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Landscape and smaller-scale effects of lugworm (*Arenicola marina*) deposit feeding on benthic bacterial assemblages

by Craig J. Plante¹

ABSTRACT

Objectives of this study were to (1) determine whether feeding by the lugworm, *Arenicola marina*, reduces abundance or alters composition of sedimentary bacterial assemblages, (2) examine recovery of “disturbed” patches of egesta, and (3) test for effects on bacterial abundance, diversity and composition at spatial scales larger than individual fecal mounds. Field comparative studies in Lubec, Maine, were conducted to test for the effects of ingestion, and manipulative experiments were done to assess rates and mechanisms of recolonization of egesta. Bacterial assemblage attributes were followed using epifluorescence microscopy and DGGE analysis of 16S rDNA. Next we examined landscape-scale effects using field addition experiments to manipulate lugworm density (0, 5, and 15 worms m⁻²). Findings indicate that (1) lugworm feeding qualitatively and quantitatively alters bacterial assemblages, (2) recovery in these biotically disturbed sediments is minimal during a single tidal emersion (~4.5 h), (3) the small-scale patchiness caused by animal feeding is homogenized by sediment movement following tidal immersion, and (4) landscape-scale effects appear to be small, e.g. with respect to bacterial abundance and “species” richness. One notable landscape-scale effect, however, was a consistent and stable increase in relative abundance of a few specific bacterial phylotypes in high lugworm density plots.

1. Introduction

Classic equilibrium mechanisms of population regulation, such as competition and predation, have received a fair amount of attention for sedimentary bacteria, although little experimental work has been done. From correlative studies, the paradigm emerged that numbers are inversely correlated with grain size and, thus, directly related to sediment-grain surface area (Dale, 1974; Deming and Baross, 1993), suggesting that “space” is limiting. In fact, a very small percentage (< 5%) of available space on grains is actually occupied by microbes (DeFlaun and Mayer, 1983). Alternatively, “spheres of diffusional influence” may intersect at bacterial densities well below that of a physical space limitation (Schmidt *et al.*, 1998).

The driving questions for studies of predation on sedimentary microbes historically have not centered on the structuring of microbial communities. For instance, from the 1970’s (to

1. Department of Biology, Grice Marine Laboratory, College of Charleston, Charleston, South Carolina, 29412, U.S.A. *email: plante@cofc.edu*

present) benthic ecologists have asked whether microbial (or specifically bacterial) biomass comprises the bulk of deposit feeders' diets. The vast majority of studies have answered in the negative (Kemp, 1987; Plante *et al.*, 1989; van Oevelen *et al.*, 2006). Somewhat more recently, microbial ecologists have asked the question of whether predation can balance the measured bacterial production in benthic habitats. Again, the answer has usually been "no" for deposit-feeding macrofauna (Bianchi and Levinton, 1981; Kemp, 1987; 1990), and also for meiofauna (Kemp, 1990; Epstein and Shiaris, 1992), and protozoa (Epstein and Shiaris, 1992; Epstein, 1997).

Rather, the high diversity (Gray and Herwig, 1996; Scala and Kerkhof, 2000) and low stability (Findlay *et al.*, 1990a, b; Rooney-Varga *et al.*, 1997) of benthic microbial communities suggests an important role for nonequilibrium determinants of community structure. Nonequilibrium models emphasize environmental disturbance and spatial heterogeneity in habitats in which a state of equilibrium is unusual. Under such conditions, community structure is largely defined by the interactions of disturbances, succession, and the patchiness of the physical environment.

Although sometimes thought of as a predator-prey interaction, deposit feeding is more appropriately treated as a disturbance. An ecological disturbance is a discrete event that removes organisms and disrupts ecosystem, community or population structure (White and Pickett, 1985), whereas predation is defined as a biotic interaction in which one participant benefits while another is harmed (Dodson, 1998). Digestion of bacteria typically provides a negligible net nutritional gain to deposit feeders (Andresen and Kristensen, 2002; van Oevelen *et al.*, 2006). Moreover, when compared to the typical predator-prey relationship, the effect of a deposit feeder on ingested microbes occurs over larger spatial scales, is less selective, and influences communities as opposed to individuals. In addition, disturbances disrupt the physical environment as well as biota. Deposit feeding alters geochemical gradients within ingested sediments and can dramatically affect grain size, sorting, water content, and compaction of sediments (Rhoads and Boyer, 1982; Katrak and Bird, 2003).

