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A geostatistical analysis of small-scale spatial variability in bacterial abundance and community structure in salt marsh creek bank sediments

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Abstract

Small-scale variations in bacterial abundance and community structure were examined in salt marsh sediments from Virginia's eastern shore. Samples were collected at 5 cm intervals (horizontally) along a 50 cm elevation gradient, over a 215 cm horizontal transect. For each sample, bacterial abundance was determined using acridine orange direct counts and community structure was analyzed using randomly amplified polymorphic DNA fingerprinting of whole-community DNA extracts. A geostatistical analysis was used to determine the degree of spatial autocorrelation among the samples, for each variable and each direction (horizontal and vertical). The proportion of variance in bacterial abundance that could be accounted for by the spatial model was quite high (vertical: 60%, horizontal: 73%); significant autocorrelation was found among samples separated by 25 cm in the vertical direction and up to 115 cm horizontally. In contrast, most of the variability in community structure was not accounted for by simply considering the spatial separation of samples (vertical: 11%, horizontal: 22%), and must reflect variability from other parameters (e.g., variation at other spatial scales, experimental error, or environmental heterogeneity). Microbial community patch size based upon overall similarity in community structure varied between 17 cm (vertical) and 35 cm (horizontal). Overall, variability due to horizontal position (distance from the creek bank) was much smaller than that due to vertical position (elevation) for both community properties assayed. This suggests that processes more correlated with elevation (e.g., drainage and redox potential) vary at a smaller scale (therefore producing smaller patch sizes) than processes controlled by distance from the creek bank.

Keywords: Spatial variability; Spatial heterogeneity; Microbial community; Community structure; Geostatistics; Salt marsh; Sediment

1. Introduction

Although individual bacterial cells function at a scale that befits their small size [1], their combined localized activities mediate processes that are important at the landscape scale. It is at this scale that microbial reactions are most often studied and a great deal of effort is currently being expended to try to relate the structure of these communities to observed functional phenomena. When studying microorganisms, the boundaries used to define a community are generally utilitarian and dictated by the required sample size, the researcher's perception of environmental variability and the overall scale of the measured property. Consequently, measurements of microbial community attributes typically represent broad-scale characterizations and rarely consider the small spatial scale at which individuals and populations may actually be interacting.

Although the broad-scale approach to studying microbial communities has proved useful for monitoring large-scale changes in microbial dynamics, studies of microbial community patch size are rare, and knowledge about the scales at which microbial interactions and associations become important is incomplete. The total capacity of microbial communities in the environment is the sum of the activity of several 'unit' communities of microorganisms [2] in distinct microhabitats, whose separate activities are pooled into what scientists observe as ecosystem function. In order to understand how these spatial units fit together

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and how the activities of the distinct units contribute to overall ecosystem function, a better understanding of the distribution of microorganisms (and microbial communities) in space is needed.

In sampling theory, spatial scale is defined by several characteristic properties: grain size, sampling interval, and extent [3]. Grain size is the size of the elementary sampling units (e.g., the volume of sample), and defines the resolution of the study [4]. Sampling interval is the average distance between sampling units. The *extent* is the total area included in the study. The dimensions of these components vary according to the ecological question being addressed and what is already known about the scale of the process of interest. For a given sampling design, no structure can be detected that is smaller than the grain size or larger than the extent of the study; in this way, the sampling design defines the observational window for spatial pattern analysis [3].

Previously, researchers have considered the spatial distribution of microorganisms in many different environments and at a variety of spatial scales. In ecological studies, the grain size is generally too large to permit analysis of the location or activity of individual organisms, although experiments have been conducted at the microscale to examine these properties [5–9]. For example, electron microscopy has been used to study the rhizosphere colonization patterns of *Pseudomonas flurorescens* using a sampling interval of 5 μm [10]. At larger scales, studies in agricultural soils have demonstrated that significant spatial heterogeneity may exist for microbiological processes [11,12], community structure [13–15] and abundance [9,16]; patch size estimates range widely from as little as 2 mm [12] to nearly 10 m [15]. Similar studies have been conducted in grassland and forest soils [17–22], in a shallow coastal aquifer [23] and in the open ocean [24,25]. For salt marsh and marine sediments, variation has been examined at small scales (< 1 m^2 [26–28]), and at intermediate (< 150 m [28,29]) and larger distances (km [28]). These studies revealed that microbial communities can be organized at a variety of spatial scales, a likely reflection of the scales of heterogeneity in the distribution of physical and chemical properties of the environment under investigation.

