Interacting effects of deposit feeding and tidal resuspension on benthic microalgal community structure and spatial patterns

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ABSTRACT: Benthic microalgae (BMA) are important primary producers constituting an essential component of nearshore food webs. BMA also release extracellular polymeric secretions that stabilize sediments. Primary objectives of our research were to assess the separate and interactive effects of biotic and abiotic disturbances on structure and spatial patterns of microalgal communities in shallow-water sediments. Using field comparative studies, we characterized the effects of 2 prominent types of disturbance: macroinvertebrate ingestion and tidal resuspension. BMA biomass and composition in fecal materials from the enteropneust *Balanoglossus aurantiacus* and sediments were followed through time using fluorometry and molecular techniques (PCR-DGGE analysis of 18S rDNA and sequencing). We also examined the effects of disturbance on spatial patterns of BMA biomass, diversity, and composition over the larger sedimentary landscape using correlative studies. Deposit-feeder ingestion was found to significantly reduce BMA biomass and alter BMA composition, although qualitative changes were comparatively less remarkable. A significant difference was also found between average biomass before and after tidal immersion. Spatial autocorrelation revealed heterogeneity in BMA distribution during low tide, and that this spatial patterning correlated with *B. aurantiacus* fecal coils. Samples taken after tidal immersion showed no patchiness, nor was there a correlation between BMA biomass and pre-immersion fecal cover. In intertidal sediments, the qualitative and quantitative impacts of deposit feeding, and the resulting landscape-scale patchiness, appear to be short-lived due to erasure by frequent tidal resuspension.

KEY WORDS: Disturbance · Spatial autocorrelation · DGGE · Diatom · Intertidal · Beach

INTRODUCTION

Benthic microalgae (BMA) are important primary producers in intertidal and shallow, subtidal sediments (Underwood & Paterson 1993, de Jonge 1994), comprising a vital food resource for heterotrophs (e.g. Miller et al. 1996). In sandy and mudflat environments, diatoms are usually the dominant representatives in BMA communities (Meadows & Anderson 1968). These benthic diatoms release extracellular polymeric secretions (EPS) that inhibit resuspension of sediments and associated biota from tidal currents or waves (Holland et al. 1974, Underwood & Paterson 1993, Miller et al. 1996). EPS also serve as a carbon source for microbes and deposit-feeding invertebrates (Decho & Lopez 1993). Consequently, the removal of EPS or the diatom producers, e.g. by invertebrate feeding, de-stabilizes sediments (Austen et al. 1999).

In marine soft-bottom habitats, disturbance can be an important factor structuring benthic communities

(Brey 1991). A disturbance removes organisms and has been defined as 'any discrete event in time that disrupts ecosystem, community or population structure and changes the physical environment' (White & Pickett 1985). Although sometimes thought of as a predator-prey interaction, we argue here that treatment of deposit-feeder ingestion as a disturbance is more appropriate because it acts over larger spatial scales (relative to BMA), influences communities as opposed to individuals, and is less selective. Moreover, deposit feeding disrupts the physical environment as well as the biota (Rhoads & Boyer 1982, Katrak & Bird 2003).

In sediments, deposit feeding can be a dominant type of disturbance for micro-organisms (Sommer 2000, Hagerthey et al. 2002, Plante & Wilde 2004). It is widely accepted that deposit feeders consume and assimilate benthic diatoms with high efficiency (Yingst 1976, Nielsen & Kofoed 1982), and numerous prior studies have documented significant BMA biomass reductions in the field due to grazing by a taxonomically diverse array of deposit feeders (Bianchi & Levinton 1981, Smith et al. 1996, Sommer 2000). Quantitative or qualitative changes in BMA due to gut passage and defecation produce patchy distributions and influence diversity patterns. As examples, grazing by *Corophium volutator* or *Hydrobia ulvae* on intertidal diatoms increased or decreased species diversity under different temperature or nutrient regimes (Hagerthey et al. 2002), and McClatchie et al. (1982) demonstrated reduced cell biomass but higher diatom species richness at highest snail (*Amphibola crenata*) densities. Hillebrand (2008) employed a meta-analysis to conclude that grazing generally increases relative spatial variability in microalgal biomass. Experiments by Sommer (2000) showed that 2 benthic invertebrate species, *Idothea chelipes* and *Littorina littorea*, differed in their spatial pattern, but not intensity or selectivity, of grazing on BMA. The spatial heterogeneity of grazing pressure may therefore be more important for maintenance of diversity than the mean intensity of grazing.

Physical disturbances are also prominently considered when evaluating BMA ecology (MacIntyre et al. 1996). In soft-bottom, intertidal, and shallow subtidal habitats, tidal resuspension can homogenize surficial sediments. However, twice-daily periods of resuspension may not be disturbances for epipelic diatoms and other BMA. Sediment transport due to tidal changes is cyclical and predictable on short time scales (relative to BMA generation times) so BMA in such habitats are adapted to these events. Both vertical migrations and adhesion to particles may reduce

the proclivity for benthic diatoms to resuspend (MacIntyre et al. 1996, Shimeta & Sisson 1999).

Common to landscape ecology studies is a focus on the reciprocal relationship between spatial heterogeneity and ecological processes. Disturbance remains prominent in landscape studies, and current research questions emphasize understanding the interactions among different kinds and scales of disturbance (Turner 2005). Interactions among small-scale biotic disturbances and larger-scale abiotic disturbance are well established for terrestrial grassland systems, and to a lesser extent, select forest ecosystems. The interactions between large mammal grazing and fire (Harrison et al. 2003, Collins & Smith 2006, Veen et al. 2008) as well as animal burrowing and fire (Rogers & Hartnett 2001) in grasslands illustrate dissimilar effects on species composition and diversity from biotic and abiotic disturbances, and associated complex interactions. To our knowledge similar studies examining multiple drivers *and their interactions* in marine intertidal habitats have not been performed, representing a critical gap in our understanding of the ecology of these important systems.