Deposit feeding can influence aspects of the ecology of benthic bacteria. Several studies that compared deposit-feeder fecal matter with ingesta have detected influences on bacterial biomass (Moriarty *et al.*, 1985; Lucas *et al.*, 2003), growth (Deming and Colwell, 1982; Plante *et al.*, 1989), and activity (Aller and Yingst, 1985; Plante and Jumars, 1992; Roberts *et al.*, 2001). What is not clear is whether these effects are manifest in the bulk sediments. Deposit feeding also can transform community composition (Dobbs and Guckert, 1988; Plante and Wilde, 2004; Grossi *et al.*, 2006) of microbes in that ingesta are dissimilar to ingested sediment. But, again, the significance to the surrounding sediment, or "sedimentary landscape," is more equivocal.

Objectives of the work described herein were to (1) test whether feeding by a model deposit feeder, *Arenicola marina*, constitutes a disturbance, resulting in removal of bacteria or alteration of community composition, (2) determine rates and mechanisms of recolonization, and (3) determine whether deposit-feeding disturbances alter bacterial community structure of the benthic intertidal landscape.

2. Methods

a. Study site and species

Samples were collected from, and experiments were performed on, sandflats near Lubec, Maine (44°48.8' N, 66°58.7' W). The model deposit feeder *Arenicola marina* (Linnaeus, 1758) was employed. This large (up to 250 mm) sedentary polychaete inhabits deep (20–40 cm) J-shaped burrows and feeds by subducting surficial sediments to its mouth. The resultant large fecal mounds (49.5 ± 32.8 (SD) cm²; N = 59) and feeding depressions of this lugworm could be visually distinguished and were dominant features of this flat. Lugworm distributions were patchy, with densities ranging between 0 and 16 worms m⁻².

b. Biotic disturbance and recolonization studies

Fecal samples were collected from an area of approximately 3600 m² (60 m × 60 m) containing a moderately high (~3–12 m⁻²) lugworm density. To collect fresh egesta, existing fecal mounds or coils (FC) were flagged and numbered to mark their locations, then brushed away. After the next egestion (t = 0 h), fresh fecal materials were collected using a clean spatula. Samples for enumeration (~0.5 g) were placed into 10 ml sterile, filtered (0.2 μm) seawater, and stored on ice (< 3 h) until processing. In addition, samples for molecular analysis were taken from ≥ 3 replicates. Approximately 0.5 g of each was collected via spatula and placed into 2 ml conical microtubes containing ~2.5 g of 0.1 mm silica/zirconia beads (Biospec Products; More *et al.*, 1994) and in liquid nitrogen until return to the Grice Marine Laboratory (after which, storage was in a -80°C freezer until processing). Surficial sediments (top ~3 mm) from ambient sediment (SED) and feeding funnels (FNL) were similarly sampled and stored.

To follow bacterial recolonization of the egesta of these deposit feeders, fecal and ambient, surficial sediment samples were collected over the 4–5 h intertidal period. After the initial egestion (t = 0 h), 100 mm diameter round polystyrene plates (cut from petri dishes) were inserted at a 45° angle into the sediment beneath the egesta to block further addition of fecal material (cf. Findlay *et al.*, 1990b). To distinguish recolonization due to *in situ* growth vs. migration from underlying sediments, I placed “latrines” (see Fig. 1 in Plante and Wilde, 2001) over burrow exits to capture and isolate egesta from sediments. Latrines, fecal materials on the sediment surface, and control areas for sediment samples were covered with 5.5-cm high × 11.5-cm diameter cylindrical glass culture dishes to inhibit evaporation (Plante and Wilde, 2001). All samples, sediment (SED), naturally incubated fecal casts (FC), and fecal casts on latrines (LAT), were sampled as described above for enumeration and molecular work.

