263**
Short <i>S. alterniflora</i>	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 <i>a</i> [0.176]	0.399
Tall <i>S. alterniflora</i>	Month [0.480]	0.480
<i>S. patens</i>	BP [0.283]+month [0.247]	0.368
Short <i>S. alterniflora</i>	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

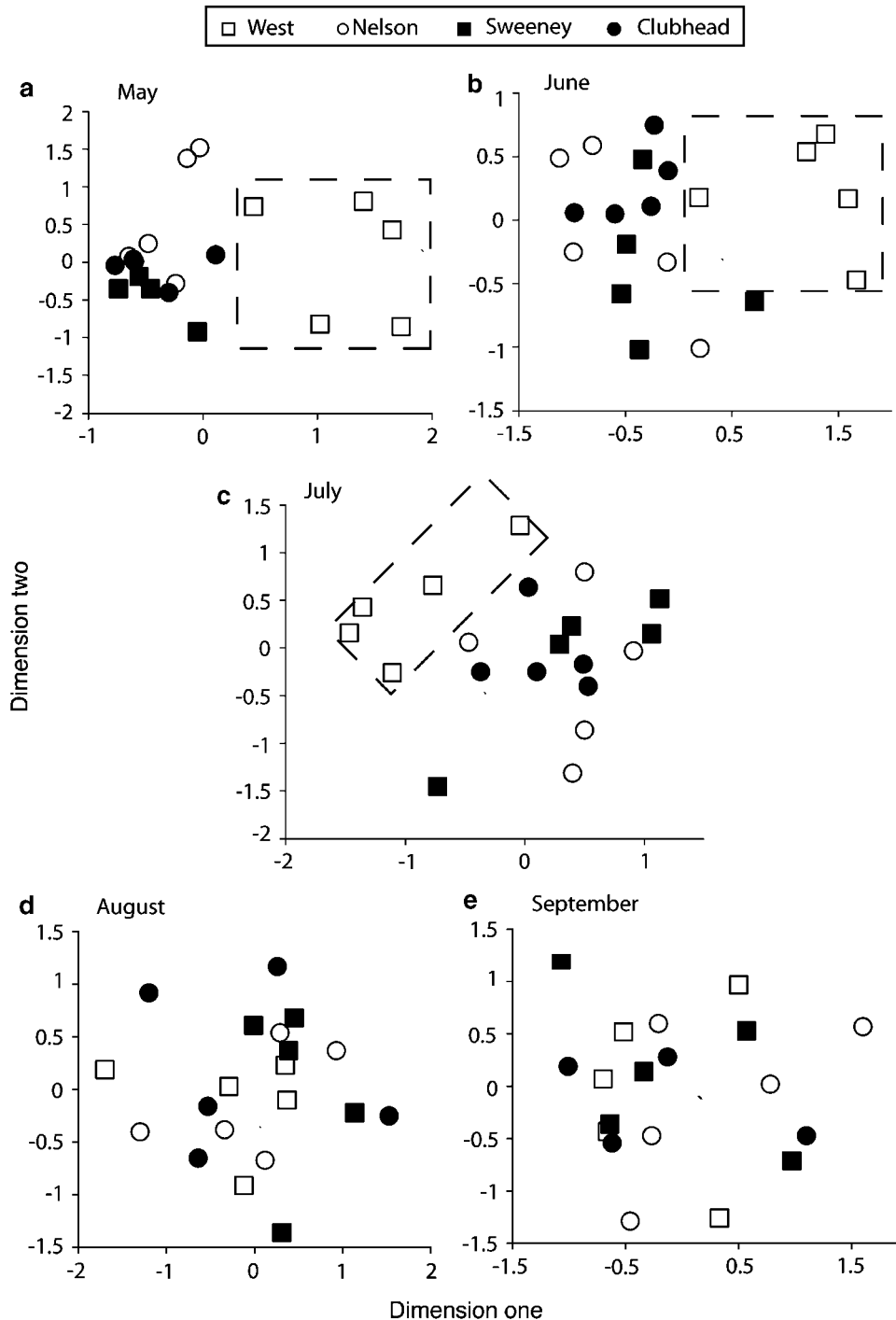


Figure 2 Non-metric multidimensional scaling plot of bacterial community composition in each month of the 2005 growing season. The different habitats are used as replicates to examine differences among the four streams included in this study. Open symbols are reference marshes and closed symbols are 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

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.