We tested whether (1) deposit feeding causes spatial heterogeneity in BMA biomass and (2) tidal resuspension homogenizes BMA biomass (eliminates patchiness caused by biotic disturbance). We also tested for differences in BMA community structure in fecal and surface sediments, both pre- and post-tidal immersion.

MATERIALS AND METHODS

Study site

Sediment and fecal samples of the golden acorn worm *Balanoglossus aurantiacus* (Hemichordata: Enteropneusta) were collected in July 2008 at Breach Inlet (32° 46′ 23″ N, 79° 48′ 53″ W), a beach on the north end of Sullivans Island, South Carolina, USA. Located between a rock jetty to the south and the foundation of a bridge to the north, the intertidal sandflat is considered a semi-protected beach. Strong currents are common throughout the semidiurnal tidal cycle due to the funneling of the ocean into a relatively narrow space between 2 barrier islands. The median grain size at the site is ~171 µm (Wilde & Plante 2002). *B. aurantiacus* is a common sediment-dweller at this site, with fecal mounds visible in the intertidal zone at low tide. These large worms (up to 1 m in length) burrow into the sand, creating a feeding funnel through which sediment is subducted into

the mouth. After the sediment has moved through the digestive system, a characteristic fecal coil is deposited at the other end of the burrow.

Field collection

To compare the structure of the BMA community in fecal coils with that in the surrounding sediment, samples were collected with either spatulas or a syringe with the Luer-end cut off (Wilde & Plante 2002). Surface sediment was collected only to 3 mm deep, as light limitations prevent diatoms from photosynthesizing deeper than 2 to 3 mm (MacIntyre et al. 1996). Surface sediment was collected 15 cm from the fecal mounds. Fecal sediment was collected directly from existing fecal coils. All of the samples (0.5 to 1.5 g of sediment each) were transferred directly into sterile 15 ml centrifuge tubes, capped tightly, and stored over ice until returned to the lab. For long-term storage, samples were kept at −20°C, covered in foil.

To further establish that physical appearance (sediment color and elevation) of fecal mounds corresponded with disturbance of BMA, 8 sediment samples were collected along 1 transect line leading from the top of each of 5 fecal mounds (where fresh fecal material was visible) out to 14 cm and subsequently analyzed for BMA biomass. Each transect line was placed at a randomly chosen angle from the water line and samples were collected at 0, 2, 4, 6, 8, 10, 12, and 14 cm. The appearance of each sampling site was recorded (i.e. the color and topography of each sampling site in comparison to surrounding sediment).

To examine physical disturbances to the BMA community, sampling was conducted at 2 times in the tidal cycle, allowing for the examination of differences in BMA community structure before and after tidal resuspension. Initial sampling of fecal and surface sediment was done at low tide, just prior to immersion. Each fecal coil was then marked using 2 pieces of PVC piping, driven into the sediment 10 cm to either side of the fecal coil, in a line parallel to the water's edge, such that water flow around the poles did not erode the sediment near the fecal coil. Sampling was repeated during early ebb tide, following a single tidal immersion. Samples were collected from the surrounding surface sediment and from the original location of the fecal coil, even if the fecal coil was no longer visible. All pre- and post-immersion fecal and sediment samples were analyzed for BMA biomass (n = 12 for each sample type), with a subset (n = 3) also subject to molecular analysis.

Spatial autocorrelation field collection

For the study of spatial heterogeneity, a modified version of the collection methods of Wilde & Plante (2002) was used. A transect was marked in the intertidal, near and parallel to the water line at low tide. A 1 × 1 m PVC quadrat was haphazardly placed along the transect line to include several fecal coils within its bounds. Within the PVC quadrat, a grid containing 8 × 8 squares (each measuring 2 × 2 cm) was placed in 2 of the corners and in the center of the opposite side (Fig. 1). Sampling within each small square allowed for samples to be taken at varied and easily determined distances from one another. To examine patterns over even larger (meter or decameter) spatial scales, a second 1 × 1 m PVC square was placed 20 m from the first (down the transect line) and samples were taken from a 64-sample grid in the center of the square. Before destructive sampling of sediment was conducted, the percent fecal cover (as recognized by sediment color and elevation) of each of the 2 × 2 cm squares in the 64-square grid was noted. Sampling for sediment chlorophyll a (chl a) was performed as described in 'Field collection'. Samples were placed into 1.5 ml microcentrifuge tubes, stored over ice until returned to the laboratory, and then stored at −20°C until analysis.

To examine effects of tidal immersion on spatial pattern, 2 additional 1 × 1 m plots were selected, 20 m apart along the transect line. At the first low tide, percent fecal cover of each of the 2 × 2 cm squares in the 64-sample grids was noted and PVC poles were placed 0.5 m from the corners of the 1 × 1 m square to mark its exact location. Following a single period of immersion, and immediately upon subsequent aerial re-exposure, samples for chlorophyll measurement were again collected, according to the procedures described in 'Field collection'.
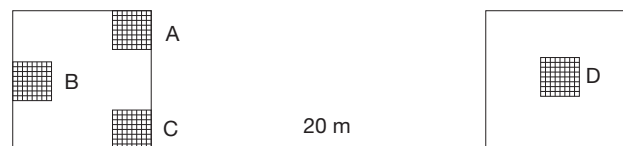

Fig. 1. Layout for spatial autocorrelation sampling consisted of 2 beach quadrats (1 × 1 m, PVC) placed at opposite ends of a 20 m transect line. One quadrat contained three 64-sample square grids (A to C); the other contained only one 64-sample square grid (D). Grids A to D were collected before tidal immersion. A similar set-up was used for Grids E to H, which were collected after tidal immersion. Not to scale

Analytical methods

Chlorophyll *a*

A modified version of the procedure described by Easley et al. (2005) was used for chl *a* measurement. Wet sediment (0.3 to 0.5 g) was placed in a vial with 10 ml of 90% acetone, wrapped in foil, and extracted at −20°C for 24 to 48 h. A fluorometer (Turner Designs TD-700) was used to measure the chl *a* content ($\mu g \ l^{-1}$). Remaining sediment was filtered onto 4.25 cm diameter glass fiber filters, dried at least 12 h at 60°C, then weighed in order to normalize chl *a* per gram of dry sediment.