In addition to the theoretical implications that motivate research into the spatial distribution of microorganisms, the results of such studies have important practical applications for scientists designing and planning experiments at the field and landscape scales. While many ecological theories and models acknowledge that elements that are close to one another in space or time are more likely to be influenced by the same generating processes, the same energy inputs, or a similar physical environment, the classical statistical procedures employed to analyze these phenomena assume independence of observations. Statisticians generally count one degree of freedom for each independent observation, which allows them to choose an appropriate statistical distribution for testing. The lack of independence that arises from the presence of autocorrelation makes it difficult (in many cases, impossible) to accurately determine the number of degrees of freedom and correctly perform tests such as correlation, regression, or analysis of variance (ANOVA). Positive autocorrelation reduces within-group variability, artificially increasing the amount of among-group variance, and often leads to the conclusion that differences among groups are significant, when in fact they are not [30].

Some procedures allow researchers to make corrections and perform statistical analyses in the presence of spatial autocorrelation (for an overview see [3,30–33]); however, the application of these techniques is often limited by constraints such as sample size or the physical distribution of sampling locations (e.g., a procedure may require sampling locations to be along a regularly spaced grid [30]). Another solution is to design a sample collection scheme so that there is little spatial structure present in the data, and then use parametric statistical hypothesis tests. In this case, samples must be collected close enough together that they represent replicates of the system under investigation, but they must be placed far enough apart to avoid autocorrelation. Regardless of which approach one chooses (correction of statistical procedures or modification of experimental design), it is first necessary to describe the type of autocorrelation present in a variable (e.g., gradient versus patches) and estimate its extent. There are several procedures available to test for the presence of spatial structure in ecological data (for reviews see [32,34–37]). Geostatistics is a powerful tool that can provide insight into the spatial structure and quantitatively describe spatial variation by expressing a measure of association, or autocorrelation, between two samples as a function of the distance between them. Geostatistical analyses are commonly used in soil and mineral science, but less so in microbial ecology [12,15,18–20,25,38–42].

The purpose of the present study was to examine the spatial structure of microbial communities in salt marsh sediments using traditional geostatistical techniques. Sampling of unvegetated creek bank sediment in a *Spartina alterniflora*-dominated salt marsh was undertaken at 5 cm intervals. The community in the samples was characterized microscopically (for total concentration of cells) and with DNA fingerprinting (to determine the overall genetic similarity between samples). These data were analyzed to determine microbial community patch size, the amount of spatial autocorrelation among the samples and the relative importance of the horizontal (distance from creek bank) versus vertical (elevation) separation of the communities. Each of the analyses performed confirmed that spatial autocorrelation existed at a relatively small scale (10–100 cm). In general, spatial structure in abundance was organized with a correlation length scale larger than that for community structure, and the patch size for the communities was greater in the horizontal direction than in the

vertical. These results suggest that the study of salt marshes at spatial scales such as these could provide insight into the structuring and distribution of microbial communities in these systems and help scientists to understand within-marsh biogeochemical process variation. Moreover, the work has important implications for researchers conducting field experiments as it indicates that sampling of these sediments at spacings less than the correlation length scale of the property of interest may result in inaccurate statistical analyses and incorrect conclusions.

2. Materials and methods

2.1. Sample collection

Sediment samples were collected from Phillips Creek marsh in the Virginia Coast Reserve Long Term Ecological Research site, on Virginia's eastern shore, during September of 1999. The area sampled (37°27.496′N, 75°50.075′W) was an unvegetated, intertidal portion of the bank of a minor tributary of Phillips Creek. For each sample, a small core was taken from the surface of the marsh to a depth of approximately 5 cm using detipped, 10 ml plastic syringes. The 44 sampling locations in this study ranged vertically over 50 cm of elevation; position was measured to the nearest 0.25 cm. The samples were not regularly spaced over the elevation gradient but the average separation between any pair of adjacent samples was 1.5 cm. The sampling locations were regularly spaced at 5 cm intervals in the horizontal direction, ranging over a length of 215 cm.