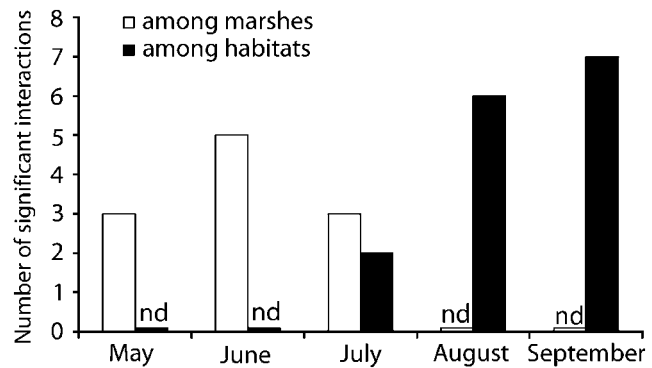


Figure 3 Spatial patterns in bacterial community composition among marshes. The number of significant differences within the bacterial community compositions among marshes and among habitats during each month of the growing season as calculated by Analysis of Similarity. ND indicates that there were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

similar and different from adjacent habitats (*R* = 0.521–1.00; Table 3).

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/

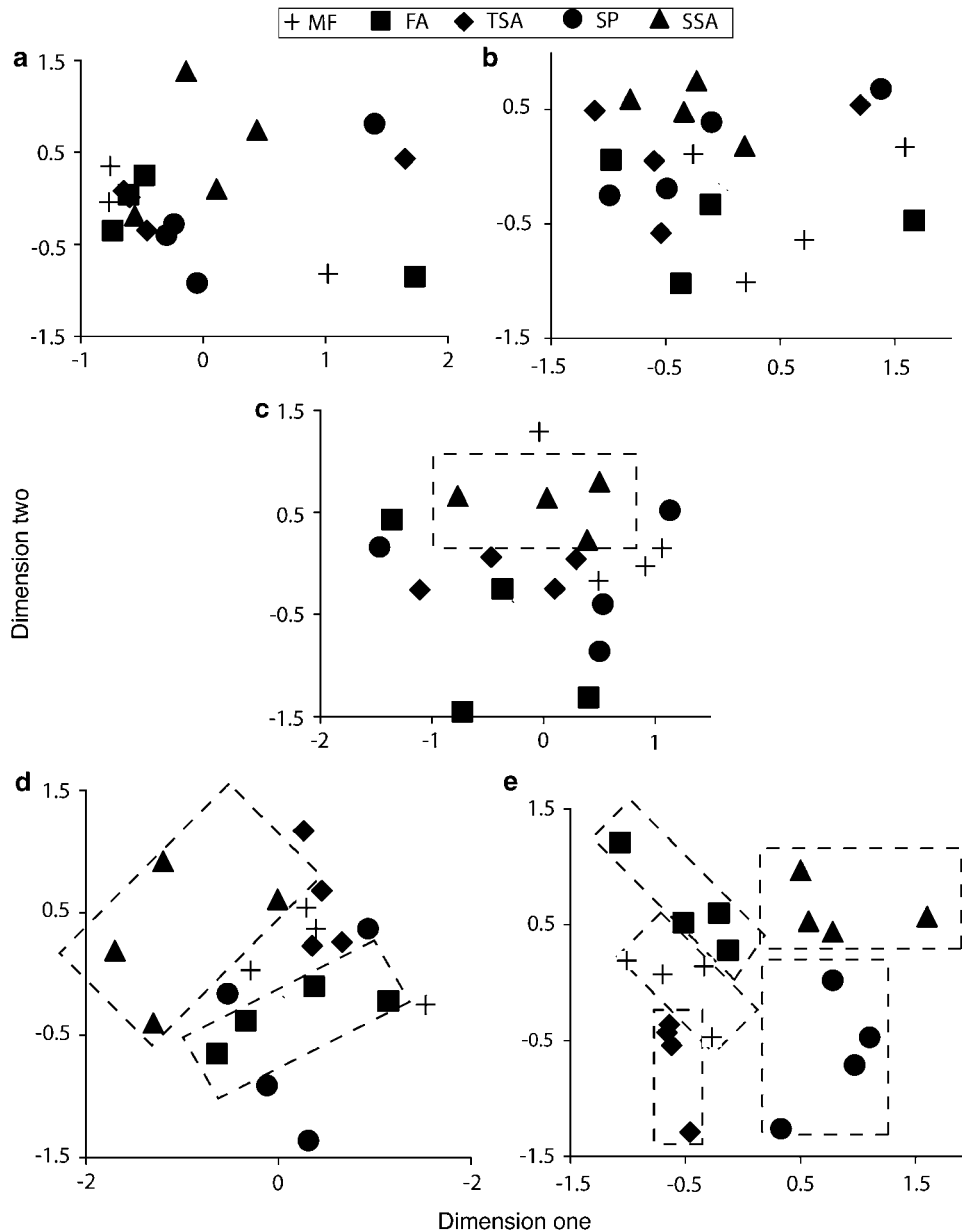


