



Controls on diatom biogeography on South Carolina (USA) barrier island beaches

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ABSTRACT: Benthic microalgae are of great significance to coastal productivity and nutrient cycling, yet determinants of their biogeography and community assembly remain unclear. We identified and compared spatial and environmental mechanisms that shape beach diatom biogeography and diversity at regional scales. We sampled at various spatial scales (from 10 cm to 120 km) from 4 barrier island beaches in South Carolina, USA, and characterized diatom assemblages using DNA metabarcoding. Twelve environmental variables from sediments, adjacent waters, and atmosphere were recorded. Despite attempts to sample from similar habitat types, environmental factors varied among islands. Correspondingly, biomass and α -diversity metrics likewise varied and were positively related to sediment nutrient levels. Community composition also varied, with each island dominated by different diatom taxa. Redundancy analysis revealed that spatial influences, in addition to environmental factors (water temperature and porewater nutrients), were significantly related to community dissimilarity (β -diversity). Variation partitioning attributed only ~5% of community variation to geographic distance alone, whereas 22% was explained by combined spatial/environmental factors. A high proportion (73%) of community dissimilarity remained unexplained, which likely implicates stochastic processes, although unmeasured physico-chemical or biological factors cannot be discounted. Weak spatial effects suggest that dispersal limitation was relatively unimportant, likely due to extensive sediment transport between beaches. Instead, spatially structured environmental variables appeared to condition beach diatom biogeography. These findings broaden our understanding of microbial biogeography to less well-studied microorganisms and marine habitats. More practically, results suggest some degree of predictability in diatom community changes in the face of anthropogenic challenges.

KEY WORDS: Benthic microalgae · Microphytobenthos · Metabarcoding · Beta-diversity · Alpha-diversity

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1. INTRODUCTION

Zoologists and botanists have studied large-scale geographic distributions since the 18th century, whereas the field of microbial biogeography is in its infancy. Biogeography, the study of the variation in species composition and diversity over geographic space and through geological time, offers insights into the mechanisms that generate and maintain biodiversity, such as species interactions, dispersal, speciation, and extinction events (Hughes Martiny et al. 2006). Due to the high dispersal rates and large population sizes of microbes, their large-scale distribu-

tion patterns and underlying mechanisms may fundamentally differ from those of macro-organisms (Hughes Martiny et al. 2006, Soininen 2012). The long-held view about micro-organisms has been that ‘Everything is everywhere, but, the environment selects’ (Baas-Becking 1934, p. 15). The essence of this hypothesis is that contemporary environmental conditions dictate community structure of microbes because microbes are not dispersal-limited. Although a handful of studies have shown that various aquatic microbes appear to be ubiquitous (Gibbons et al. 2013, Whittaker & Ryneerson 2017), most recent studies reveal that microbial taxa have limited geo-

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graphic distributions (e.g. Pommier et al. 2007, Sul et al. 2013, Hoarfrost et al. 2020). Today the Baas-Becking model has largely been supplanted by the 'moderate endemism model' of microbial biogeography (Foissner 2006), i.e. the idea that some microbes may have cosmopolitan distributions but most have restricted distributions, and that sometimes the environment selects and sometimes it does not (van der Gast 2015).

Today the focus of the field has shifted from testing microbial taxa for ubiquity to identifying the mechanisms that shape patterns of spatial or temporal distribution. The most common approach has endeavored to partition the relative roles of contemporary environmental factors and historical contingencies (e.g. dispersal limitation). A number of recent studies have indicated that the relative importance of environmental and spatial variables on microbes depends on study scale (Martiny et al. 2011, Soininen et al. 2011, Vilmi et al. 2016), but also on disparate biological traits (e.g. body size, dispersal mode; Fenchel & Finlay 2004, Hughes Martiny et al. 2006, Lindström & Langenheder 2012). Because most microbial biogeography studies to date have focused on bacteria or phytoplankton, there is need to expand to other taxonomic groups and different life modes such as benthic microalgae (BMA).

BMA are omnipresent in shallow-water coastal ecosystems and play important ecological roles, e.g. contributing significantly to primary production (Cahoon 1999, Underwood & Kromkamp 1999), serving as vital food resources for higher trophic levels (e.g. Miller et al. 1996, Pinckney 2018), and facilitating nutrient exchange across sediment–water interfaces (Thornton et al. 2002). BMA are a diverse group and include diatoms, cyanobacteria, dinoflagellates, chlorophytes, and euglenophytes (Cahoon 1999, Rejil 2012). Of these, diatoms typically are dominant representatives in BMA communities (Semcheski et al. 2016), with epipsammon (BMA attached to sand grains) generally dominating coarser sediments, while epipelon (free-living) dominate finer sediments. The ubiquity of benthic diatoms, coupled with their short life spans and sensitivity to environmental variation, also make them prime candidates for assessment of water quality in coastal habitats (Desrosiers et al. 2013).

Although a wealth of literature pertaining to spatio-temporal variation in BMA biomass and productivity already exists (see MacIntyre et al. 1996, Cahoon 1999 for reviews), studies focused on species composition and community assembly processes have lagged due to methodological constraints (e.g. dependence on labor-intensive microscopic examination

for species identification). Despite these constraints, BMA community assembly at small (m to km) spatial scales is fairly well understood compared to that at larger geographic scales. Physical factors, such as salinity (Sahan et al. 2007, Rovira et al. 2012), sediment composition (Sabbe & Vyverman 1991, Paterson & Hagerthey 2001), and tidal elevation (Sullivan 1982, Davis & McIntire 1983) are generally thought to be dominant influences over local (m to km) scales, although biotic interactions also have been shown to impact BMA ecology (Sahan et al. 2007, Plante et al. 2010, 2011). However, we know relatively little about spatial patterns and community assembly processes at regional and global spatial scales.

The biogeographic study reported here focused on BMA inhabiting barrier island beaches. Barrier islands, the dominant geomorphic features along the US Atlantic and Gulf Coasts, provide replicated units of area, isolated from other habitats. Additionally, the spatial ecology of barrier beaches is relatively well understood. For instance, an extensive literature exists regarding the main physico-chemical gradients in these habitats, as well as the spatial distributions of macrobiota and the ecological processes that underlie these distributions (McLachlan & Defeo 2017). Temperature increase and altered seawater chemistry associated with climate change are expected to profoundly impact these coastal ecosystems (Bindoff et al. 2019). Moreover, barrier islands are especially vulnerable to storm-surge flooding and erosion due to low elevations and movement of waves and sand. Notably, recent research has established that BMA community structure is sensitive to seawater temperature (Scholz & Liebezeit 2013, Cartaxana et al. 2015), pH (Andrén & Jarlman 2008), elevated CO₂ (Johnson et al. 2013), as well as changes in depth (Sahan et al. 2007, Jesus et al. 2009) and sediment flux (Sanilkumar et al. 2011). Thus, understanding fundamental ecology of the dominant autochthonous primary producers is essential to predicting vulnerability and resilience of coastlines in the face of climate change.

Our goals were to describe large-scale spatial patterns and elucidate community assembly processes of BMA in marine coastal waters. Toward this end, mensurative studies and high-throughput DNA amplicon sequencing were performed using South Carolina (USA) barrier-island beaches to address fundamental biogeographic theory about microalgal diversity and species composition. Null hypotheses were: (1) BMA community structure of beach sediments does not differ among barrier islands, (2) BMA β -diversity is not correlated with geographic distance

between samples over the spatial scales of the study (10 cm to 120 km), and (3) BMA β -diversity is not correlated with environmental differences.