After collection, the sediment samples were transported to the lab on dry ice and stored at −20°C until acridine orange direct counts (AODC) [43,44] DNA extraction could be performed.

2.2. DNA extraction and quantification

DNA was extracted from the sediment samples using the MoBio UltraClean soil DNA isolation kit (Solana Beach, CA, USA). The amount of sediment used in each extraction ranged from 0.25 to 0.83 g; it was varied according to cell counts (AODC), so that the quantity of DNA obtained from each sample would be approximately equal. Cells were lysed using the vortex procedure described in the kit documentation. Purified DNA was resuspended in 10 mM Tris buffer and stored at −20°C. The concentration of DNA from each sample was measured using the PicoGreen dsDNA quantification kit (Molecular Probes, Eugene, OR, USA).

2.3. RAPD community fingerprinting

2.3.1. RAPD conditions

Randomly amplified polymorphic DNA (RAPD) reactions were performed as previously described [45]. Briefly, 5 μl of a DNA solution (approximately 350 pg DNA) was added to a 20 μl reaction mixture containing: 10 mM Tris–Cl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 100 μM each of dATP, dCTP, dGTP, and dTTP, the primer at 0.2 μM (Operon Technologies, Alameda, CA, USA), and 1 U of *Taq* DNA polymerase. The PCR program comprised 45 cycles of: 1 min at 94°C, 1 min at 36°C, and 2 min at 72°C. Amplification products were separated by electrophoresis in 1.75% agarose gels (containing ethidium bromide (0.25 μg ml^{-1})), and photographed under UV light. Each sample was amplified using several different RAPD primers (see below), and the individual results pooled to represent a single community fingerprint.

2.3.2. Primer selection

Previous work that characterized the pelagic microbial communities in Phillips Creek [45] was used to guide primer selection in this study. Each primer successfully used in the earlier study was tested on a subset of the sediment samples; the number, clarity and distinctness of bands, and the reproducibility of the RAPD fingerprints were used to select the best primers to profile the entire set of samples. Primers C4, F1, F3, F4, F5, F7 and T7 were chosen for use (sequences available in Franklin et al. [45] and from Operon Biotechnologies (www.operon.com)). These primers are short (10 bp), random sequences that can anneal at numerous locations throughout a genome and they are not selective for individual organisms, groups of organisms, or genes. Because of this, RAPD can detect sequence variation that is distributed throughout the microbial DNA pool, theoretically producing a fingerprint of the genetic composition of the entire community.

2.3.3. RAPD data analysis

The raw data from RAPD represent a series of bands in an agarose gel, each being a DNA fragment of a certain size. For each primer, each amplification band was treated as a unit character and scored as present or absent in each sample (i.e., recorded as 1 or 0). The results for all of the primers were then pooled into a single large dataset. Collectively, these primers produced a total of 87 bands and individual samples contained between 11 and 34 bands. The average number of bands produced for a given sample was 21.

2.4. Variogram analysis of spatial autocorrelation

In this study, the variation in bacterial abundance (as measured by AODC) and in community structural similarity (as measured by RAPD fingerprinting) was compared using a geostatistical semi-variogram analysis to study autocorrelation as a function of distance. The correlation between spatial separation and each community parameter was modeled considering the two distance components (horizontal and vertical) separately, rather than

using the Euclidean distance between each set of samples, as it was not expected that the scale of variability in the horizontal and vertical directions would be the same. Moreover, as the samples were taken along a slope, the vector connecting many of the points (using Euclidean distance) passed through the air, rather than the sediment, so it did not seem reasonable to use this separation distance to model the spatial autocorrelation of the microbial communities. Comparing each sampling location with each of the other 43 resulted in a total of 946 points to analyze (this is the number of points in the upper (or lower) triangle matrix between each sampling location).