Figure 4 Spatial patterns in bacterial community composition among habitats. Non-metric multidimensional scaling plots of bacterial community composition in each month of the 2005 growing season. Here the different streams are treated as replicated to examine difference among habitats. Stress for each 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 <i>a</i> [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.

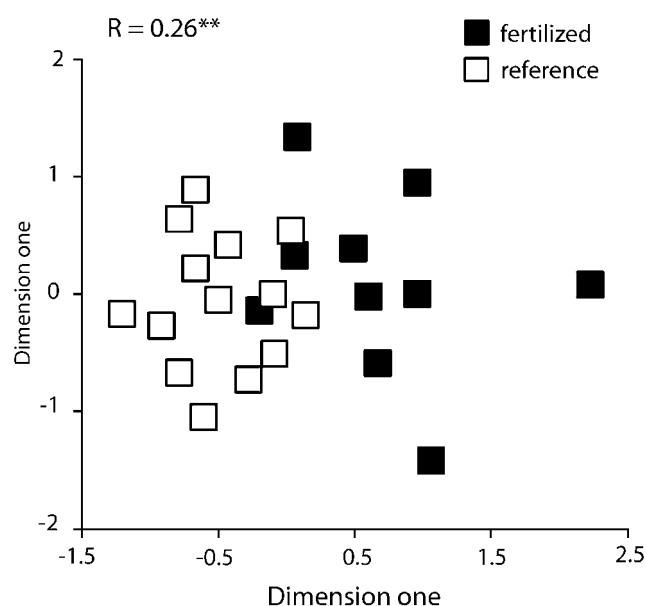


Figure 5 Non-metric multidimensional scaling plots of bacterial community composition in fertilized and reference marshes within the filamentous algal marsh zone (stress = 0.20). **Significant at $P < 0.01$.

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.