DNA extraction

DNA was extracted using a modified version of the bead-mill method (Aguilera et al. 2006): ~1 g of sample was combined with 0.1 mm zirconia–silica beads (~2 g), 250 µl of extraction buffer (1M NaCl, 70 mM Tris, 30 mM EDTA), and 500 µl of 100 mM HNa_2PO_4 (pH 8) prior to two 20 s runs at 2500 fpm on a Mini Beadbeater (Biospec Products). DNA was precipitated (3 M sodium acetate:isopropanol = 1:6, v/v), cleaned (series of ethanol washes), resuspended (50 µl sterile water), and then stored at 4°C for >12 h. Concentration and size was determined on a 1% agarose gel in conjunction with a low DNA mass ladder (BioRad EZ Load Ladder). If needed, a DNA purification kit (Promega Wizard PCR Preps DNA Purification System) was used according to the manufacturer's instructions.

PCR-DGGE

Because numerous studies have demonstrated that epipelic diatoms dominate in this type of habitat (McClatchie et al. 1982, Underwood & Paterson 1993, Grippo et al. 2010), diatom-specific primers STRAM 9F (5′-CTGCCAGTAGTCATACGCTC-3′) and 517 Rgc-clamp (5′-GCCCGGGGCGCGCCCCGGGCGG GGCGGGGGACCAGACTTGCCCTC-3′) were used to amplify the 18S rRNA gene according to the PCR protocols of Cary et al. (2006). Gel electrophoresis in 1.0% agarose stained with ethidium bromide was used to visualize the amplified DNA. The products were quantified using the Quantity One (Bio-Rad Laboratories) software package and a low mass ladder. When final concentration of the amplified product was below target levels, the PCR product was used as template for an additional 7 cycles of the standard protocol (with accompanying initial denaturing and extension steps).

For DGGE, a 6% polyacrylamide gel (acrylamide: bisacrylamide = 37.5:1) with a denaturing gradient of 25 to 40% (100%: 7 M urea and 40% v/v deionized formamide) was loaded with 200 ng DNA per well. When necessary, PCR products were combined and concentrated using ethanol precipitation. A Versa Doc Imaging System and accompanying Quantity One software were employed for DGGE gel acquisition and analysis. After removing background intensity from all lanes (via the rolling disk method), the software detects different bands, their density in the lanes, and matches them to similar bands in other lanes on the same gel. This software was also used to identify and analyze the bands present in each lane, as described by Stocum & Plante (2006).

Bands of interest were extracted from the gel using a sterile pipette tip and stored in 10 µl Tris-HCl (10 mM, pH 8.5) at 4°C until sequence analysis was performed. DGGE primers were used for sequencing (Beckman Coulter CEQ 8000 Genetic Analysis System) minus the GC-clamp. Test samples, consisting of the brightest bands from a fecal and surface sediment samples (both pre- and post-tidal immersion), were examined to verify source DNA as diatom in nature. Reads approximately 300 to 400 nucleotides long were evaluated using the GenBank blastn database (Altschul et al. 1990), and results with >96% maximum identities were considered.

Statistical analysis

ANOVA was used to test for differences in biomass (chl *a*), DGGE band number (*S*), and species diversity between sample type (fecal and sediment), and between samples before and after tidal immersion. Shannon-Wiener diversity indices (*H'*), incorporating band number and relative band intensities, were calculated using PRIMER v6 (Primer-E). The JMP 7.0 software package (SAS Institute) was used to perform the ANOVA calculations. Further, the DGGE banding patterns were used to create an unweighted similarity matrix using the Quantity One software package, which was then analyzed by nonmetric multidimensional scaling (NMDS) using PRIMER v6 software. Applied to DGGE data, an NMDS plot shows every banding pattern as a single point from which relative changes in community structure can be visualized (van Hannen et al. 1999). Additionally, analysis of similarity (ANOSIM) tests were used to

compare community composition among sample types (Rees et al. 2004, Powell et al. 2005). Both 2-way (crossed design, with sample type and time as factors) and 1-way ANOSIM as described by Clarke & Green (1988) were performed on each gel similarity matrix. Within the PRIMER v6 tutorial, Clarke & Gorley (2006) interpret values of $R > 0.75$ as well separated, >0.5 as overlapping but different, >0.25 as barely separable, and <0.25 (including negative values) as indistinguishable. Both R-statistics and significance levels generated from ANOSIM were used to assess differences among communities; however, with small numbers of replicates, and thus few possible permutations, p-values can be less useful than R-statistics. Spatial autocorrelation calculations were performed to test for spatial heterogeneity as described by Wilde & Plante (2002). SAM v3.0 was used to perform spatial autocorrelation and Pearson's correlation analysis (Rangel et al. 2006). The latter allows for correction of degrees of freedom due to spatial autocorrelation. The coefficient Moran's I was used to construct correlograms between chlorophyll values and inter-sample distances (Sandulli & Pinckney 1999).