To test for potential recolonization cues associated with *A. marina* egesta, I also followed recovery in artificial, bacteria-free fecal coils (cf. Plante and Stinson, 2003). Briefly, this consisted of boiling sediments in 10 volumes of 30% peroxide, then vacuum-filtering (0.2 μm bottle-top filter). Peroxide was removed by washing with 40 volumes of sterile filtered seawater, then sediments were again vacuum-filtered. Following complete removal of the seawater wash, 0.5 volumes more of sterile seawater were added

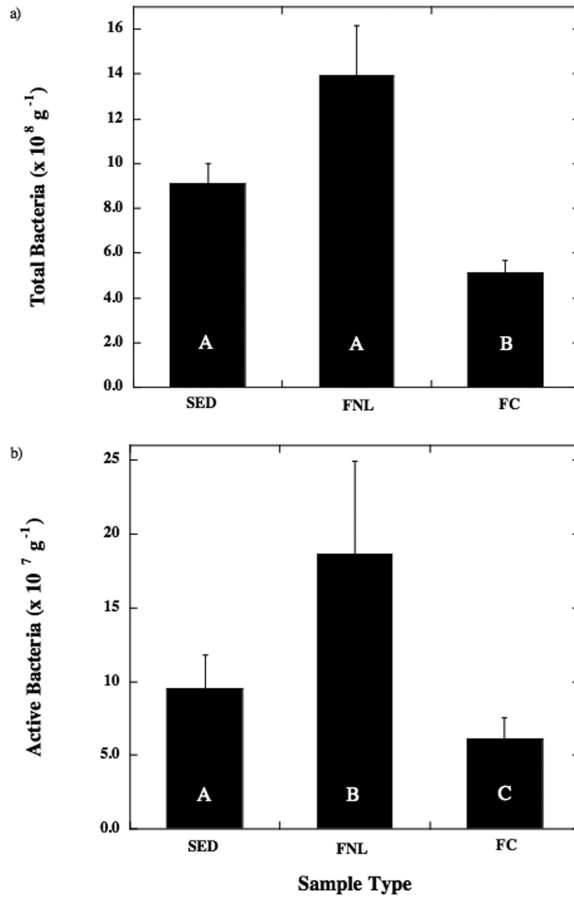


Figure 1. Mean \pm SEM of (a) total, and (b) active bacterial densities. SED: surface sediment, FC: fecal matter, FNL: sediments in feeding funnels. Different letters indicate significant ($\alpha = 0.05$) difference among sample types.

to the dried sediments for re-saturation. A 1 cc syringe (Luer end cut off) was used to extrude 'mock' fecal coils that were similar in size and shape to fresh fecal coils of *A. marina*. These artificial fecal coils were placed onto surficial sediments within the area of the experiments described above. Mock coils were sampled by spatula just after placement ($t = 0$ h) and just before being flooded with the rising tide ($t = 4$ h), and prepared for total bacterial direct counting as described above.

c. Landscape study

Lugworms were collected from areas of relatively high worm abundance, then transplanted to an adjacent area of low abundance. Within this area, three 10-m x 10-m plots were marked with flags, within each of which nine replicate 1 m x 1 m plots were randomly

located. On each of three consecutive days, three replicates of three treatments (0, 5, and 15 lugworms m^{-2}) were started by transplanting lugworms. Corners of each 1 m^2 plot were marked with PVC pipe (~ 2.5 cm diameter cylinders of 30 – 35 cm length). After introduction of worms, injured worms or those not burrowing within 1 h were replaced with additional worms. Each experimental unit was sampled for bacterial enumeration and for molecular analysis at 0, 2 and 13 d, as described above. For sampling, a 1 m \times 1 m square with 1 cm graduations along each side was placed over the four corner posts. Meter sticks were placed over the square along randomly chosen x and y coordinates, and each sample was taken from a 1-cm circle around the chosen point of intersection. Throughout the duration of the experiment and three days afterwards (16 d), worm abundances were estimated by enumerating fecal mounds in and immediately adjacent (within 15 cm) to each of the 1 m^2 quadrats.