Prior to constructing a variogram, it was necessary to segregate the data into distance classes (bins). The purpose of binning the data was to obtain the best resolution (maximum detail) at small distances without being misled by structural artifacts resulting from whatever particular size class was chosen. To determine the appropriate number of bins for each analysis, Sturge's rule [3] was applied, which states that the appropriate number of classes = $1+3.3 \log_{10} m$, where m is the number of points in either the upper or lower triangle matrix (in this case, 946); the calculation suggested that 10 size classes were appropriate for this data set. Furthermore, variograms are generally not valid beyond half of the maximum distance between samples, and so the appropriate lag distance (the distance increment for each class) was calculated as the maximum pair distance divided by 2 and then subdivided into 10 equal classes [46]. For the vertical direction, this resulted in a 2.5 cm lag distance; for the horizontal direction, a lag distance of 10 cm was established.

To calculate a variance term for the AODC, each value was first normalized by dividing it by the average concentration of cells among all samples. The inverted covariance was then calculated between each pair of samples using GEOEAS [46]. A traditional variance term could not be calculated for the RAPD results (multivariate binary data); instead, the relative similarity between each pair of samples was determined using the Jaccard coefficient (which is based upon the proportion of positive bands shared by each sample pair [47]). This similarity matrix was then converted to a dissimilarity matrix (dissimilarity = 1 − similarity), which represents the relative difference in community genetic structure between each pair of samples. Plots of relative dissimilarity versus separation distance should take the form of a typical variogram.

Experimental variograms are often fitted with a continuous function to smooth out sample fluctuations and estimate useful model parameters (e.g., the correlation length scale (range) and the spatial dependence). Several types of functions are available including the linear, Gaussian, exponential and spherical models. A linear variogram would indicate a linear spatial gradient and would represent a situation where the samples are spatially autocorrelated at all distances measured (the sampling distance is not large enough to capture all of the spatial variability at that scale). Gaussian, exponential and spherical models are bounded in that they level off, either at a given range value (spherical model) or asymptotically (exponential and Gaussian models). Differences between these functions lie mostly in the shape of the left-hand portion of the curves, near the origin; in practice, the spherical and exponential models do not differ by much [3]. Several authors have warned against the risk of numerical instability associated with the Gaussian model, and it is rarely used at this point [34].

For each parameter (community structure and AODC), separate variograms were constructed for each direction (horizontal and vertical). Data were then fitted with an exponential model, as suggested by Legendre and Legendre [3]:

$$y = C_0 + C_1[1-\exp(-3x/a)]$$

where y is the variance term [either inverted covariance (for AODC) or dissimilarity (for genetic community structure)], and x is the spatial separation distance. From the model, C_0, C_1, and a were estimated; C_0 is a parameter quantifying the nugget effect (the amount of variability at distance = 0), C_1 is a spatially structured component of the model, and a is the range (the distance beyond which variance is no longer a function of spatial separation). The sill (C) is the y value at which the variogram levels off and was calculated as: $C = C_0 + C_1$.

One difficulty with using equal distance classes when constructing a variogram is that the number of pairs of points in large distance classes is often too small for valid testing; to avoid this problem, only the spatial structure of the first two thirds of the variogram was modeled [35]. All regressions were performed in SigmaPlot (Version 5.0) and R^2 was used to measure the goodness of fit of the model to the data.

2.5. Determination of group differences

2.5.1. Community structure

In addition to the geostatistical analyses, the data were also analyzed to determine if any group differences existed between samples positioned along the elevation gradient. Samples were divided into four groups (Fig. 1) based on elevation, horizontal position and frequency of inundation. The four groups were: group 1 (samples 1–16), sediment always saturated; group 2 (samples 17–26), sediment frequently flooded; group 3 (samples 27–31), sediment occasionally flooded; and group 4 (samples 32–44), sediment rarely flooded. A Mantel test [48,49] was used to evaluate whether overall microbial community structure was significantly different among the various groups. In general, a Mantel test determines the amount of correlation between two matrices, and a permutation procedure is used to assess the significance of this correlation. In this application, the two matrices being compared were (1) the observed dissimilarity matrix calculated from the RAPD data, and