RESULTS

Fecal sediments contained far less chl a than did surrounding sediment (Fig. 2). In samples taken prior to tidal immersion, a significant reduction (~91.7%) was found between microalgal biomass (chl a) in fecal versus surface sediment samples ($p < 0.001$). Tidal immersion also strongly affected chl a content.
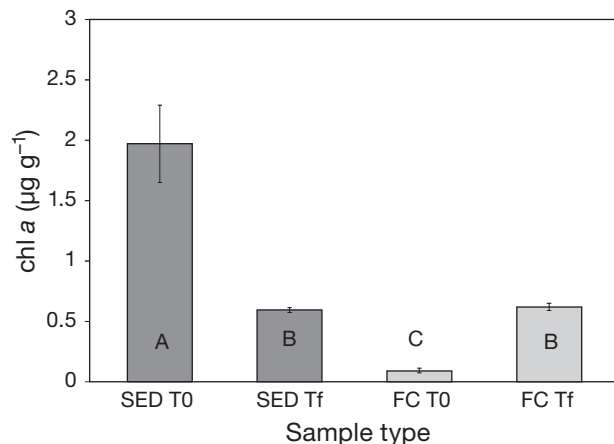
Significant differences in microalgal biomass were observed between surface sediment pre- and post-tidal immersion ($p < 0.001$), and between fecal material pre- and post-tidal immersion ($p < 0.001$). No significant difference existed between fecal and surface sediment biomass after tidal immersion ($p = 0.388$).

Samples taken at 2 cm intervals along transects across fecal patches showed significant global differences ($p < 0.001$). A Tukey-Kramer honestly significant difference test revealed that there was no significant difference between chl a content of the patch center (0 cm) and 2 cm ($p = 0.980$; Fig. 3), but these 2 levels were significantly different than those collected at 6, 8, 10, 12, and 14 cm, all of which were indistinguishable from one other ($p > 0.263$ for all such comparisons). The chl a value at 4 cm was not significantly different from either of the other 2 groups ($p > 0.188$ for all such comparisons). The reduced chl a content of the sediment at 0, 2, and 4 cm corresponded to the visual boundaries of the fecal mounds.

Autocorrelation analysis revealed spatial heterogeneity in BMA biomass at low tide (Fig. 4A). A significant positive autocorrelation existed at the smallest spatial scale (~2.4 cm, $p < 0.0001$). Significant negative autocorrelation existed at ~43.7 cm ($p < 0.001$), ~73.6 cm ($p < 0.001$), and ~78.6 cm ($p = 0.043$) between sampling grids. As sampling sites became further apart, a negative correlation was seen, though not always significant. Autocorrelation values again were positive at larger distances between sampling sites (Fig. 4A). Patch size was approximated to be ~7 cm in radius (inter-sample distance at which correlogram switches from positive to negative Moran's I; Sandulli & Pinckney 1999).
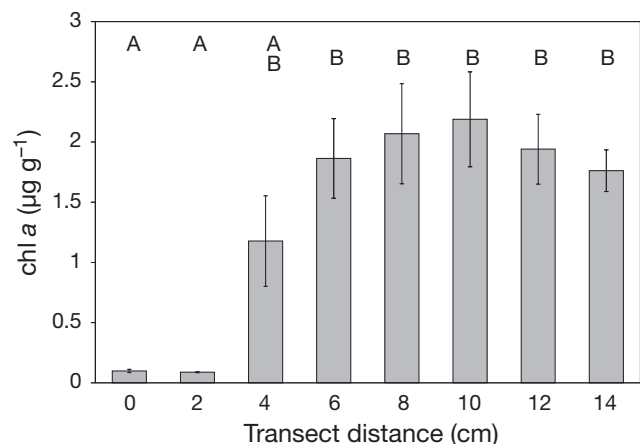

Fig. 2. Chlorophyll a content (mean ± SEM, n = 12) of surface sediment (SED, dark grey bars) and fecal (FC, light grey bars) samples pre- (T0) and post-tidal (Tf) immersion. Different letters on or above bars indicate statistically different ($\alpha = 0.05$) mean values

Fig. 3. Sediment chlorophyll a content (mean ± SE, n = 5) at 2 cm intervals along transects, starting at center of *Balanoglossus aurantiacus* fecal mounds. Different letters indicate significantly different ($\alpha = 0.05$) mean values
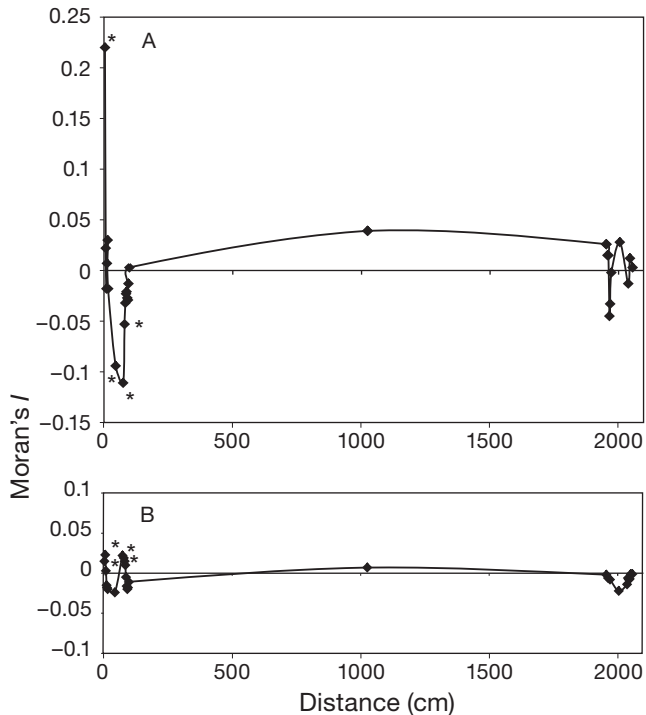
Fig. 4. Moran's *I* values for sediment chlorophyll *a* content (A) before and (B) after tidal immersion. *Significant values (α = 0.05)

After tidal immersion, a different spatial pattern was observed (Fig. 4B). Significant positive auto-correlations were detected at diverse spatial scales: ~2.4 cm (p = 0.047), ~6.0 cm (p = 0.004), ~73.3 cm (p = 0.047), and ~78.6 cm (p = 0.047). While these correlations were significant, the p-values closely approached α = 0.05 in several cases, and the auto-correlation coefficients were small relative to the pre-immersion values, indicating weaker relationships.