2. MATERIALS AND METHODS

2.1. Study area and design

To test whether isolation by geographic distance influences community composition, it is important to control for contemporary environmental factors by sampling from similar habitat types. Although sampling in this way cannot eliminate all environmental variation among sample sites, with good replication (sampling the same habitat types in several different geographic locations), the distance effect can be tested (Hughes Martiny et al. 2006). We carefully selected beach areas of similar character from the north–northeast end of 4 barrier islands in South Carolina, USA — Kiawah Island (32.610° N, 80.029° W), Folly Beach (32.682° N, 79.889° W), Isle of Palms (32.801° N, 79.741° W), and Pawleys Island (33.436° N, 79.117° W)—from which sediment and seawater samples were collected. These barrier islands are geomorphologically and biologically typical for the Atlantic coast of the southeastern USA (Fig. 1; Lennon et al. 1996). Longshore currents result in a primarily north-to-south longshore transport of sediment along these dissipative beaches. Mean tidal range for all islands is ~ 1.7 m. Beachfront homes lined the sampled beaches on all 4 islands, although a golf course was also near the Kiawah sampling site. A narrow strip of sand dunes and shrub forest separated homes from the beach. Man-made obstructions to currents (piers, groins, jetties) were all ‘down-stream’ of sample sites, with one exception: 2 small groins were located ~ 500 and 700 m north of our Folly Beach sampling site. Although sand renourishment is conducted intermittently on these islands, the most recent renourishment on the relevant parts of any of these islands was at least 7 yr prior (a portion of Folly Beach was renourished in 2014 but was south of our sampling area).

Samples were collected in the afternoon 1 h before low tide at each location within the same week (1–6 July 2015) to control for tidal and temporal variation in BMA community structure. On all 4 sampling dates, initial conditions were partly sunny and warm (25–29°C). Skies became more overcast during collections from Kiawah and Pawleys Islands, although sampling was completed before rains commenced. According to tide charts, all samples were taken at

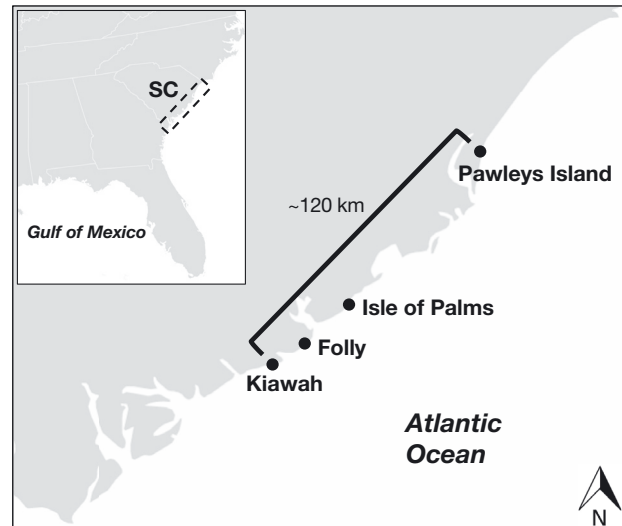


Fig. 1. Geographic location of each of the 4 studied beaches in South Carolina (SC), USA (source: freevectormaps.com)

+0.15–0.30 m (relative to mean low water) elevation. This tidal height was confirmed at Folly Beach using a Trimble R8 model 4 GNSS receiver. Replicate samples within each island were taken at various spatial scales (decimeter, meter, and decameter scales) (Fig. 2). Distances between islands were calculated using Google Earth v. 7.1.5 with coordinates obtained from a handheld GPS unit; distances ranged between 15 and 124 km. At each beach, we collected samples along a 200 m transect, which was parallel to the edge of the water, at 0, 100, and 200 m (Fig. 2). At each of these locations, we randomly chose 3 sites: a starting site 57.7 cm from center at a randomly chosen angle, and 2 other sites situated 1 m distant at 60° angles (Fig. 2). At the 100 and 200 m locations, sediment samples were taken for DNA, chlorophyll *a* (chl *a*), as well as nutrient and other sedimentological analyses at each triangle ver-

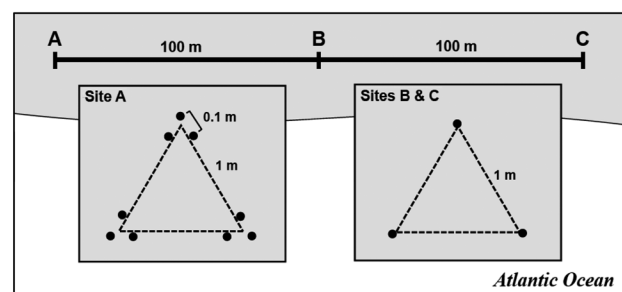


Fig. 2. Sampling scheme at each beach. A 200 m transect (black horizontal line) was run parallel to the shoreline. Samples were collected 1 h before low tide from 3 sites along the transect at 0, 100, and 200 m (marked A, B, and C, respectively). Black circles represent where samples were collected at each site

tex ($n = 3$ for each analysis at each location). However, at the 0 m location, 3 sub-sites were selected randomly around each vertex for collection of additional samples at the 10 cm scale (i.e. $n = 9$ for each analysis at the 0 m location, Fig. 2). Only the top 1 cm of sediment was collected at each site using cut-off 10 cc syringe samplers (Plante et al. 2011) for most samples, although 60 cc syringes (repeated 7–10 times) were used to expedite collection of larger amounts of sediment (~40 ml) for nutrient analysis. Samples were placed into sterile 15 or 50 ml plastic centrifuge tubes and held on ice until return to the laboratory for immediate processing, or frozen (-20°C) until later analysis.

2.2. Environmental factors

Sediment temperature was recorded at each of the 15 sampling sites on each island, and air temperature was recorded at each location. Seawater conditions (dissolved oxygen, temperature, and salinity) were recorded using a YSI Professional Plus, and turbidity was measured using a Secchi disk at a water depth of ~0.5 m (wave trough) perpendicular to each location. Seawater was also collected at a water depth of ~0.5 m (wave trough) and was placed on ice until returned to the lab, where pH and salinity (refractometer) were measured. Moisture and organic content were calculated from collected sediments by subtraction following drying (60°C for 24 h) and ashing (13 h at 400°C) in the lab. Dry sediment samples were filtered using a 1 mm sieve, and the mass of sediment and/or shell hash over 1 mm was recorded and discarded. Remaining sediment was analyzed using a Malvern Mastersizer 3000 Laser Diffraction-Particle Size Analyzer fitted with a red and blue light laser ($\lambda = 632.8$ and 470 nm, respectively) following the manufacturer's protocol. Ten measurements were taken for each sediment sample. Median grain size of each sample was used for further analyses. For nutrient analysis, porewaters were extracted from beach sediments using a method adapted from Saager et al. (1990). Syringes were centrifuged at 2000 rpm ($315 \times g$) for 10 min to filter water through GF-F filters into collection tubes. Sediment samples were diluted 1:1 with purified distilled water to obtain necessary volume for analysis. Samples were sent to the South Carolina Algal Ecology Laboratory (SCAEL) at the Marine Resources Research Institute (Charleston, SC, USA) for analysis of orthophosphate (PO_4^{3-}), nitrate/nitrite ($\text{NO}_2^-/\text{NO}_3^-$), and ammonium (NH_4^+) using a Lachat Quick-Chem 8000 nutrient auto-analyzer.

2.3. Microbial community analysis

BMA biomass was estimated using chl *a* as a proxy, measured via fluorometry (Hill-Spanik et al. 2019). Chl *a* and pheophytin *a* were extracted in 90% HPLC-grade acetone for 72 h at -20°C in the dark, and concentrations were determined using a Turner Designs TD-700 fluorometer according to the non-acidification method of Welschmeyer (1994). Concentrations were then normalized by the mass of dry sediment.

DNA was extracted from 0.5 g of sediment using an Omega E.Z.N.A Soil DNA kit following a modified version of the manufacturer's protocol to increase DNA yields (Bézy et al. 2014). Amplicon libraries were generated by following the Ion Amplicon Library Preparation (Fusion Method) protocol (Life Technologies). Primers D512for (5'-ATT CCA GCT CCA ATA GCG-3') and D978rev (5'-GAC TAC GAT GGT ATC TAA TC-3') (Zimmermann et al. 2011) were used to amplify a portion of small subunit ribosomal DNA that contains the V4 region. The forward and reverse primers included a 5' tail for Ion Torrent sequencing (adapter A: 5'-CCA TCT CAT CCC TGC GTG TCT CCG AC-3', adapter TrP1: 5'-CCT CTC TAT GGG CAG TCG GTG AT-3', respectively). The forward primers also contained the 4 bp library key, an individual barcode for each sample (Ion Xpress Barcode Adapters 2, 5, 8, 9–26, 28–31; Life Technologies), and a barcode adapter. These primers produce a ~520 bp amplicon (including adapters, barcodes, and keys). PCR was carried out in a 50 μl reaction volume using 2 or 4 μl of DNA template, 25 μl of Q5 Hot Start High-Fidelity 2 \times Master Mix (New England BioLabs) with a final concentration of 4 mM MgCl_2 , 0.5 μM of each primer, and 0.16 mg ml^{-1} bovine serum albumin (New England BioLabs). The PCR cycling parameters were 98°C for 2 min, then 21 cycles of 98°C for 10 s, 67°C ($-0.3^{\circ}\text{C cycle}^{-1}$) for 30 s, 72°C for 30 s, then 16 cycles of 98°C for 10 s, 61°C for 30 s, 72°C for 30 s, followed by 72°C for 2 min. PCR products were electrophoresed on 1% agarose gels stained with GelRed (Biotium) and visualized under UV light.