d. Effects of tidal immersion

Although recolonization during aerial exposure is limited to potential mechanisms of regrowth and migration, during tidal immersion “recruitment,” likely passively with sediment transport, could contribute to recovery. To test for recolonization during immersion, fresh *A. marina* fecal coils were sampled during low tide, then the area was re-sampled after one period of tidal immersion (7-8 h) and again after 2 d (4 periods of immersion, ~ 44 h). After the initial sampling, 100 mm diameter round polystyrene plates were inserted at a 45° angle into the sediment beneath the coils to block subsequent addition of new egesta, as described above. Fecal materials and sediments were collected using a clean spatula. Samples were placed into sterile, filtered seawater, and stored on ice (< 3 h) until processed for active and total bacterial counts, as described above.

e. Enumeration of bacteria

Bacteria were dislodged from the sediment using a short burst (20 s) of sonication with a 3-mm sonic probe (Branson Sonifier 250) at setting 4 on the output control (amplitude = 306 μm , power output = 65 W). Metabolically active bacteria were enumerated using the fluorogenic redox dye 5-cyano-2,3-ditolyl tetrazolium chloride (CTC; Polysciences, Inc.) (Rodriguez *et al.*, 1992). Aliquots (1.8 ml) were taken from the diluted, sonicated sample and transferred to 3 ml centrifuge tubes for the CTC reduction assay. CTC (180 μl of 25 mmol L^{-1}) was added to each tube and incubated (3 h) at 25°C in the dark while shaking (200 rpm). Samples were fixed in formalin (2% final concentration) for subsequent direct counts of active and total bacteria.

Total counts were made using a modified version of the protocol of Hymel and Plante (1998). Briefly, fixed samples were centrifuged at 5000 \times g for 15 min (15°C) then resuspended in Trizma buffer (0.05 mol L^{-1} , pH 8.10) and a dispersing agent (0.5% Triton-X 100) and sonicated for 20 s with a 3 mm sonic probe at 65 W. Samples were then stained with SYBR Gold (5×10^{-4} final concentration) for 20 min, and concentrated onto

0.2 μm black polycarbonate membranes. For each sample and filter set, 20 grids (or more) were counted to include > 200 total cells per slide.

Both total and active counts were made from each slide using a Nikon epifluorescence scope at $1000\times$. SYBR-staining bacteria were counted using a wide-blue filter set (excitation 450 to 490 nm, 510 cut-off; emission 520 nm). Active bacteria (CTC staining) were counted using a rhodamine (green) filter set (excitation 510 to 560 nm, 580 cut-off; emission 605 nm). Samples were then dried and weighed so as to normalize to dry g sediment⁻¹.

f. DNA extraction, PCR and DGGE

Bacterial cells in sediments were physically lysed using bead-mill homogenization, then extracted as described in Plante and Wilde (2004). The Wizard PCR DNA purification system (Promega) was used for final purification, using manufacturer's instructions.

PCR was performed using the primer set (314f-GC and 517r) for amplification of the V3 region of the 16S rDNA. PCR conditions followed those described by Plante and Wilde (2004). PCR products were purified and concentrated using the Wizard PCR DNA purification system. Multiple (2-4) 50 μl amplification reactions of the same sample were typically combined and reconstituted in 35 μl TE for a 3- to 6-fold concentration. DNA was quantified fluorometrically using the PicoGreen dsDNA quantitation kit (Molecular Probes).