Surface plots of chl *a* content and percent fecal cover values visually represent the correspondence between these 2 variables (Fig. 5). The inverse relationship is especially evident in Grid A (Fig. 5A). Although the absolute loss in BMA biomass is less remarkable, the negative relationship between chl *a* and fecal cover is also evident in Grids B and D (Fig. 5B,D), whereas no chl *a* trough is seen in the grid that contained no *Balanoglossus aurantiacus* fecal mounds (Fig. 5C). Before tidal immersion, a significant negative correlation between chl *a* and percent fecal cover was observed (r = −0.199, p = 0.014). Following tidal immersion, no significant correlation was found (p = 0.697). Visually, no correspondence is evident in surface plots between pre-immersion fecal cover and post-tidal immersion chl *a* contents (Fig. 6).

DNA sequencing of select DGGE bands seemed to confirm primer specificity. In each case (n = 5), highest percent similarity (96 to 98%) when compared to the GenBank blastn database was found to be with diatom species. A mean of 11 DGGE bands resulted from pre-immersion fecal samples, whereas post-inundation fecal samples had 15.3 bands, and pre- (13.7) and post- (13.3) immersion sediment samples exhibited intermediate band numbers; however, band number did not differ significantly either for sample type (p = 0.792) or time (p = 0.141). Similarly, mean Shannon-Wiener diversity indices ranged between 2.17 (pre-immersion fecal samples) and 2.51 (post-immersion fecal samples), with pre- and post-immersion sediment values in between (2.48 and 2.41, respectively). These diversity indices did not differ significantly, either by sample type (p = 0.323) or time (p = 0.238). Although the majority of DGGE bands were common to all sample types, banding patterns were clearly distinguishable by time (R = 0.519, p = 0.004; ANOSIM), but less clearly separable by sample type (R = 0.278, p = 0.120). NMDS analysis revealed that pre-immersion fecal samples were distinct from all other sample types (Fig. 7). When analyzed using 1-way ANOSIM, pairwise comparisons within the ANOSIM corroborated these distinctions. Pre-immersion fecal samples were distinct from all other sample types (R > 0.481 for all comparisons), whereas fecal samples after inundation were not distinct from sediment before or after tidal immersion (R = 0.074 for both comparisons), nor did the sediment samples differ (R = 0.111).

DISCUSSION

In the present study we observed ~92% BMA removal due to *Balanoglossus aurantiacus* gut passage. This compares well to 83 and 94% removal by the same species at the same beach in the summers of 2006 and 2007, respectively (Plante et al. 2010). This reduction between ingesta and egesta falls at the high end of the range noted for other deposit-feeding taxa (47 to 93%; Fenchel 1972, Hargrave 1970, Kofoed 1975, Yingst 1976, Plante et al. 2010). Generally, studies that have compared BMA biomass in plots with and without diverse deposit feeders have demonstrated the capacity to substantially reduce BMA biomass over larger sedimentary landscapes (Bianchi & Levinton 1981, Miller et al. 1996, Hagerthey et al. 2002).

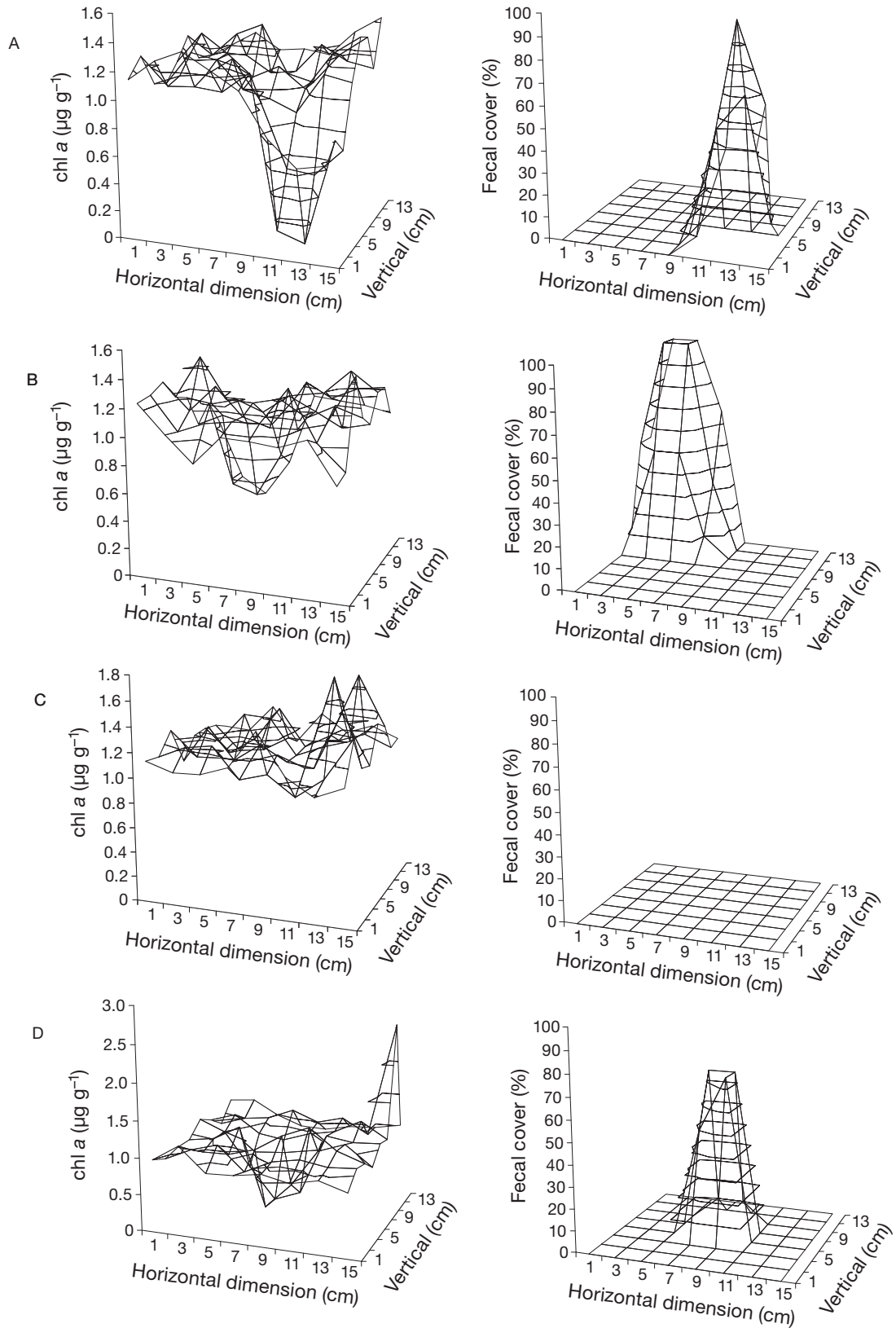Spatial autocorrelation analysis demonstrates that this biological disturbance results in BMA biomass