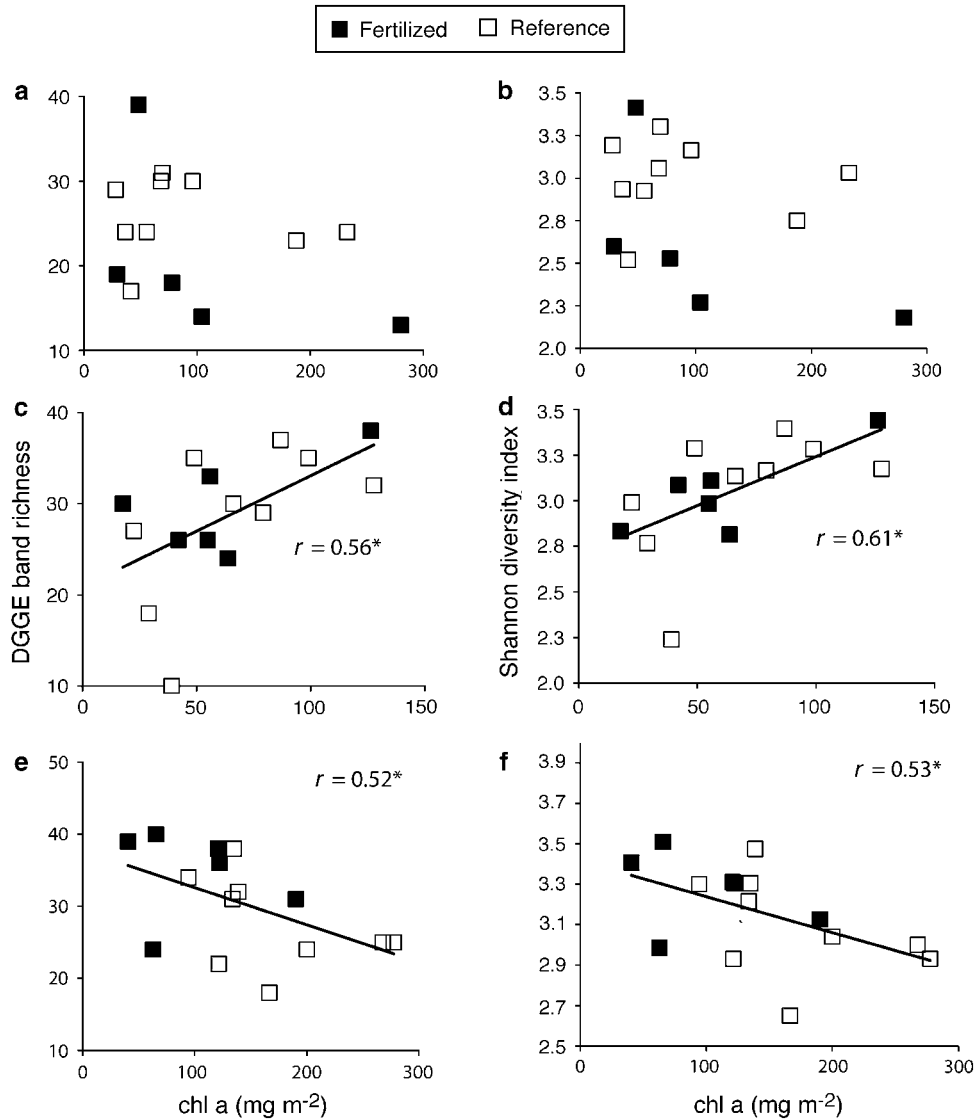


Figure 6 DGGE band richness (panels a, c and e) and Shannon's Diversity Index (panels b, d and f) calculated from DGGE band data plotted as a function of benthic chl *a* concentrations for three marsh habitats: the filamentous algal zone (panels a and b), the mudflat zone (panels c and d) and the tall 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.

Acknowledgements

Funding for this research came from a National Science Foundation Postdoctoral Fellowship in Microbial Biology to JLB. Funding for the marsh fertilization experiment (TIDE) came from The National Science Foundation (grants DEB 0213767, OCE 9726921 and OCE 0423565). We thank J Michael Johnson, Christian Picard, Deanne Drake, Kari A Galván, D Samuel Johnson, Lynsey E Lemay and Erin Miller for maintaining the enrichment experiment and for field assistance. Two anonymous reviewers greatly improved the quality of this manuscript.

References

- Bass-Becking LGM. (1934). *Geobiologie of Inleiding tot de Milieukunde*. WP Van Stockum and Zoon: The Hague, the Netherlands.
- Blum LK, Roberts MS, Garland JL, Mills AL. (2004). Distribution of microbial communities associated with the dominant high marsh plants and sediments of the United States east coast. *Microb Ecol* **48**: 375–388.
- Bowen JL, Crump BC, Deegan LA, Hobbie JE. (2009). Increased supply of ambient nitrogen has minimal effect on salt marsh bacterial production. *Limnol Oceanogr* **54**: 713–722.
- Buesing N, Gessner MO. (2003). Incorporation of radiolabeled leucine into protein to estimate bacterial production in plant litter, sediment, epiphytic biofilms, and water samples. *Microb Ecol* **45**: 291–301.
- Cho JC, Tiedje JM. (2000). Biogeography and degree of endemicity of fluorescent *Pseudomonas* strains in soil. *Appl Environ Microbiol* **66**: 5448–5456.
- Chrost RJ, Siuda W. (2006). Microbial production, utilization, and enzymatic degradation of organic matter in the upper trophogenic layer in the pelagial zone of lakes along a eutrophication gradient. *Limnol Oceanogr* **51**: 749–762.
- Clarke KR. (1993). Non-parametric multivariate analysis of changes in community structure. *Aust J Ecol* **18**: 117–143.
- Clarke KR, Warwick RM. (2001). *Changes in Marine Communities: An Approach to Statistical Analysis and Interpretation*, 2nd edn. Primer-E Ltd.: Plymouth, Marine Laboratory, Plymouth UK, 177pp.
- Córdova-Kreylos AL, Cao Y, Green PG, Hwang HM, Kuivila KM, LaMontagne MG *et al.* (2006). Diversity, composition, and geographical distribution of microbial communities in California salt marsh sediments. *Appl Environ Microbiol* **72**: 3357–3366.