Denaturing gradient gel electrophoresis (DGGE) employed the D-Code™ Mutation Detection System (Bio-Rad Laboratories). An 8% polyacrylamide gel with a 40 to 60% gradient of denaturant was used. Sample volumes were varied so that 400 ng of PCR product was loaded for each sample and the gel was run at 70 V for 16 h at 60°C. Gels were stained with 1X SYBR Gold (Molecular Probes) for 30 min and visualized in a Model 1000 VersaDoc imaging system (Bio-Rad Laboratories).

g. DNA sequencing for bacterial identification

DGGE bands of interest were excised, eluted, and purified using the Wizard PCR DNA purification kit. PCR was again performed, using the protocol described above, with the same primers except that the GC clamp on the 314f primer was excluded. Cycle sequencing of the PCR products was completed using a Beckman Coulter dye terminator cycle quick start kit, employing the same primers. Samples were sequenced by capillary electrophoresis on a Beckman Coulter CEQ 8000 automated DNA sequencer. For presumptive identification, sequences were compared to those in GenBank using the BLAST function of the National Center for Biotechnology Information server (<http://www.ncbi.nlm.nih.gov>).

h. Data analysis

Two-way ANOVA were used to test for effects of time and sample type (sediment, isolated or naturally incubated fecal casts) on total and active bacterial abundances. Data were tested for normality (Lillefor's test) and heteroscedasticity (Levene's test) and natural

log transformed as required. If main effects were significant, planned pairwise multiple comparison tests employed Fisher's LSD correction; if main effects were not significant, the more conservative Bonferroni's adjustment was used (Milliken and Johnson, 1984). In one case, heteroscedasticity could not be removed by transformation (ln, sqrt, or arcsin). In this case, Kruskal-Wallis (with Scheirer-Ray-Hare extension) and Wilcoxon Sign Rank tests were employed for main factor and pairwise comparisons, respectively.

Quantity One software (Bio-Rad) used for DGGE gel image acquisition was also employed for analysis. The software performs a density profile through lanes, detects individual bands, and matches bands occupying the same position in the different lanes. Similarity indices were then calculated using band position and intensity (weighted) or band position only (unweighted) via the method of the Dice Coefficient. The complements of these similarity values were calculated to generate a dissimilarity matrix, which was then analyzed by nonmetric multidimensional scaling (NMDS). 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, 1- and 2-way analysis of similarity (ANOSIM) tests were used to compare community composition among time points and treatments (Rees *et al.*, 2004; Powell *et al.*, 2005). One-way ANOSIM as described by Clarke and Green (1988) was performed on each gel similarity matrix. Pairwise comparisons among treatment types and times within each treatment were also performed. Clarke and Gorley (2006) interpret values of $R > 0.75$ as well separated, > 0.5 as overlapping but different, and > 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.

Two-way ANOVA were also employed to compare "species richness" (# of phylotypes or bands) among sample types and times.

3. Results

a. Disturbance and recolonization of patches

SYBR Gold counts revealed removal of bacteria whether densities in fresh egesta were compared to surficial sediments (44%, $p = 0.031$) or to sediments in feeding funnels (63%, $p < 0.001$) (Fig. 1a). Densities in surficial sediments and funnels did not differ significantly ($p = 0.071$), although some evidence for concentration in funnels was apparent. Similarly, densities of active bacteria were reduced in fecal casts compared to both surficial sediment (36%, $p = 0.046$) and funnel sediments (67%, $p = 0.028$). Densities of active bacteria in funnels were significantly greater than those in sediments ($p = 0.046$) (Fig. 1b).

When total bacterial numbers in the recolonization experiment were compared, densities among treatments varied ($p = 0.001$), although neither time effect ($p = 0.190$) nor the time x treatment interaction ($p = 0.755$) was significant (Fig. 2a). Neither was there recovery in artificial coils over ~4 h of intertidal exposure. The peroxide treatment to remove bacteria was efficient as shown by $> 98\%$ removal relative to natural sediments (mean total