Fig. 5. Surface plots showing the sediment chlorophyll *a* content and corresponding fecal cover of four 16 cm × 16 cm sampling grids before tidal immersion (Grids A to D here and in Fig. 1). Horizontal and vertical dimensions refer to along-transect and perpendicular (up-beach) aspects of each grid, respectively
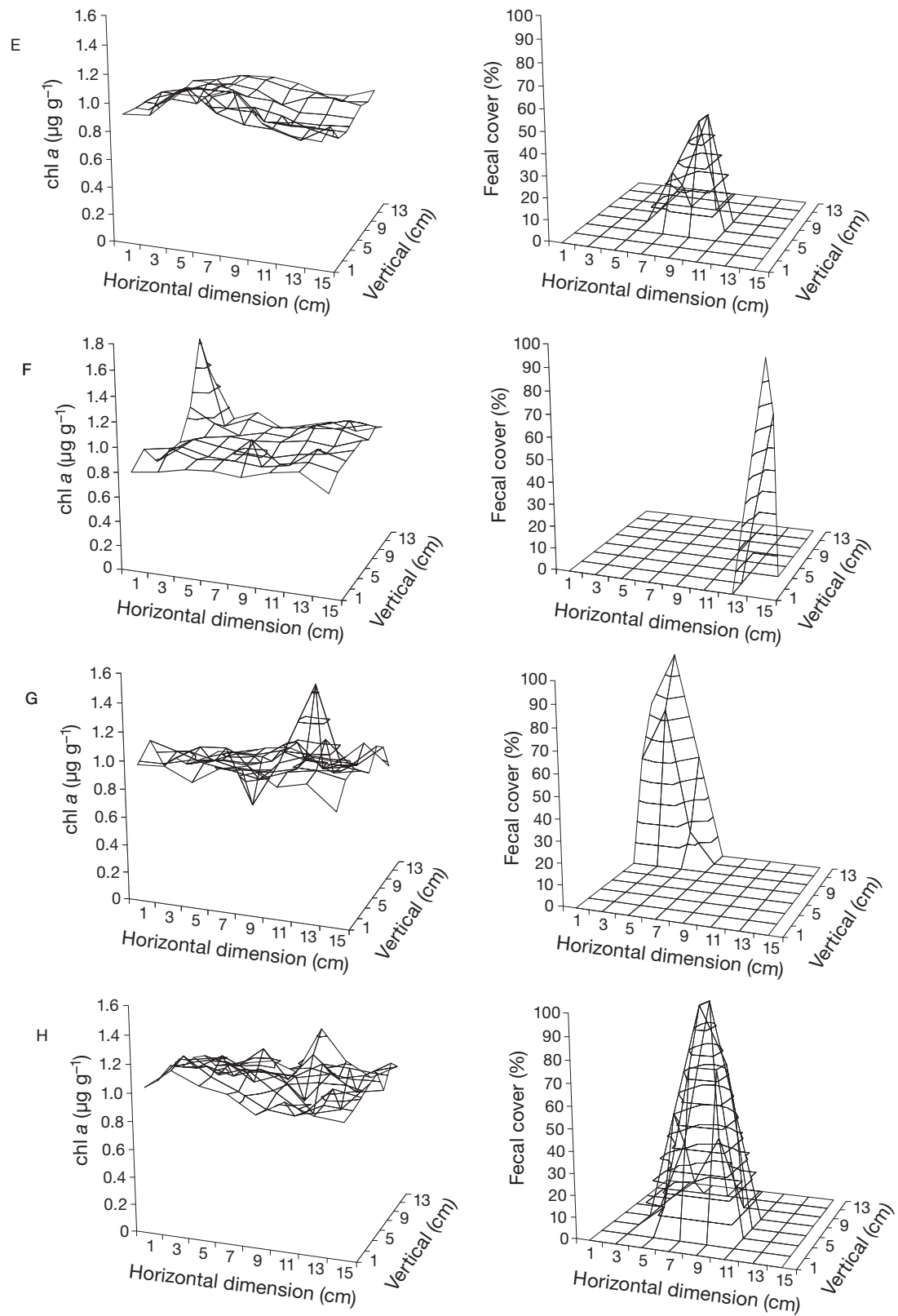
Fig. 6. Surface plots showing the sediment chlorophyll *a* (post-immersion) content and corresponding fecal cover (pre-immersion) of 16 cm × 16 cm sampling Grids E to H (as mentioned in Fig. 1). Horizontal and vertical dimensions refer to along-transect and perpendicular (up-beach) aspects of each grid, respectively
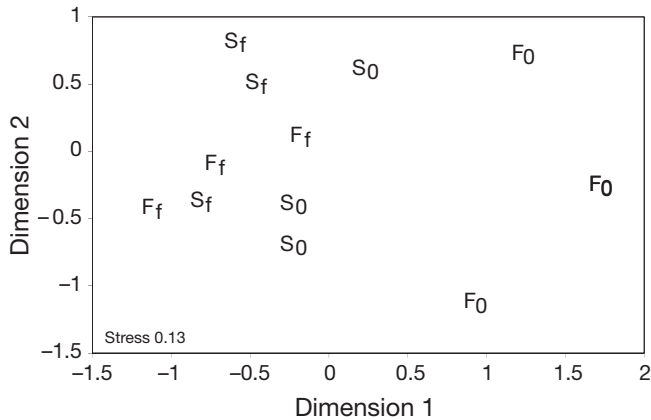
Fig. 7. Non-metric multidimensional scaling plot for the 18S rDNA DGGE analysis, pre-immersion (subscript 0) and post-immersion (subscript f) fecal (F) and surface sediments (S)