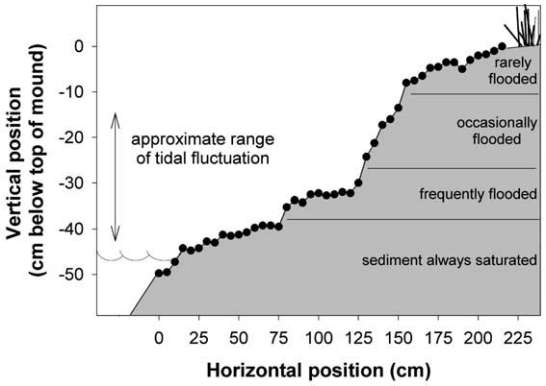

Fig. 1. Map of creek bank showing sampling locations (indicated with ●), position of Phillips Creek, and approximate range of tidal influence along the slope. Sampling locations were divided into four groups (1 to 4, bottom to top) based on their flooding frequency and elevation.

(2) a conceptual model matrix that defined group identity. Data were analyzed to test the null hypothesis that there was no difference within- and between-group genetic similarities of the communities using the Mantel–Struct program [50]. A Monte Carlo procedure (with 5000 permutations) was then used to control for the impact of spatial autocorrelation among the sampling locations and to evaluate the significance of the group differences.

2.5.2. Bacterial abundance (AODC)

A Mantel test was also used to compare bacterial abundance in the different elevation groups. First, a dissimilarity matrix was calculated by determining the absolute value of the difference between each sample pair, divided by the maximum difference for all pairs. This matrix, along with the group identity for each sample, was analyzed using the Mantel–Struct program [50]; as above, a Monte Carlo procedure (with 5000 permutations) was used to determine statistical significance.

3. Results

3.1. Variogram analysis of spatial autocorrelation

Fig. 2 shows the results of the geostatistical analyses for each parameter (bacterial abundance and community structure) for each direction (horizontal and vertical). The data were fitted with an exponential model, and the model output is presented in Table 1. In general, the model fitted the data quite well (Table 1, Fig. 2); R^2 varied between 0.62 and 0.96, and all correlations were significant, with $P < 0.005$. For AODC, the spatial dependence (the proportion of variance in the data that was accounted