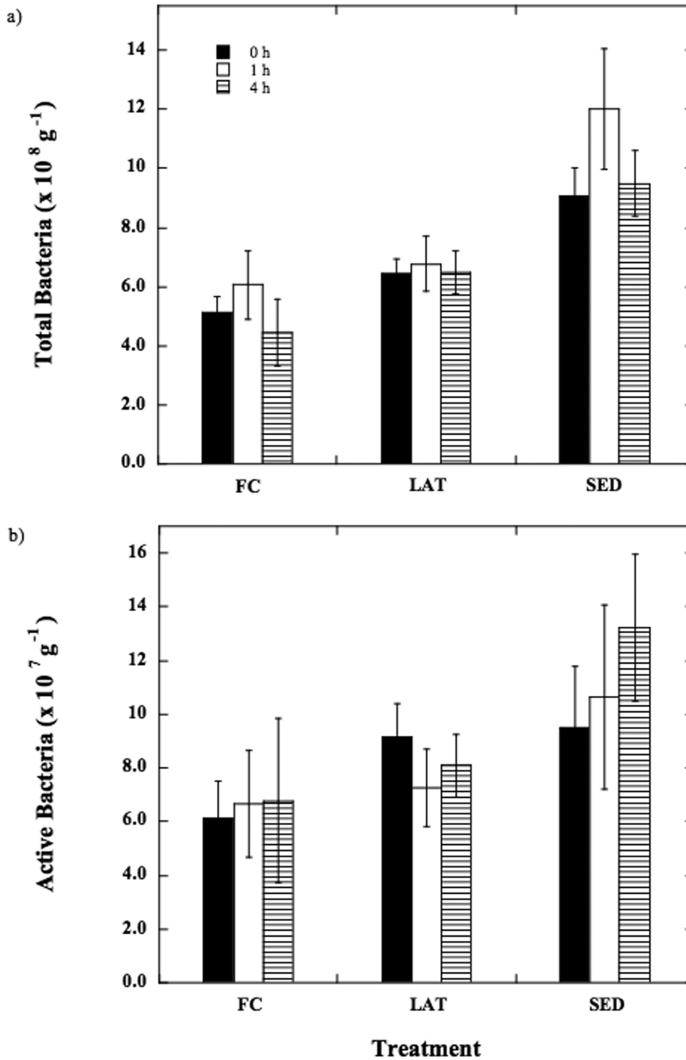


Figure 2. Mean \pm SEM of (a) total, and (b) active bacterial densities through time in recolonization experiments. SED: surface sediment, LAT: latrine-isolated fecal matter, FC: naturally-incubated fecal matter. Asterisks indicate significant ($\alpha < 0.05$) temporal change.

bacterial density of $15.7 \times 10^6 \text{ g}^{-1}$ at $t = 0 \text{ h}$). After 4 h, total bacterial densities remained low ($18.2 \times 10^6 \text{ g}^{-1}$), showing no significant increase ($p = 0.662$). An absence of numerical recovery in natural coils was likewise noted when just active bacteria were examined (Fig. 2b). Although the treatment effect was again significant ($p = 0.047$), the effect of time was not ($p = 0.746$), nor was the interaction significant ($p = 0.747$).