- Crump BC, Adams HE, Hobbie JE, Kling GW. (2007). Biogeography of bacterioplankton in lakes and streams of an arctic tundra catchment. *Ecology* **88**: 1365–1478.
- Crump BC, Hobbie JE. (2005). Synchrony and seasonality in bacterioplankton communities of two temperate rivers. *Limnol Oceanogr* **50**: 1718–1729.
- Crump BC, Kling GW, Bahr M, Hobbie JE. (2003). Bacterioplankton community shifts in an arctic lake correlate with seasonal changes in organic matter source. *Appl Environ Microbiol* **69**: 2253–2268.
- Deegan LA, Bowen JL, Drake D, Fleeger JW, Friedrichs CT, Galván CT *et al.* (2007). Susceptibility of salt marshes to nutrient enrichment and predator removal. *Ecol Appl* **17**: S42–S63.
- Drake DC, Peterson BJ, Deegan LA, Harris LA, Miller EE, Warren RS. (2008). Plant nitrogen dynamics in fertilized and natural New England salt marshes: a paired ¹⁵N tracer study. *Mar Ecol Prog Ser* **354**: 35–46.
- EPA (2002). Mid-Atlantic Integrated Assessment (MAIA) *Estuaries 1997–1998. Summary Report*. U.S. Environmental Protection Agency. Office of Research and Development. National Health and Environmental Effects Research Laboratory, Atlantic Ecology Division. Narragansett, RI 02882. 115 pp.
- Fierer N, Jackson RB. (2006). The diversity and biogeography of soil bacterial communities. *Proc Natl Acad Sci USA* **103**: 626–631.
- Fierer N, Morse JL, Berthrong ST, Bernhardt ES, Jackson RB. (2007). Environmental controls on landscape-scale biogeography of stream bacterial communities. *Ecology* **88**: 2162–2173.
- Franklin RB, Blum LK, McComb AC, Mills AL. (2002). A geostatistical analysis of small-scale spatial variability in bacterial abundance and community structure in salt marsh creek bank sediments. *FEMS Microbiol Ecol* **42**: 71–80.
- Fuhrman JA, Hewson I, Schwabach MS, Steele JA, Brown MV, Naeem S. (2006). Annually reoccurring bacterial communities are predictable from ocean conditions. *Proc Natl Acad Sci USA* **103**: 13104–13109.
- Green J, Bohannan BJM. (2006). Spatial scaling of microbial biodiversity. *Trends Ecol Evol* **21**: 501–507.
- Hewson I, Jacobson/Meyers ME, Fuhrman JA. (2007). Diversity and biogeography of bacterial assemblages in surface sediments across the San Pedro Basin, Southern California Borderlands. *Environ Microbiol* **9**: 923–933.
- Horner-Devine MC, Lage M, Hughes JB, Bohannan BJM. (2004). A taxa–area relationship for bacteria. *Nature* **432**: 750–753.
- Hughes Martiny JB, Bohannan BJM, Brown JH, Colwell RK, Fuhrman JA, Green JL *et al.* (2006). Microbial biogeography: putting microorganisms on the map. *Nat Rev Microbiol* **4**: 102–112.
- Johnson DS, Fleeger JW. (2009). Weak response of salt-marsh infauna to ecosystem-wide nutrient enrichment and fish predator removal: A four year study. *J Exp Mar Biol Ecol* **373**: 35–44.
- Kan J, Crump BC, Wang K, Chen F. (2007). Bacterioplankton community in Chesapeake Bay: predictable or random assemblages. *Limnol Oceanogr* **51**: 2157–2169.
- Kent AD, Yannarell AC, Rusak JA, Triplett EW, McMahon KD. (2007). Synchrony in aquatic microbial community dynamics. *ISME J* **1**: 38–47.
- Legendre P, Legendre L. (1988). *Numerical Ecology*. Elsevier: Amsterdam, The Netherlands.