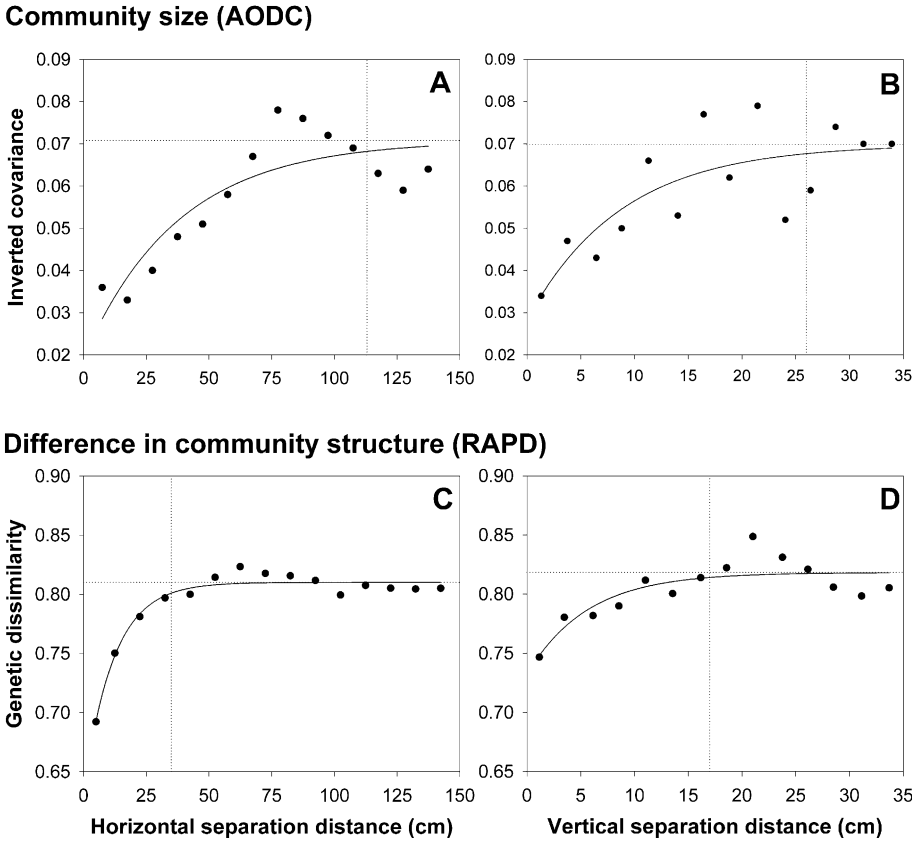

Fig. 2. Variograms constructed to model spatial autocorrelation in bacterial abundance (A and B) and difference in microbial community structure (C and D) along the creek bank. Directional variograms were constructed for each parameter based upon either the horizontal (A and C) or vertical (B and D) separation of the samples. Best-fit lines, constructed using an exponential model, are presented, as are the sill (asymptotic value of the curve) and range (separation distance where y is 95% of the sill).

Table 1
Summary of model parameters obtained from fitting an exponential equation to the experimental variograms

Parameter	Direction	Nugget (C_0)	Sill ($C = C_0 + C_1$)	Relative nugget effect (C_0/C)	Spatial dependence (C_1/C)	R^2	Range (cm)
Bacterial abundance (AODC)	horizontal	0.019	0.071	0.27	0.73	0.76	113
	vertical	0.028	0.070	0.40	0.60	0.62	26
Difference in community structure (RAPD)	horizontal	0.631	0.810	0.78	0.22	0.96	35
	vertical	0.732	0.818	0.89	0.11	0.79	17

for by the spatial model) was quite high. In the horizontal direction, the spatial model could account for 73% of the variance in microbial abundance; for the vertical direction, 60% of the variation in bacterial abundance could be correlated with the spatial separation of samples. The range (the distance beyond which data exhibit no spatial autocorrelation) was 26 cm in the vertical direction and 113 cm in the horizontal. The sill (the value of the variogram at distances beyond the range) was the same for both directions.

In geostatistics, anisotropy is said to be present in data when the autocorrelation function is not the same for all geographic directions. In the case of bacterial abundance, the analyses produced the same sill for the two different directions, but different ranges; this phenomenon is referred to as geometric anisotropy [51]. A geometric anisotropy ratio can be calculated as the ratio of the larger range to the smaller range; here that ratio was 4.3 (horizontal/vertical). This means that, on average, the same amount of variability occurred over 4 horizontal distance units as occurred in 1 vertical distance unit. It is interesting to note the similarity between this value and the aspect ratio (horizontal distance/vertical distance = 4.3).

For similarity in community structure, spatial dependence was lower (horizontal: 22%, vertical: 11%), and most of the variation in community structure was not accounted for by simply considering the spatial separation of the samples. The range of spatial extent for overall community structural similarity was smaller than that calculated for bacterial abundance: 35 cm for the horizontal direction and 17 cm for the vertical. As with bacterial abundance, the sills for the two directions were similar and a geometric anisotropy ratio could be calculated. In this case, the anisoptropy value was 2, a value smaller, but in the same direction as for bacterial abundance (horizontal/vertical).

3.2. Analysis of group differences

An analysis of group differences was performed to evaluate whether the different flooding zones (Fig. 1) contained communities whose structure and abundance were significantly different. A Mantel test was performed and a Monte Carlo procedure (using 5000 permutations) was employed to assess the significance of the test statistic. A Bonferoni-type correction was used to adjust the α level, depending on the number of comparisons made [35]; a modified α level of 0.008 ($\alpha = 0.05$ divided by six comparisons) was used.

Overall, the sample groups were found to contain significantly different microbial communities as assayed by RAPD fingerprinting ($P = 0.0002$). Specifically, group 4 (rarely flooded/high marsh) was found to differ from all of the other groups, and group 3 was significantly different from group 1 (Table 2).