PCR products were purified using Agencourt AM-Pure XP reagent (Beckman Coulter) as per the Ion Torrent Amplicon Library Preparation (Fusion Method) User Guide. Concentrations of purified PCR products were determined using the DNA 1000 Kit and a 2100 Bioanalyzer (Agilent Technologies). An equimolar solution of 25 samples was then pooled and subsequently quantified, again using the DNA 1000 Kit and a 2100 Bioanalyzer. The pooled amplicon library was sent to the Medical University of

South Carolina's Progenomics Facility (Charleston, SC) for further processing and sequencing on an Ion Torrent Personal Genome Machine using an Ion 314 Chip Kit v2 (Life Technologies).

2.4. Processing of DNA sequencing data

Quantitative Insights Into Microbial Ecology (QI-ME) v. 1.9.1 was used for all sequence analyses (Caporaso et al. 2010). Raw sequence data were filtered to remove any sequences less than 300 bp, greater than 600 bp, and with average quality scores less than 20. Sequences were then clustered into operational taxonomic units (OTUs) at 98% sequence similarity using de novo clustering with UCLUST (Edgar 2010). The 98% sequence similarity threshold was chosen because it has been found to best represent species-level differences in benthic diatoms (Zimmermann et al. 2011, Luddington et al. 2012). A representative subset of sequences for each OTU was then aligned and filtered using PyNAST with the SILVA database (release 119) as a reference (Caporaso et al. 2010) and each was assigned taxonomic identity using BLAST (Altschul et al. 1990) and the SILVA database with a cut-off of at least 80% confidence in the classification. A phylogenetic tree was constructed using Fasttree 2.1.3 (Price et al. 2010). After removal of singletons, further filtering was used to exclude all sequences other than those of known diatoms (i.e. we retained only Diatomea, Bacillariophyta, Coscinodiscophyta, Bacillariophyceae, Fragilariales, Mediophyceae, Melosirids, and Rhizosolenids).

2.5. Statistics

Biomass (chl *a*) was tested for differences among islands. Assumptions of normality or homogeneity of variance were not met for several environmental variables, and violations were not corrected with natural log-transformations; therefore, nonparametric (Kruskal-Wallis) tests were used to compare these factors among islands. A non-parametric statistic (Kendall's τ) was likewise used to test for correlations between environmental variables and biomass. Most analyses of diatom community structure were implemented in R version 3.5.2 (R Core Team 2018) using the 'phyloseq' (McMurdie & Holmes 2013) and 'vegan' (Oksanen et al. 2019) packages. OTU abundance data were transformed to fractional abundance and standardized to median sequencing depth. Mean α -diversity indexes (OTU richness, Shannon-

Weiner, and Pielou's evenness) were calculated for each island and compared using ANOVA. We used the Bray-Curtis dissimilarity statistic to quantify the compositional differences among sites. Non-metric multidimensional scaling (NMDS) and permutational multivariate analysis of variance (PERMANOVA) were used to visualize and test significance of differences in diatom community structure among islands, respectively.

We also tested for significant differences in within-island variation among islands using the test of β -dispersion homogeneity (Anderson 2006). Similarity percentage (SIMPER) analysis was used to identify taxa primarily responsible for the differences observed between islands. Mantel tests were used to test for correlations between β -diversity of the diatom community and geographic distance or environmental distance. Partial Mantel tests were employed to analyze the relationship between environmental variables and diatom community structure with geographic distance controlled and vice versa. Multiple regression on distance matrices was used to reveal the environmental variables that were significantly ($p < 0.05$) correlated with variation in community structure. This reduced number of factors was then subjected to BIOENV analyses (Clarke & Ainsworth 1993), and those variables in the best resulting models were fitted into redundancy analyses. Partial distance-based redundancy analysis (db-RDA) was applied to examine relationships between community structure and environmental variables, while controlling for geographic distance. The principal coordinates of neighbor matrix (PCNM; Borcard & Legendre 2002) eigenfunctions, which represent the spectral decomposition of the spatial relationship across sampling locations, were considered as the spatial variables in the ordination-based analyses. The PCNM was constructed using the 'pcnm' function in R. Variation partitioning enabled us to determine the various unique and combined fractions of variation in the diatom community structure explained by the spatial and environmental data, with the unexplained proportion representing unexplained or unmeasured variables.

3. RESULTS

3.1. Environmental variables

Eight of 12 physical parameters varied among islands; the 4 exceptions were salinity, dissolved oxygen, turbidity, and water temperature (Table 1). In

Table 1. Mean environmental conditions ($\pm 95\%$ CI) of each island at the time of sampling. Values in **bold** indicate significance (at $\alpha = 0.05$), with different uppercase letters indicating significant ($p < 0.05$) differences in pairwise comparisons. DO: dissolved oxygen; ND: no data

Environmental factor	Global p	Folly	Isle of Palms	Kiawah	Pawleys
Ammonium ($\mu\text{M l}^{-1}$)	<0.0001	4.67 \pm 0.35 A	6.63 \pm 0.82 B	12.47 \pm 4.16 B	46.64 \pm 9.64 C
DO (% saturation) ^a	0.165	94.2 \pm 4.9	96.9 \pm 3.5	94.0 \pm 4.5	ND
Median grain size (μm)	<0.0001	244.9 \pm 8.9 A	202.8 \pm 7.1 B	193.9 \pm 3.1 C	215.7 \pm 1.4 D
Moisture (% mass)	<0.0001	24.4 \pm 1.2 A	27.8 \pm 0.4 B	27.7 \pm 3.5 B	26.9 \pm 0.3 C
Organic matter (% mass)	<0.0001	0.3 \pm 0.05 AB	0.2 \pm 0.02 C	0.3 \pm 0.03 A	0.4 \pm 0.03 B
pH	0.015	8.25 \pm 0.07	8.33 \pm 0.05	8.45 \pm 0.17	8.36 \pm 0.02
Phosphate ($\mu\text{M l}^{-1}$)	<0.0001	3.24 \pm 0.13 A	2.39 \pm 0.17 B	3.13 \pm 0.42 A	9.78 \pm 0.58 C
Salinity	0.424	32.7 \pm 3.0	33.3 \pm 2.0	33.7 \pm 4.0	34.2 \pm 1.9
Temperature, air ($^{\circ}\text{C}$)	0.015	28.3 \pm 0.7	24.5 \pm 0.7	29.7 \pm 1.5	26.1 \pm 1.3
Temperature, sediment ($^{\circ}\text{C}$) ^a	<0.0001	30.5 \pm 0.3 A	26.6 \pm 0.3 B	31.4 \pm 0.2 C	ND
Temperature, water ($^{\circ}\text{C}$)	0.068	28.4 \pm 0.7	28.2 \pm 0.2	30.1 \pm 0.2	28.3 \pm 0.4
Turbidity (cm from Secchi)	0.192	30.5 \pm 12.7	33.6 \pm 27.3	24.0 \pm 0.2	37.2 \pm 8.9

^aNo data for Pawleys Island

Table 2. Mantel tests for correlations between environmental variables and geographic distance with 999 permutations. Values in **bold** indicate significance at $\alpha = 0.05$. DO: dissolved oxygen

Environmental factor	R	p
Ammonium ($\mu\text{M l}^{-1}$)	0.736	0.001
DO (% saturation) ^a	0.413	0.001
Median grain size (μm)	-0.037	0.525
Moisture (% mass)	-0.073	0.343
Organic matter (% mass)	0.150	0.006
pH	0.041	0.473
Phosphate ($\mu\text{M l}^{-1}$)	0.919	0.001
Salinity	0.244	0.001
Temperature, air ($^{\circ}\text{C}$)	0.256	0.001
Temperature, sediment ($^{\circ}\text{C}$) ^a	0.854	0.001
Temperature, water ($^{\circ}\text{C}$)	0.120	0.039
Turbidity (cm from Secchi)	0.174	0.002
Combined environmental	0.570	0.001

^aNo data for Pawleys Island

particular, factors associated with sediments tended to show stronger significance relative to measurements from water and air (Table 1). In addition, 9 of 12 environmental variables were significantly correlated with geographic distance, as was the combined environmental effect (Mantel tests, Table 2).