patchiness in surface sediments. We observed a positive autocorrelation at a small spatial scale (~2.4 cm between sampling sites), indicating that samples taken at that distance tended to be similar in biomass. Patch radius was estimated to be ~7 cm (as indicated by the line crossing the *x*-axis on the correlogram, our Fig. 4A; Sandulli & Pinckney 1999). Assuming circular patch shape, we estimate an average patch size of ~154 cm^2. Similar studies employing spatial autocorrelation analyses likewise detected microalgal patches in temperate intertidal sediments; Blanchard (1990) noted an average patch size of 79 cm^2 and Sandulli & Pinckney (1999) found microalgal patch sizes ranging between 30 and 191 cm^2. Jesus et al. (2005) likewise detected centimeter-scale patchiness in intertidal BMA using pulse-amplitude modulated (PAM) fluorometry. At their sandy site (their site most similar to ours), patch sizes ranged between 7.3 and 40.2 cm^2 (Jesus et al. 2005). Our patch size estimate falls into these broad ranges, tending toward the upper end. In the present study, the observed patchiness appears to be due to *Balanoglossus aurantiacus* fecal coils, areas of lower microalgal biomass. According to our transect study, the average radius for fecal mounds approximated 6 cm, or a total patch size of about 113 cm^2. Given the discontinuous nature of both the transect measurements (every 2 cm) and the samples for spatial autocorrelation, this represents good general agreement with the patch size estimate from the autocorrelation analysis. These estimates also correspond with the distinct appearance (physical elevation and color difference) of the fecal coils. This agreement is important because percent fecal cover data for our correlative analyses were visually determined.

At intermediate spatial scales (40 to 80 cm), negative spatial autocorrelation was detected during late low tide, indicating that transitions from high to low BMA biomass occurred within the main sampling unit (1 m^2). Although previous studies of this nature have observed significant spatial patterning over larger spatial scales (≥1 m), attributed to sediment characteristics (Paterson et al. 2003) or sand waves (Plante et al. 1986), we did not detect significant autocorrelation at scales over ~0.8 m.

ANOSIM results illustrate that the BMA community composition of fresh *Balanoglossus aurantiacus* egesta was distinguishable from that of surrounding sediments. Clarke & Warwick (2001) point out that sample types can be distinguished by ANOSIM due either to differences in mean or by marked difference in variance. Our NMDS plot suggests that the fecal-sediment separation is due primarily to the latter. A few explanations might account for the greater BMA compositional variability in fecal coils. First, sediments from different depth strata, with disparate BMA communities, could be ingested. Duncan (1987) suggests that *B. aurantiacus* subducts surficial sediments to depths of 3 to 10 cm before ingesting, and also that these deposit feeders shift the position of their burrows and feeding funnels on a daily basis. Thus, deeper sediments are likely ingested both when the animal relocates, and to a lesser degree, in normal feeding modes. Alternatively, it is possible that coils may have been in different stages of succession. Prior studies at this site showed significant BMA recovery in as little as 3 h (to ~20% of ambient chl *a* levels; Plante et al. 2010). Vopel et al. (2007) likewise observed rapid recovery of benthic phototrophs following disturbance associated with heart urchin burrowing in subtidal sediments, potentially in response to a favorable nutrient supply in freshly reworked sediment.

Despite removing individuals, disturbance can increase species evenness (and diversity) at the scale of individual patches through compensatory mortality, in which competitively dominant species suffer disproportionately following a disturbance (Connell 1978). Although differences in community structure between egesta and background sediment were indicated by DGGE and ANOSIM in the present study, diatom species richness (band number) and diversity did not differ. In part, the inability to detect significant differences despite lower absolute *S* and *H'* in pre-immersion egesta could be due to the high variability in community structure among fecal samples (see previous paragraph and Fig. 7). Methodological limitations also may hinder the ability to

detect differences in species richness among communities. Prior studies have demonstrated that specific template DNA must represent 1 to 2% of total DNA to be detected by the PCR-DGGE approach (Muyzer et al. 1993, Murray et al. 1996), thus rare species are missed and total richness is underestimated. Despite these possible limitations, differences in the relative intensity of bands and composition were distinguishable by ANOSIM. The changes in relative band intensity observed in the present study may be especially important, as it has often been observed that disturbances normally have little effect on species richness—more likely, major shifts in relative dominance and species evenness result (Reice 1994). It is better established that disturbances generate diversity at spatial scales larger (i.e. landscape scale) than that of individual patches (Sousa 2001). A different sampling design than employed in the present study, e.g. comparison of landscapes with and without deposit feeders, would be needed to test hypotheses about diversity at larger spatial scales.