DGGE banding patterns revealed clear differences among compositions of bacterial

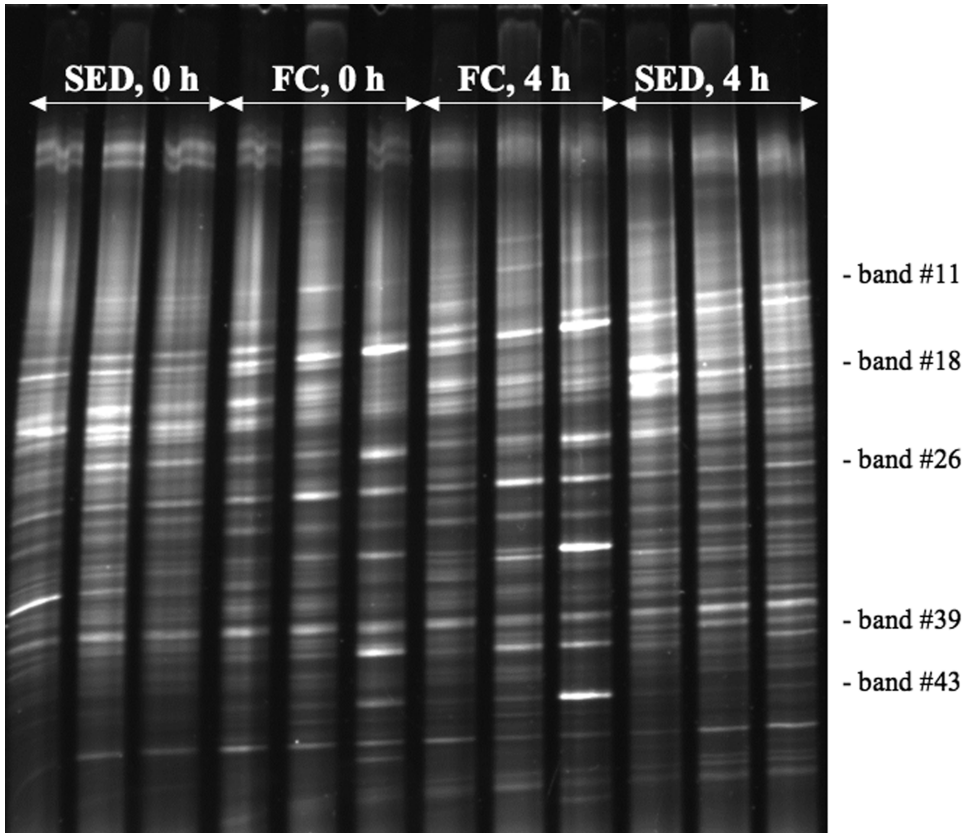


Figure 3. Representative DGGE gel profiles (unretouched) for fecal and sediment samples at 0 and 4+ h incubation. SED: surface sediment, FC: fecal matter. Indicated bands are unique or are more intense in particular sample types, and are discussed in text.

assemblages in fresh fecal and sediment samples (Fig. 3). It also was clear that communities were more variable in fecal samples as compared to sediments (Figs. 3 and 4). When numbers of bands were compared, no significant effect of time ($p = 0.068$), or treatment ($p = 0.305$) was observed, although the interaction was significant ($p = 0.040$), with sediments after 4.5 h showing a greater mean number of bands (42.3) than 0 h sediment (36.3, $p = 0.053$), 0 h fecal coils (33.3, $p = 0.012$) and 4.5 h coils (35.3, $p = 0.036$). A more qualitative examination of gels indicated that a few bands (i.e., bacterial phylotypes) were unique to, or at least relatively more important in, a particular sample type. For instance, bands #26, 39 and 43 were more intense in egesta, while bands #11 and 18 represent phylotypes that were relatively less abundant in egesta, and likely removed by digestion (Fig. 3). However, the majority of bands were present in both fecal and sediment samples, with similar intensities (Fig. 3).

Although temporal shifts in community profiles of ambient sediments were detectable