3.2. Benthic microalgal biomass

BMA biomass also varied among islands as indicated by chl *a* values ($F_{3,56} = 3803.37$, $p < 0.0001$; Kruskal-Wallis). Highest average biomass was observed in Pawleys Island sediments ($1.70 \mu\text{g g}^{-1}$ chl *a*), while Folly ($0.08 \mu\text{g g}^{-1}$) exhibited the lowest

biomass (Fig. 3). Biomass was positively correlated with several sediment characteristics, including ammonium and phosphate levels, organic matter, pH, and sediment temperature (Table 3).

3.3. Diatom community structure

Sequencing resulted in a total of 165 286 useable reads with an average of 2755 sequences per sample. After quality control (primer and adapter removal, chimera removal, removal of quality scores less than 20) there was an average of 1285 sequences per sample (range = 456–3625) with an average product

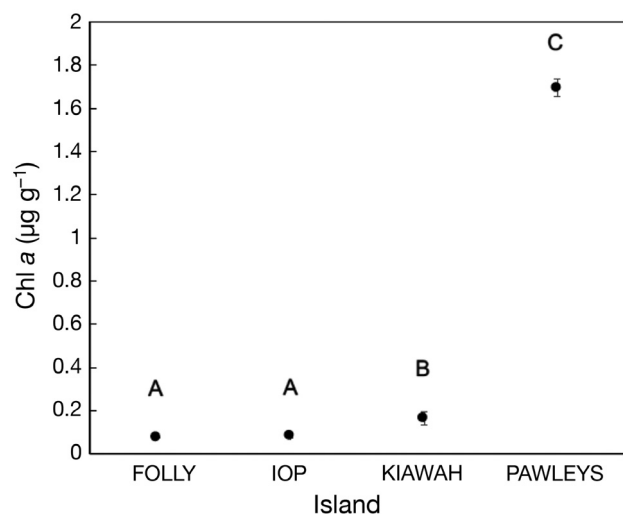


Fig. 3. Mean chlorophyll *a* (chl *a*) concentrations for beach sediments for the 4 barrier islands. Error bars indicate $\pm 95\%$ confidence intervals. Different uppercase letters indicate significantly different (at $\alpha = 0.05$) means (ANOVA with Tukey HSD correction). IOP: Isle of Palms

Table 3. Correlations (Kendall's τ test) between environmental variables and microalgal biomass (chlorophyll *a*). Values in **bold** indicate significance at $\alpha = 0.05$. DO: dissolved oxygen

Environmental factor	τ	p
Ammonium ($\mu\text{M l}^{-1}$)	0.654	<0.001
DO (% saturation) ^a	-0.220	0.349
Median grain size (μm)	-0.162	0.070
Moisture (% mass)	-0.008	0.929
Organic matter (% mass)	0.342	<0.001
pH	0.523	0.019
Phosphate ($\mu\text{M l}^{-1}$)	0.383	0.001
Salinity	0.288	0.209
Temperature, air ($^{\circ}\text{C}$)	0.078	0.730
Temperature, sediment ($^{\circ}\text{C}$) ^a	0.354	0.001
Temperature, water ($^{\circ}\text{C}$)	0.110	0.627
Turbidity (cm from Secchi)	0.046	0.837

^aNo data for Pawleys Island

length of 467 bp. After filtering and singleton removal, the diatom dataset was composed of 1279 OTUs with an average of 967 sequences per sample (range = 364–3540). Although Shannon and evenness rarefaction curves plateaued, observed OTU curves were not saturated (Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m661p017_supp.pdf), thus richness calculations may be underestimated. Approximately 75.2% of total sequences were from diatoms. Much of the remaining sequencing data belonged to ciliates and other members of the Alveolata superphylum (2.2%), chlorophytes (1.7%), non-diatom stramenopiles (14.0%), and unclassified members of the SAR supergroup (6.3%), but were excluded from our analyses.

Sequences from the Bacillariophyceae and Mediophyceae diatom classes were prominent components of the community on all 4 islands (Fig. 4). Bacillario-