Like deposit feeding, tidal immersion also appears to cause significant reduction in sedimentary microalgal biomass because chl *a* levels were much reduced in surface sediments collected just after tidal emersion as compared to exposed sediments during the flood stage of the previous low tide. However, this conclusion is complicated by the cyclical vertical migrations performed by epipelic diatoms and other BMA that can cause considerable variation of biomass in surface sediments over short time scales. It is thought that these migrations are confined to the uppermost sediment layers, the exact depth of which is dictated by light penetration and therefore sediment characteristics. Because we sampled to 3 mm, if the migrations were restricted to the uppermost few millimeters, this activity would not complicate our interpretations. Fortunately, Easley et al. (2005) documented BMA vertical migrations at this study site. Although they found changes in chl *a* down to depths of 10 mm over a complete tidal cycle (including periods of sediment resuspension when inundated), they observed BMA migrations only within the top 1 to 2 mm of sediments (see their Fig. 2). Thus, loss of chl *a* between periods of emersion would appear to constitute a true disturbance. It is possible that removal was due to mortality, although translocation (i.e. to the water column, deeper waters, or deeper sediment strata) seems more likely. Although lab studies have indicated that abrasion can result in BMA loss (Miller 1989, Delgado et al. 1991), realistic levels of sediment transport in the field typically have failed to show significant BMA destruction (Miller 1989).

Even more apparent is that sediment transport or resuspension during periods of immersion obliterates the spatial heterogeneity in BMA biomass caused by *Balanoglossus aurantiacus* feeding. After tidal immersion, BMA biomass was significantly higher where fecal mounds had been, roughly matching the now lower levels in adjacent sediments. Jesus et al. (2005) noted high temporal autocorrelation in BMA biomass values during emersion, but the correlation broke down after tidal inundation, suggesting reorientation of patch dynamics during immersion. Furthermore, in the present study, BMA compositional differences in egesta were no longer detectable following tidal immersion. Visual absence of fecal coils corroborates the notion that BMA patchiness caused by *B. aurantiacus* feeding was erased.

BMA patchiness has long been recognized as an important factor to be considered in sampling design (Blanchard 1990). Variability in photosynthetically active biomass in intertidal sediments has also been recognized as an important consideration in productivity models (Perkins et al. 2001, Serodio et al. 2001). At higher trophic levels, dispersion patterns might be patchy if BMA are a limiting resource. For instance, patchiness of meiobenthic copepods has been shown to be correlated with BMA biomass (Decho & Fleeger 1988, Sandulli & Pinckney 1999). Other studies, however, revealed no correlations in other meiobenthic taxa or at different locales (Blanchard 1990, Pinckney & Sandulli 1990), perhaps because BMA biomass is at times so high that it is not a limiting factor for consumers (Santos et al. 1995). BMA biomass may also create cues for invertebrate larval settling (Butman et al. 1988). For sedentary organisms, such as sediment-dwelling worms, the environment in which the larvae settle is vital to adult success. Chemical signals derived from diatom biofilms, such as EPS, could be of particular importance to species that feed heavily on BMA (Harder et al. 2002). More indirectly, BMA patchiness due to deposit feeding may influence sediment stability. Previous work has shown that erosion rates are negatively correlated with sediment chlorophyll content (Sutherland et al. 1998). The EPS produced within diatom biofilms allow them to form mat-like structures on the surface of sediment, which increase erosion thresholds and stabilize sediment (Yallop et al. 1994, Sutherland et al. 1998). Thus, heterogeneity in diatom distributions due to deposit feeding contributes to EPS patchiness; consequently, erosion is more likely to occur (Lanuru 2004, Tolhurst et al. 2006).

However, at our intertidal site, BMA biomass heterogeneity due to deposit feeding is an ephemeral

phenomenon. Although sampling schemes conducted at low tide need to account for patchiness and spatial autocorrelation, as do total productivity models, many of the above-mentioned processes are focused during periods of tidal immersion, thus the homogenization observed in the present study suggests that patchiness at low tide holds limited significance. For instance, invertebrate recruitment, sediment resuspension, and animals grazing on BMA may be little impacted by the patchiness during aerial exposure at our study site and in similar intertidal environments. That the incoming tide homogenizes but also removes BMA biomass does, however, suggest that a significant portion of intertidal BMA production is redistributed, e.g. to the water column or deeper sediments, thereby subsidizing these adjacent ecosystems (de Jonge & van Beusekom 1992, Malet et al. 2008).

In summary, our results demonstrate that *Balanoglossus aurantiacus* feeding removes BMA biomass and alters community structure, and also corroborate earlier studies showing that deposit feeding produces spatial heterogeneity. We also show that tidal inundation of our semi-protected beach reduces sediment BMA biomass and homogenizes benthic diatoms such that patchiness caused by deposit feeding is erased. To determine whether these results are generalizable, studies conducted with higher abundances or a wider taxonomic range of deposit feeders are needed. In addition, studies similar to those reported herein, but conducted in situations with minimal sediment transport, are needed to assess the significance of more persistent BMA patchiness. Our future work will employ such studies in adjacent subtidal waters. In addition, we will attempt to employ manipulative experiments to prevent resuspension within intertidal sediments at Breach Inlet or similar intertidal sites.

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