For microbial abundance, the average concentration of cells ($\times 10^{10}$ cell ml^{-1} sediment) was: group 1, 7.4; group 2, 5.6; group 3, 6.8; group 4, 7.8. Overall, these differences were significant ($P = 0.03$), with group 4 (rarely flooded/high marsh) being the most distinct (Table 2).

Table 2
Results from a series of Mantel tests comparing community properties for the different elevation groups; P values were obtained using a Monte Carlo procedure (5000 permutations)

	Group			
	1	2	3	4
				abundance
1: always saturated	–	0.62	0.99	0.003*
2: frequently flooded	0.088	–	0.007*	0.0008*
3: occasionally flooded	0.0008*	0.018	–	0.39
4: rarely flooded	0.004*	0.0002*	0.0006*	–
	community structure			

The lower (left) half of the matrix contains the results for community structure (RAPD) and the upper (right) portion of the matrix contains the results from the comparison of bacterial abundance (AODC).
* Significant P values, after α was corrected to 0.008 for multiple comparisons (see text).

4. Discussion

The overall goal of this study was to quantify spatial autocorrelation among the microbial communities residing in salt marsh creek bank sediments. In particular, we sought to determine the extent to which the spatial distribution of samples contributes to overall between-sample variability and to estimate microbial community patch size. In general, the results demonstrated that small-scale variability exits, both in terms of total bacterial abundance (AODC) and in terms of community structure (RAPD fingerprinting results). The patch size of the microbial communities found in these sediments varied somewhat, depending on the parameter used to assay the organisms and on the way that the spatial separation of the sampling units was calculated; patch size estimates ranged between approximately 10 and 100 cm. These values are similar to those obtained by other researchers in other environments, as outlined in the introduction.

The correlation length scales for bacterial abundance (AODC) were larger than the values obtained when considering community structure, for both directions (vertical: 113 cm versus 35 cm; horizontal: 26 cm versus 17 cm). This finding suggests that the environmental factors controlling these two community attributes may be different, and may vary at different spatial scales in the environment. Alternately, these results could reflect a single environmental parameter influencing the two microbial attributes differently (or to different extents). Simultaneous measurement of the distribution of microbial communities and physicochemical properties (e.g., organic matter concentration, sediment moisture content, or redox status) at small spatial scales would add greatly to our understanding of how environmental heterogeneity can influence microbial community development and, conversely, of how microbial communities may alter their microenvironment. The samples collected for this study were too small to permit measurement of the microbial communities as well as physicochemical parameters.

Most of the studies of microbial community variability at small spatial scales have considered more general properties (e.g., total abundance, biomass, or activity [18,24,29]), while relatively few studies have examined the distribution of microbial community structure [13,22,23,25]. Of those that have, the range over which the communities were spatially autocorrelated was generally smaller than the range established for more broad microbial community properties. However, there are very few studies that have compared these two aspects of the same community [17,25]. Likewise, studies that have examined the distribution of guilds (e.g., denitrifiers [28]) or specific groups of organisms [12,14,22] in the environment, tended to find smaller correlation length scales than those studies that assayed for more general microbial parameters. This discrepancy may in part be due to the fact that researchers who study specific functions or particular groups of organisms are more likely to conduct experiments at small spatial scales, while researchers who study more general parameters often collect larger samples that can limit the resolution of a study.

In this study, the spatial dependence (the percent of the total variance in the data that can be explained by considering the spatial separation of the sampling units) was much less for the analyses that considered community structure (horizontal: 22%, vertical: 11%) than for the analyses that considered bacterial abundance (horizontal: 73%, vertical: 60%). Most of the variability in community structure must come from variation at other scales, experimental error, or the influence of other environmental parameters. The reproducibility of the RAPD procedure is sensitive to a number of experimental factors [52,53] and experimental error might partially explain the relatively large nugget effect observed in the geostatistical analyses of the RAPD data, in comparison to the AODC data. However, recent work has demonstrated the reproducibility of RAPD fingerprinting with microbial community DNA samples [45,54], and it is unlikely that methodological problems contributed significantly to the results presented here.