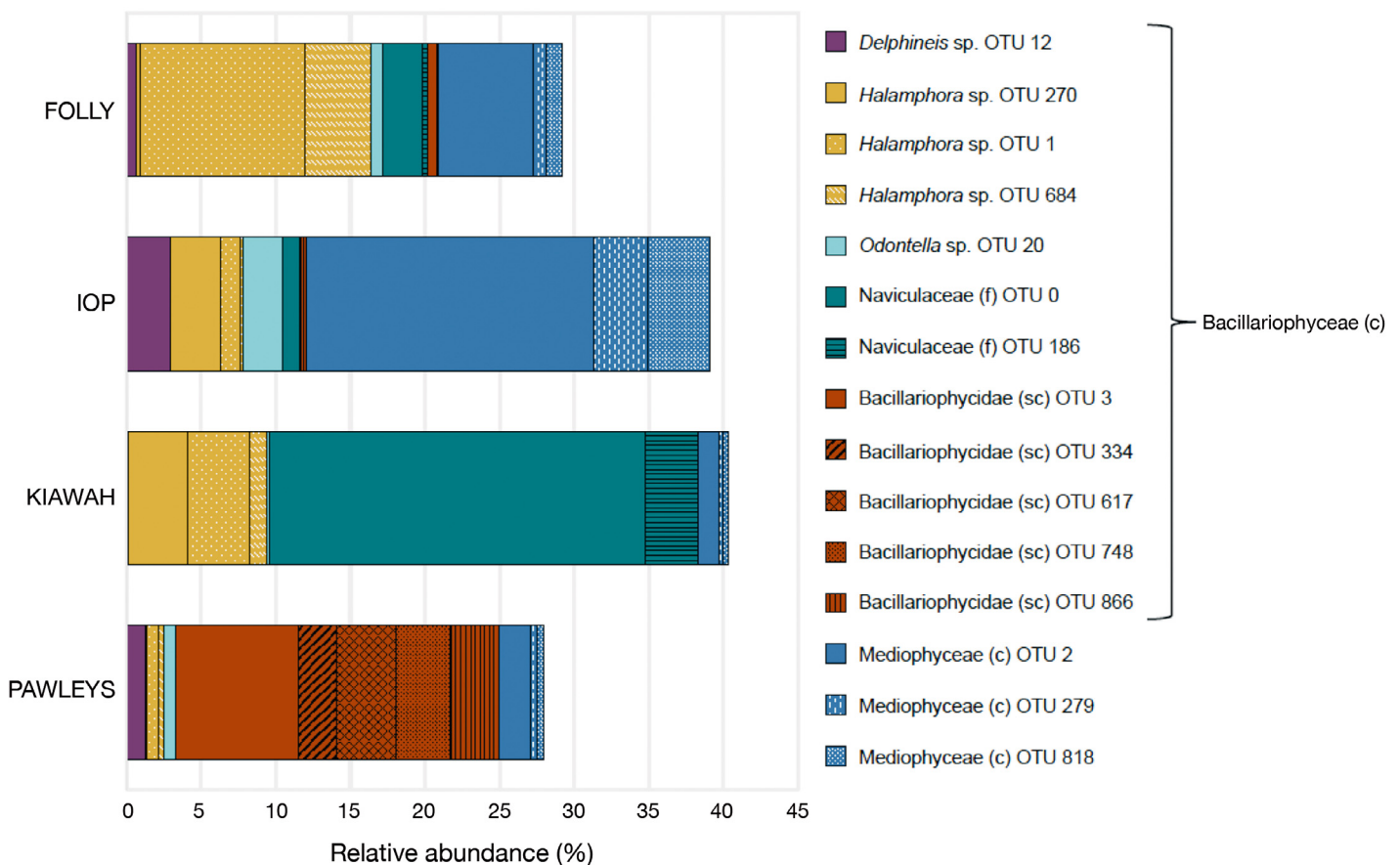


Fig. 4. Average relative abundance of dominant operational taxonomic units (OTUs) found at each beach (IOP: Isle of Palms). Only OTUs with relative abundances >3% (from at least 1 beach) were included to allow for clear visualization of the data (thus, relative abundances do not sum to 100%). Sequences of OTUs were compared to GenBank using BLAST (Altschul et al. 1990) to determine taxon identities to the lowest level possible: if >98% ID, then genus was assigned, but if <98% ID, family (f), subclass (sc), or class (c) was assigned based on BLAST results and AlgaeBase taxonomy (Guiry & Guiry 2020). Taxa with the same classification (e.g. genus, family, class) are represented by the same color; distinct OTUs within that classification have differing patterns

phyceae sequences comprised more than half of the relatively abundant (>3%) OTUs on Folly, Kiawah, and Pawleys Islands (Fig. 4). Relative abundances within Bacillariophyceae did vary however, with *Hal-amphora* spp. (family Amphipleuraceae) sequences dominating on Folly, sequences from members of the Naviculaceae making up the bulk of the relatively abundant diatom sequences on Kiawah, and several less-defined OTUs of the subclass Bacillariophycidae, which were distinct from those common on Folly and Kiawah, dominating Pawleys Island. In contrast, 3 OTUs within the class Mediophyceae together dominated (~70%) the relatively abundant diatom sequences on the Isle of Palms (Fig. 4).

Several α -diversity metrics varied significantly among islands (Fig. 5). Least diverse was Kiawah

Island or the Isle of Palms, depending on the metric employed (Fig. 5). Pawleys Island consistently exhibited the highest α -diversity metrics. Fisher's α and Simpson's index likewise revealed Pawleys to be most diverse (data not shown). Overall, richness, i.e. OTU number, was correlated with biomass ($\tau = 0.230$, $p = 0.010$), whereas the Shannon index and Pielou's evenness were not ($p > 0.05$).

Despite much overlap in the dominant taxa (Fig. 4, Table S1), an NMDS ordination revealed a clear distinction in community structure among the 4 islands (Fig. 6). A high proportion (48%) of all 1280 OTUs were unique to one island, whereas only 11% were common to all 4 islands (Fig. S2). The patterns were further corroborated by PERMANOVA, which demonstrated that the island sampled was a significant

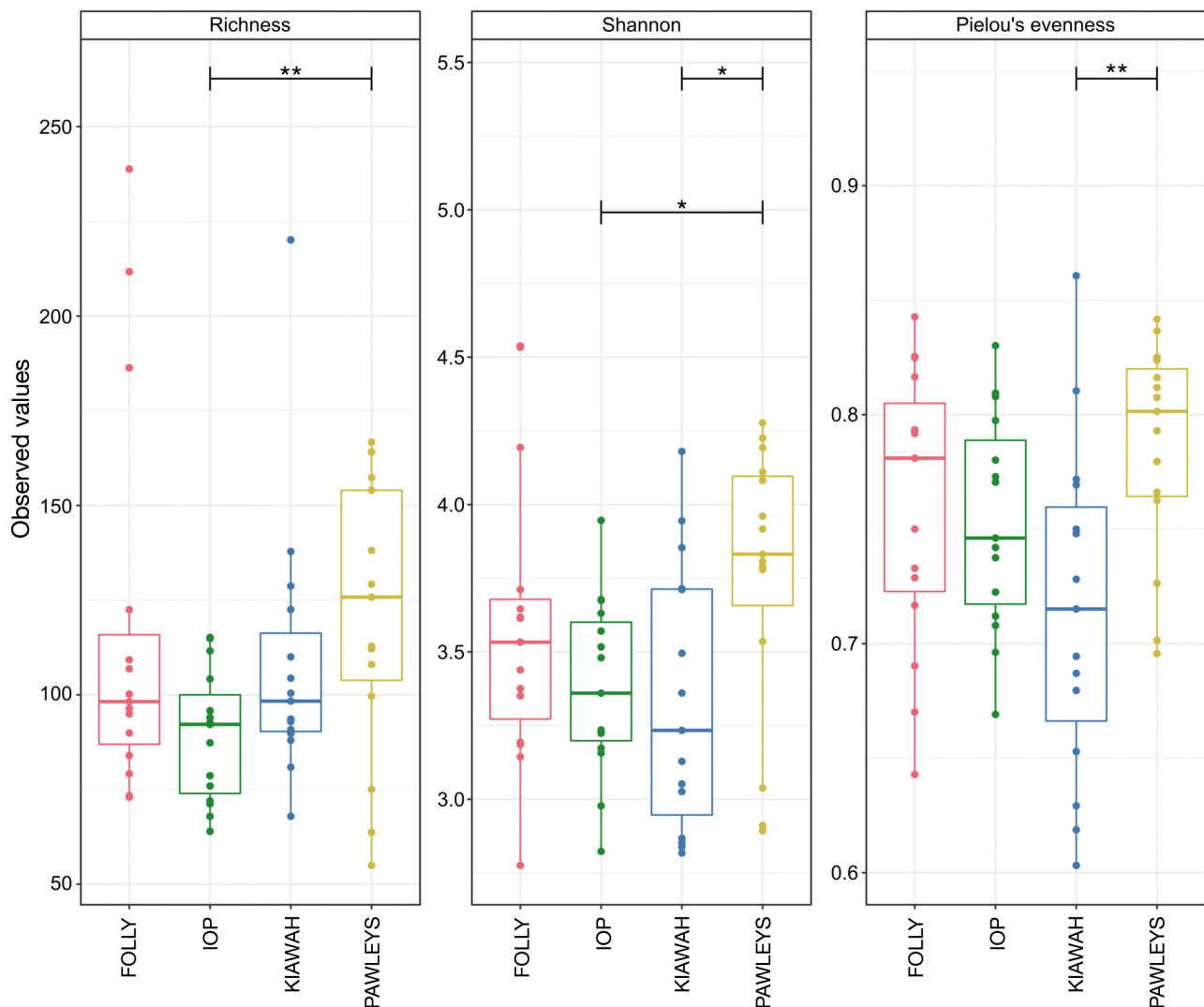


Fig. 5. α -diversity measures of observed number of operational taxonomic units ('richness'), Shannon Index, and Pielou's evenness for each location. The median is represented by the horizontal bar within each box, while the top and bottom of box show the interquartile range, and whiskers extend to 1.5 times the interquartile range. * $p < 0.05$, ** $p < 0.01$. Symbols are color-coded by island. IOP: Isle of Palms

determinant of community structure ($F_{3,56} = 9.89$; $R^2 = 0.346$, $p = 0.001$). Multiple comparisons (using the Bonferroni correction) showed that all 6 pairwise island comparisons were likewise significant ($p < 0.006$ for all) and that Kiawah and Pawleys were most distinct ($R^2 = 0.329$) while the Isle of Palms and Folly were the least different from one another ($R^2 = 0.182$). Beta dispersion, or spread among samples, also differed among islands ($F_{3,56} = 3.13$; $p = 0.038$). In particular, pairwise comparisons showed that diatom communities were less variable on the Isle of Palms as compared to Pawleys ($p = 0.010$) and Folly Islands ($p = 0.010$), the latter difference possibly explaining the significant result for the Isle of Palms–Folly PERMANOVA comparison despite the considerable visual overlap in the NMDS plot (Fig. 6).