- Lorenzen GL. (1967). Determination of chlorophyll and phaeo-pigments; spectrophotometric equations. *Limnol Oceanogr* **12**: 343–346.
- Magalhaes CM, Joye SB, Moreira RM, Wiebe WJ, Bordalo AA. (2005). Effect of salinity and inorganic nitrogen concentrations on nitrification and denitrification rates in intertidal sediments and rocky biofilms of the Douro River estuary, Portugal. *Wat Res* **39**: 1783–1794.
- Papke RT, Ramsing NB, Bateson MM, Ward DM. (2003). Geographical isolation in hot spring cyanobacteria. *Trends Ecol Evol* **8**: 86–90.
- Ramette A. (2007). Multivariate analyses in microbial ecology. *FEMS Microbiol Ecol* **62**: 142–160.
- Ramette A, Tiedje JM. (2007). Multiscale responses of microbial life to spatial distance and environmental heterogeneity in a patchy ecosystem. *Proc Natl Acad Sci USA* **104**: 2761–2766.
- Rees GN, Baldwin DS, Watson GO, Perryman S, Nielsen DL. (2004). Ordination and significance testing of microbial community composition derived from terminal restriction fragment length polymorphisms: application of multivariate statistics. *Antonie van Leeuwenhoek* **86**: 339–347.
- Sarda R, Valiela I, Foreman K. (1996). Decadal shifts in a salt marsh macroinfaunal community in response to long-term experimental nutrient enrichment. *J Exp Mar Biol Ecol* **205**: 63–81.
- Stepanuskas R, Moran MA, Bergamaschi BA, Hollibaugh JT. (2003). Covariance of bacterioplankton composition and environmental variables in a temperate delta system. *Aquat Microb Ecol* **31**: 85–98.
- Sullivan MJ, Currin CA. (2000). Community structure and functional dynamics of benthic microalgae in salt marshes. In: Weinstein MP, Kreeger DA (eds). *Concepts and Controversies in Tidal Marsh Ecology*. Kluwer Academic Publishers: Dordrecht, The Netherlands, pp 81–106.
- Urakawa H, Maki H, Kawabata S, Fujimara T, Ando H, Kawai T *et al.* (2006). Abundance and population structure of ammonia oxidizing bacteria that inhabit canal sediments receiving effluent from municipal wastewater treatment plants. *Appl Environ Microbiol* **72**: 6845–6850.
- Valiela I, Howes B, Howarth R, Gibin A, Foreman K, Teal JM *et al.* (1982). Regulation of primary production in a salt marsh ecosystem. In: Gopal B, Turner RE, Wetzel RG, Whigham DF (eds). *Wetlands: Ecology and Management*. International Scientific Publications: Jaipur, India, pp 152–168.
- Valiela I, Teal J. (1974). Nutrient limitation in salt marsh vegetation. In: Reimold RJ, Queen WH (eds). *Ecology of Halophytes*. Academic Press: New York, USA, pp. 547–563.
- Valiela I, Teal JM, Allen SD, Van Etten R, Goehring D, Volkman S. (1985). Decomposition in salt marsh ecosystems: the phases and major factors affecting disappearance of above-ground organic matter. *J Exp Mar Biol Ecol* **89**: 29–54.
- Van Raalte CD, Valiela I, Carpenter EJ, Teal JM. (1974). Inhibition of nitrogen fixation in salt marshes measured by acetylene reduction. *Estuar Coast Mar Sci* **2**: 301–305.
- Van Raalte CD, Valiela I, Teal JM. (1976). Production of epibenthic algae: light and nutrient limitation. *Limnol Oceanogr* **21**: 862–872.
- Woodcock S, Curtis TP, Head IM, Lunn KM, Sloan WT. (2006). Taxa–area relationships for microbes: the unsampled and the unseen. *Ecol Lett* **9**: 805–812.