Given the relatively small proportion of the variance in community structure that was spatially dependent, it seemed reasonable to analyze the RAPD data to see if there were any differences between groups of samples located at different elevations (Fig. 1). Groups were defined on the basis of vertical position and frequency of inundation, and roughly corresponded to four areas between low and high marsh. The RAPD profiles obtained for the group 4 samples (rarely flooded/high marsh) were significantly different from those obtained in any of the other zones (Table 2). Similarly, the communities inhabiting group 3 (occasionally flooded) were significantly different from the group 1 (saturated sediment/low marsh) communities. For bacterial abundance (AODC), the group differences were not as strong, although group 4 was again distinct from the other samples. The flooding regime along the creek bank could influence the microbial communities in a number of ways. Besides the direct effect of inundation on microbial community structure (e.g., flooding could add or remove organism types), there are a number of different environmental parameters (e.g., sediment moisture content, redox status) that may vary in response to the patterns of water movement. Further study would be necessary to establish whether any of these parameters are important in generating the community differences observed here.

One of the main problems when working with samples that are spatially autocorrelated is that parametric statistical procedures are not appropriate for data analysis. As part of this study, we wanted to estimate the range of spatial influence of microbial abundance within these sediments, and then to determine whether sampling beyond this range provided a different estimate of mean bacterial

concentration, compared to sampling that was conducted at separation distances smaller than the correlation length scale. To test whether the estimate of abundance one would obtain by sampling two spatially autocorrelated locations was significantly different from the value one would obtain by sampling two locations that were spatially independent, we calculated the average AODC between each pair of samples (to simulate several sampling efforts), and then categorized each average as having been obtained using spatially autocorrelated samples or as being spatially independent. After sorting the averages into these two groups, an ANOVA was used to determine whether the estimates of abundance obtained using spatially autocorrelated samples were significantly different from those obtained using pairs of independent samples. Overall, the estimate of mean AODC was significantly lower using spatially autocorrelated samples [$6.9\times10^{10}\pm4.2\times10^{8}$ (S.E.)] compared to spatially independent samples [$7.6\times10^{10}\pm5.8\times10^{8}$ (S.E.)] (ANOVA results: d.f. = 945, $F=88.96$, $P<0.0009$). This exercise demonstrates the important impact spatial autocorrelation can have on the estimate of mean environmental properties in a given region or habitat.

Another major trend revealed in this analysis was the anisotropy associated with the distribution of microorganisms in the sediments. For both community parameters, the patch size was always greater when horizontal as opposed to vertical separation was used as the distance measure. This finding could partly be an artifact of the sampling design – our sample density was much higher for the x direction than for the y direction (44 samples per 50 cm vertical elevation versus 44 samples per 215 cm horizontal expanse). The higher sampling density meant that there was a smaller average separation distance when considering elevation (1.5 cm) compared to the horizontal direction (5 cm), and this smaller separation distance increased the opportunity to detect smaller-scale variability. Since microbial communities are organized at a hierarchy of spatial scales, it is possible that the sample design used here simply captured community variation at two different levels. It is also possible that there are different environmental parameters influencing community development in the two directions and that the processes more correlated with elevation (e.g., drainage and redox potential) varied at a smaller scale than the processes controlled by distance from the creek bank. It is important to note that throughout most of this discussion, the horizontal and vertical components of space have been discussed as if they were independent, though they are not.

Within marsh ecosystems, microbial communities serve many critical functions, including the decomposition of organic material and the biogeochemical cycling of minerals and nutrients. Researchers generally see a great deal of variation in microbial community structure and processes, both within and between marshes. Often, biogeochemical process variation within visibly homogenous environments of a single marsh is greater than that between marshes [55–58]. The results presented here suggest that community structure and microbial abundance can vary at small scales (<1 m) in these systems, and that this information needs to be incorporated into the experimental design when sampling these habitats. It is reasonable to expect the patch size of the microbial community to vary in different environments, and some care must be taken when trying to extrapolate the results of this work to other systems.

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