3.4. Spatial and environmental determinants of diatom community structure

To test whether spatial distance was an important factor influencing disparities in community structure, a distance decay of similarity model was employed. Community similarity was significantly negatively correlated ($r = -0.257$; Pearson product-moment coefficient) with geographic distance (Fig. 7). A Mantel test for correlation between geographic distance and Bray-Curtis dissimilarity also supported the distance effect ($r = 0.634$, $p = 0.001$). Partial Mantel tests also supported the spatial effect in that geographic distance was correlated with Bray-Curtis dissimilarity while controlling for each of the 12 environmental variables or the combined environmental factor (Table S2). In contrast, when those same environmental variables were tested for correlation with community dissimilarity while controlling for geographic distance, only air temperature, water temperature, grain size, and turbidity were significant (Table S3).

BIOENV analysis revealed that the best subset of environmental variables producing the strongest correlation ($\rho = 0.701$) with variation in community composition included 3 variables: water temperature, ammonium, and phosphate levels. db-RDA indicated that environmental variables played a significant role ($F_{3,49} = 2.42$; $p = 0.001$; Fig. 8) in structuring the diatom communities, although water temperature and ammonium concentration were

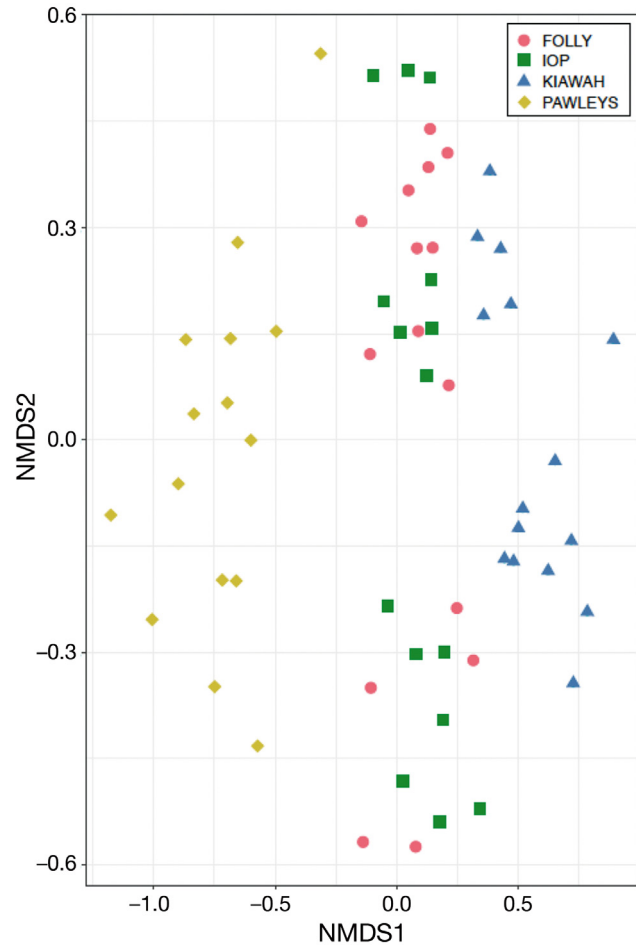


Fig. 6. Nonmetric multidimensional scaling (NMDS) ordination of the dissimilarity (Bray-Curtis distance) in diatom community structure (NMDS stress = 0.198). Symbols are color-coded by island. IOP: Isle of Palms

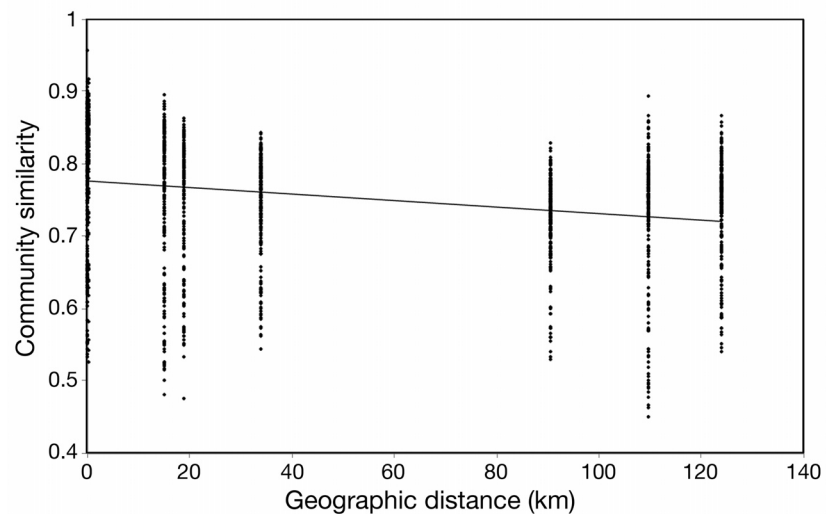


Fig. 7. Distance decay of Bray-Curtis diatom community similarity with increasing geographic distance between sampling locations ($r = -0.257$, $n = 1770$, $p < 0.001$)

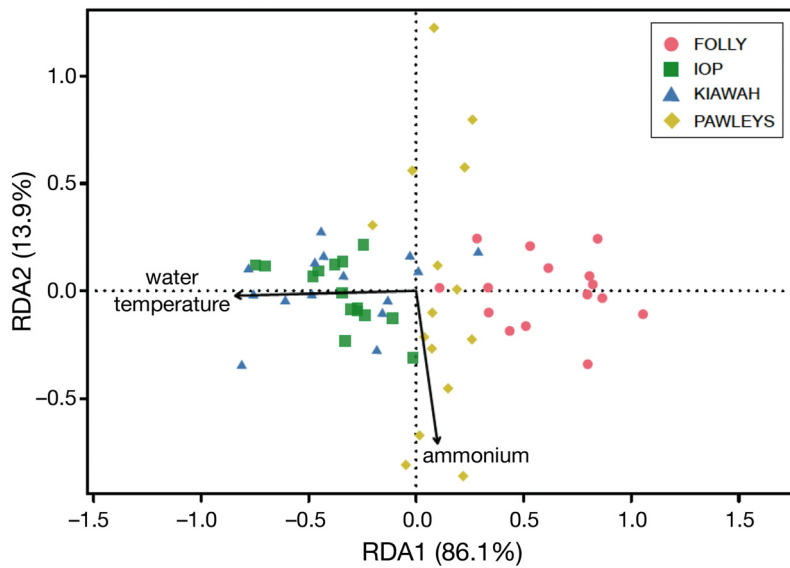


Fig. 8. Distance-based redundancy analysis (db-RDA) ordination showing significant environmental variables that influenced the composition of beach diatom communities. Symbols are color-coded by island. IOP: Isle of Palms

the only significant individual factors. A comparable RDA model with water temperature and phosphate as the only significant variables could be constructed, due to a high correlation between ammonium and phosphate ($\rho = 0.84$). Variation partitioning showed that environmental and spatial variables could explain only 27% of the variation in the diatom community composition in total (Fig. 9a). Pure spatial effects explained approximately 5% of the variation, whereas the pure environmental effect was essentially zero. Approximately 22% of the variation was attributed to spatially structured environmental variation (i.e. the fraction jointly explained by environmental and spatial factors).

We also performed the above-described constrained ordinations after omitting Pawleys Island in order to test the role of dissolved oxygen and sediment temperature (which were not measured on Pawleys due to unforeseen circumstances). The best BIOENV model ($\rho = 0.594$) included the 2 variables sediment temperature and water temperature. RDA again indicated that environmental variables played a significant role ($F_{2,38} = 3.63$; $p = 0.001$) and that both sediment and water temperature were significant individually ($p = 0.005$ and 0.001 , respectively). Similar to the complete 4-island analysis, variation partitioning showed that environmental and spatial variables could explain 28% of the variation in the diatom community composition in total (Fig. 9b), with spatially structured environmental variation accounting for nearly all of this portion.

4. DISCUSSION

Microbes play critical roles in biogeochemical cycling and trophic dynamics in estuaries and the coastal ocean. Thus, increased understanding of the factors that determine spatial variation in microbial communities, and how natural and anthropogenic factors affect them, is urgently needed. Here, we focused on spatial and environmental drivers of BMA communities at regional spatial scales.

Despite our efforts to sample from similar beach habitats, many of the measured environmental factors exhibited significant variation among islands. With the exception of temperature, differences in air and water conditions were generally small, whereas all sedimentological factors

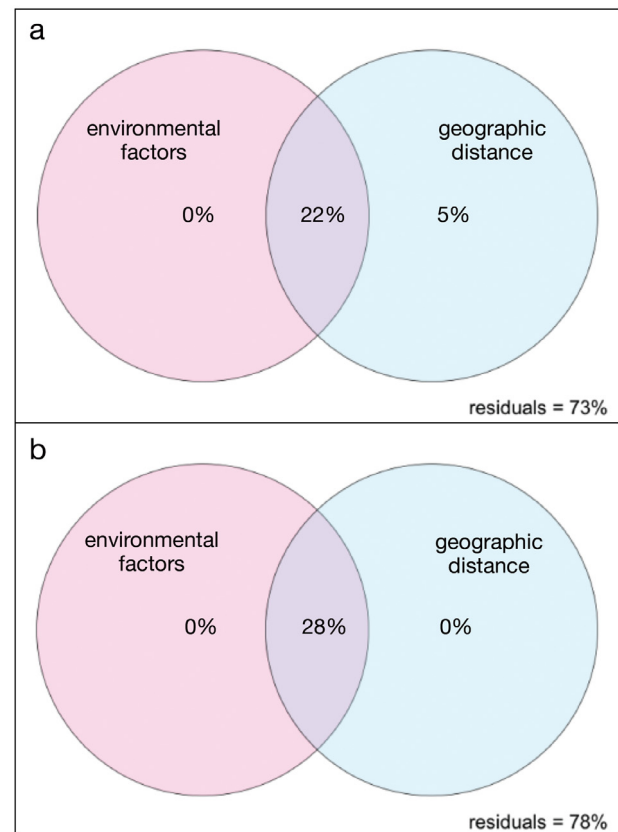


Fig. 9. Partitioning of diatom community structure in beach sediments of (a) all 4 studied barrier islands and (b) only 3 of the islands (Pawleys Island removed from the analysis). The explanatory power of pure and shared fractions of environmental and spatial factors are indicated as adjusted R^2

differed markedly. Most notable were the remarkably higher nutrient levels on Pawleys Island, with both ammonium and phosphate exceeding the other islands by at least 3-fold. In general, ammonium and phosphate levels at our study sites overlap with those found in porewaters of other high-energy beaches (Reckhardt et al. 2015 and references within). Although no previously published porewater nutrient data from Pawleys Island exist for direct comparison, our findings of high ammonium and phosphate appear to corroborate the high nearshore nutrient levels and frequent coastal hypoxia along this stretch of the South Carolina coast. Long Bay, in which Pawleys Island is located, is thought to experience regional oceanographic processes that constrain water masses to the nearshore, thus preventing the dispersion of local terrestrial inputs from the Myrtle Beach metropolitan area, a highly urbanized resort community (Sanger et al. 2012).

BMA biomass differed significantly among islands, which was not surprising given the variation in many of the environmental factors on the 4 islands. Our range of mean values from the islands ($0.08\text{--}1.7\ \mu\text{g g}^{-1}$ chl *a*) were in general agreement with the few prior studies focused on high-energy beaches (chl *a* concentrations $<2.0\ \mu\text{g g}^{-1}$; Kendrick et al. 1996, Cahoon et al. 2012, Hill-Spanik et al. 2019) and, as expected, were low relative to most measurements of BMA biomass in estuarine intertidal sediments (e.g. up to $>50\ \mu\text{g g}^{-1}$ chl *a*, MacIntyre et al. 1996). We found correlations between biomass and several sedimentological factors (i.e. ammonium, phosphate, temperature, and pH). The relationship with ammonium and phosphate agrees with several prior experimental and observational studies demonstrating nutrient-limitation and increased BMA productivity and/or biomass with nutrient enhancement (e.g. Uthicke & Klumpp 1998, Scholz & Liebezeit 2012). The much higher chl *a* concentration that we observed at Pawleys Island (10–20 times higher than the other islands) likely was due to the elevated nutrient levels (3–10 times) also noted there. The relationship with temperature is less straightforward, with some studies suggesting a positive relationship (e.g. Vieira et al. 2013), while others report decreased productivity with elevated temperatures (Hicks 2011, Cartaxana et al. 2015), which altogether suggest a unimodal relationship. The positive relationship observed in the present study suggests that temperature tolerances were not exceeded at any of our study sites. Although biomass was also correlated with pH, mean values differed only by 0.2 pH units among islands. Furthermore, inferences

about direct pH effects are tenuous given the difficulty separating pH from possible CO_2 -limitation effects.

All 3 α -diversity metrics were higher for the Pawleys Island BMA. Diversity for the other 3 islands differed in rank order depending on the specific metric, but overall were similar. These diversity patterns mirror those for biomass, with which OTU number was correlated. Few studies have reported on the relationship between BMA biomass and diversity, although Vyverman et al. (2011) observed an inverse relationship between biomass and species diversity in estuarine BMA communities. Slightly more common have been studies examining the relationship of productivity and diversity, although results have been somewhat contradictory. Hillebrand & Sommer (1997, 2000) noted decreased species diversity with higher productivity, whereas Vyverman et al. (2011) reported either a positive or unimodal (or 'humped') relationship in different locations. Much more thoroughly studied in vascular plant communities, a unimodal diversity–productivity relationship has frequently been observed (e.g. Waide et al. 1999, Rajaniemi 2003). Likewise, evidence suggests that phytoplankton might also exhibit a humped productivity–diversity relationship (Vallina et al. 2014). Although it is tempting to assign the positive relationship between richness and biomass observed in this study to low rates of production (i.e. being on the ascending part of the diversity–productivity curve), the relationship between biomass and productivity is complex and dictated by many other factors that we did not evaluate (e.g. grazing pressure).

We rejected our first null hypothesis that diatom community structure of beach sediments would not differ. Distinct assemblages were obvious statistically (based on PERMANOVA), with each beach dominated by different taxa. Folly, Kiawah, and Pawleys Island communities comprised primarily sequences from pennate diatoms of the class Bacillariophyceae. These results are in accord with several prior studies that likewise noted a preponderance of Bacillariophyceae, especially of the family Naviculaceae, in beach sands (Plante et al. 2010, An et al. 2017, Hill-Spanik et al. 2019). In contrast, the Isle of Palms beach was dominated by diatoms of the class Mediophyceae. The Mediophyceae are polar Centrales (Medlin 2015) and are mostly considered to be planktonic diatoms. It is well-known that planktonic microalgae often accumulate in sediments (Ribeiro 2010), thus a fraction of the diatoms in our sediment samples, especially on the Isle of Palms, almost certainly comprised non-resident phytoplankton. Re-

ardless of relative planktonic vs. benthic contribution, the diatom communities of these 4 barrier islands were dramatically different.

Geographic distance had a significant but relatively small influence on community structure. Diatom community similarity was negatively correlated with geographic distance between sample sites on the 4 islands, and partial Mantel tests revealed that this correlation held even after controlling for environmental factors. Such a distance effect indicates that processes other than present-day selection are affecting spatial variation in community structure. At least some dispersal limitation is required for past events to leave a contemporary signature, otherwise differentiation due to past selection or drift would be counter-acted. As a result, a significant distance effect can be interpreted as evidence of dispersal limitation (Hanson et al. 2012). However, variation partitioning indicated that spatial factors alone accounted for only 5% of the variation in community composition at this spatial scale (and ~0% when Pawleys Island was excluded from the analysis). Although several studies of microbial biogeography have found no dispersal limitation (e.g. Cermeño & Falkowski 2009, Rodríguez-Martínez et al. 2013, Chen et al. 2017), our results agree with the majority of such studies that found a significant but small pure spatial effect. For instance, Hanson et al. (2012) found that roughly 68% of such studies reported a significant pure distance effect and that, on average, 10.3% of variation in microbial composition could be explained by geographic distance alone.

Body size and dispersal mode influence dispersal, such that passively dispersed organisms of larger size should exhibit more dispersal limitation and clearer spatial patterning (De Bie et al. 2012). In accord with these predictions, dispersal limitation has been more important to community variation in diatoms than it was for bacteria when compared within the same aquatic habitats (De Bie et al. 2012, Vilmi et al. 2016). To date, most studies designed to disentangle spatial from environmental effects in marine microbes have focused on planktonic bacteria and microeukaryotes. However, when benthic microbes have been examined, typically both bacteria (Schauer et al. 2010, Xiong et al. 2014; but see Hewson et al. 2007) and microeukaryotes (Gong et al. 2015, Chen et al. 2017) have exhibited significant dispersal limitation. A clearer signal of dispersal limitation among benthic microorganisms agrees with the findings of Zinger et al. (2011), whose global synthesis demonstrated the pure spatial effect for benthic bacteria to be more than twice that of pelagic

bacteria. This discrepancy is likely due to less horizontal flow at the seafloor compared to the water column or attachment of benthic microbes to sediment particles (Zinger et al. 2011). Although biogeography studies on beach diatoms have been rare, the 2 available studies conducted by W. Zhang et al. (2018) and Pan et al. (2020) indicate relatively high pure spatial effects (15 and 18%, respectively). Unfortunately, the nature of beach habitat (e.g. dissipative vs. reflective) in those studies was not reported (W. Zhang et al. 2018, Pan et al. 2020). In our study, the high degree of sediment transport (van Gaalen 2004) seems to have outweighed the effects of body size and particle attachment, such that dispersal limitation was minor.

Prior studies have suggested that the spatial scale of the study influences the relative influence of local environmental and spatial effects in driving community variation (Hughes Martiny et al. 2006, Vilmi et al. 2016). In general, large geographic distances should produce stronger correlations between community variation and spatial variables (Nekola & White 1999, Soininen et al. 2011). For instance, several studies of freshwater and marine BMA have demonstrated dispersal limitation at continental or global spatial scales (e.g. Verleyen et al. 2009, Martiny et al. 2011), whereas environmental factors have been shown to be more influential at local scales (Verleyen et al. 2009). Our results suggest that dispersal limitation of BMA on high-energy beaches is minimal, at least at regional (up to 120 km) spatial scales. The aforementioned studies of W. Zhang et al. (2018) and Pan et al. (2020) further illustrate the importance of scale in that they found relatively high spatial effects on diatom biogeography across beaches over global (12 000 km) spatial scales.

The primary drivers of diatom community structure on the beaches from our study appear to be spatially structured physico-chemical factors, specifically water temperature and sediment nutrient levels. Although several environmental conditions were correlated with β -diversity metrics, collinearity among many of these variables was observed. As a result, RDA revealed that water temperature and ammonium and/or phosphate (which were highly correlated with one another) were the primary environmental factors related to diatom community structure. Water temperature previously has been observed as a key driver of benthic microeukaryotic communities (W. Zhang et al. 2018, Pan et al. 2020). Nutrient levels have also been shown to be dominant influences on the community structure of microphytobenthos (Du et al. 2016, 2017). In particular, Chen et al. (2017) found that BMA β -diversity was corre-

lated with total nitrogen and total phosphorus (and phosphate specifically), and Pan et al. (2020) likewise identified total nitrogen as a key driver of community variation. However, interpreting the actual role of individual physico-chemical factors in community assembly is confounded because environmental variables appeared to be spatially structured. Variation partitioning demonstrated that the pure effect of environmental factors was effectively zero, whereas the combined effect of spatial and environmental factors (~22%) was the greatest identifiable contributor to community dissimilarity. Several of the significantly different environmental variables were correlated with geographic distance, suggesting that those factors were spatially shaped to varying degrees. The combined effect of environmental and spatial factors, rather than pure spatial or environmental factors, was also found to dominate community structure of microphytobenthos over regional scales in the East China Sea (H. Zhang et al. 2018). This is not a universal finding, however, as similar studies have found relatively small combined effects (Chen et al. 2017, Pan et al. 2020). A possible explanation for the dominant combined effect in our study is that widely dispersed diatoms encounter large variation in physico-chemical conditions among beaches and are filtered by physical conditions or competition with resident taxa, resulting in the preponderance of physiologically better-adapted diatoms. Accordingly, we speculate that spatially structured environmental variation may be driving the biogeography of BMA on South Carolina beaches.

However, the majority (73%) of variation among diatom assemblages in our variation partitioning analysis was left unexplained. Hanson et al. (2012) found that on average 50% of variation in microbial composition was unexplained, although that proportion ranged widely among studies. In studies specific to benthic marine microeukaryotes, unexplained variation ranged between 35 and 46% (Chen et al. 2017, H. Zhang et al. 2018, Pan et al. 2020). Unexplained variation in the present study may be due to unmeasured environmental factors (e.g. silicate; H. Zhang et al. 2018) or biotic interactions (e.g. grazing pressure; Azovsky et al. 2005). In addition, unexplained variation in microbial communities may be due to neutral processes (Hubbell 2001). For instance, ecological drift (stochastic processes of birth, death, colonization, and extinction), even in the absence of selection, can create geographic distance effects (Slatkin 1993, Hutchison & Templeton 1999). As with selection, drift strengthens the distance-decay relationship. Importantly, drift must interact

with dispersal to create a distance-decay pattern. With little or no dispersal, drift would create a patchy distribution of organisms and, hence, variation in composition that is not spatially autocorrelated (Hutchison & Templeton 1999, Hubbell 2001). At least one previous study concluded that neutral factors played a significant role in structuring microalgal communities in both sandy and muddy habitats (Plante et al. 2016). Thus, factors we did not consider here may also be important and, if included, conclusions about the relative importance of environmental, spatial, and neutral processes in assembly of diatom communities may change.

Additional aspects of study design and methodologies constitute limitations to our study. The 'snapshot' nature of our study over just 6 summer days constrains generalizability to other seasons. However, we found the spatial factor (i.e. dispersal limitation) to be minor relative to environmental variables, likely due to high sediment transport between beaches. Given that transport is normally lowest in summer, it is probable that high dispersal also holds for other seasons. On the other hand, we were unable to collect samples contemporaneously, so it is possible that the BMA communities could have changed over our 6 d sampling period. Seasonal changes in BMA community structure are well established (e.g. Semcheski et al. 2016), and a smaller number of studies show that communities can vary over the daily tidal phase (Easley et al. 2005). However, there is almost no evidence that community structure varies over daily or weekly time scales if time of day and tidal phase are controlled. For instance, no changes in biomass (Cahoon et al. 2012, Hill-Spanik et al. 2019) or community structure (Hill-Spanik et al. 2019) were observed over days to weeks. To our knowledge, only 1 study, by Orvain et al. (2012), noted biomass changes over the scale of spring-to-neap tidal cycles, but only for 1 of 2 months examined. It should also be noted that our samples were limited to the top 1 cm of beach sand. It is well known that epipelagic diatoms migrate vertically within sediments. Normally, active BMA are restricted to the top few millimeters of sediment due to light attenuation, although in areas well mixed by waves or currents, BMA can be found to depths of greater than 10 cm (Mitbavkar & Anil 2002). Thus, tidally induced vertical migrations can influence community structure (Underwood et al. 2005). However, given that we consistently sampled at the same point in the daily tidal phase (1 h prior to low tide), and sampled to the same depth at each beach, these potential changes were controlled for by our sampling design.

New insights into the spatial distribution and community assembly processes of microphytobenthos in high-energy benthic ecosystems contribute to our theoretical understanding of microbial biogeography, and more practically, suggest potential use of benthic diatoms as biological indicators in these ecosystems at least up to regional spatial scales. Assessing the environmental integrity of coastal environments is central to their successful management. There are several theoretical and practical reasons to suggest that BMA might be useful bioindicators. Practically speaking, modern molecular biological approaches can be used for microbial community analysis with relative ease. Further, an ideal indicator species or community would be associated with a given habitat due to local physical conditions or biotic interactions (i.e. 'environmental filtering'), not due to dispersal limitation. Our results suggest the promise of employing diatom communities as bioindicators in beach habitats because we observed little dispersal limitation, whereas environmental factors (including anthropopressures, temperature, and nutrients) were key drivers of community structure. However, because a large fraction of β -diversity remained unexplained, more research is needed to determine whether relevant data are lacking or if residual variation is simply an implicit feature of the beach ecosystem. Furthermore, additional studies of this type conducted over greater spans of time and at different locales are needed to test the temporal and spatial generalizability of our findings.

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