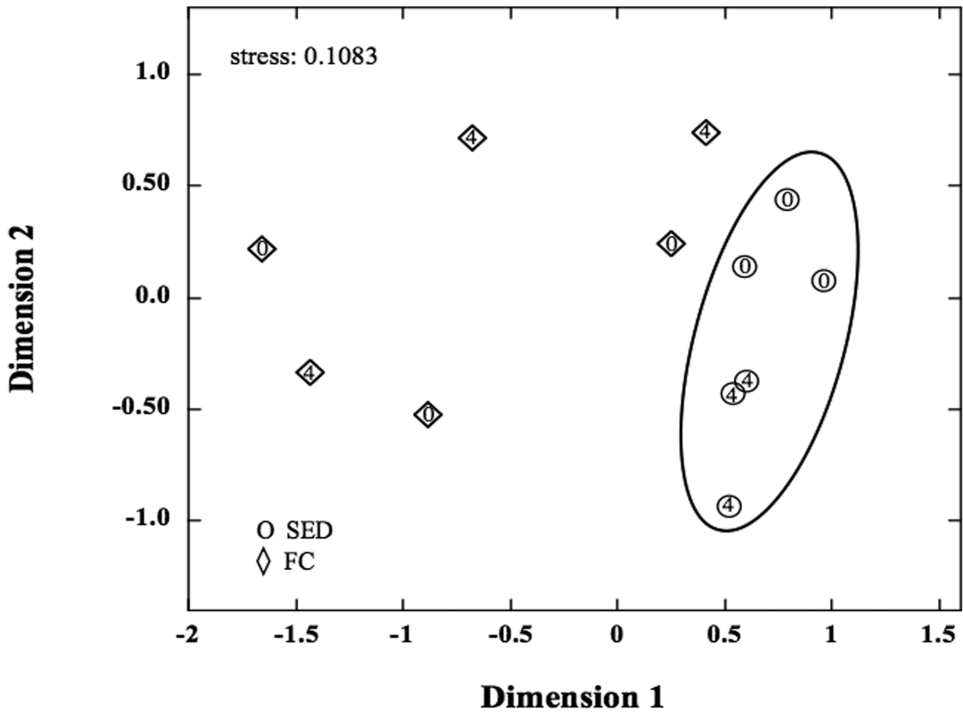


Figure 4. NMDS map showing the changes in the structure of bacterial assemblages through time. The numbers inside the symbols refer to hours of experiment. SED: surface sediment, FC: fecal matter. The oval shows clustering of sediment assemblages.

(Fig. 4), they were not dramatic. Community composition of fecal materials was distinguishable from those of sediments ($R = 0.519, p < 0.010$; 2-way ANOSIM), but a temporal shift over 4-5 h was not detected in these fecal samples ($R = 0, p = 0.400$ for 0 vs. 4.5 h; Fig. 4). Although a few bands exhibited clear differences in intensity in 0 vs. 4.5 h fecal samples, such patterns were generally not consistent among replicates. These inconsistencies likely were due to the greater variability observed in fecal samples, as compared to those from ambient sediment.

b. Influence of tidal immersion

After a single tidal immersion, fecal coils marked at the previous low tide could no longer be visually distinguished. In almost all cases, however, fresh fecal coils could be found nearby (5-10 cm), likely produced by the same worm that produced the original coil. In most cases, these new coils were located at the edge of the round polystyrene plates (placed beneath the original egesta), indicating that these plastic inserts successfully precluded subsequent egestion at the original site by forcing the worms to re-locate their tail shafts.

The effects of both sample type ($p < 0.001$) and time ($p < 0.001$) on total bacterial density were significant, whereas their interaction was not ($p = 0.176$). At the initial pre-immersion sampling, total bacterial densities differed between fresh egesta and adjacent sediment ($p < 0.001$). After one period of immersion, bacterial densities in egesta increased ($p = 0.027$) but showed no further increase over the next four tidal cycles ($p = 1.000$) (Fig. 5a). Although bacterial densities in egesta and sediment still differed after a single period of immersion ($p < 0.001$), after four tidal cycles differences were no longer significant ($p = 0.082$). Over this two-day period, total bacterial densities in surficial sediments did not increase significantly ($p = 0.282$) (Fig. 5a).

The effects of sample type ($p = 0.035$), time ($p < 0.001$) on active bacterial densities were significant, whereas the treatment*time interaction again was not ($p = 0.363$; Kruskal-Wallis test). Wilcoxon Signed Rank tests revealed that densities of active bacteria in egesta were significantly greater after both a single ($p = 0.005$) and four ($p = 0.008$) tidal cycles as compared to initial densities in fresh egesta, although the increase between one and four immersion periods was not significant ($p = 0.086$). At $t = 0$, densities in egesta and sediment differed ($p = 0.013$), but after immersion this was no longer true ($p = 0.721$ and 0.066 for comparisons after one and four cycles, resp.). Densities of active bacteria also increased over four tidal cycles in sediment samples ($p = 0.009$), although increases between initial and one tidal immersion or between one and four tidal cycles were not significant ($p > 0.05$) (Fig. 5b).

c. Landscape-scale effects

Lugworm densities were successfully manipulated and maintained over the duration of the experiment (Fig. 6), with the low-, medium- and high-worm treatment plots maintaining densities near initial levels.

The effects of treatment ($p = 0.192$), time ($p = 0.539$), and the treatment*time interaction ($p = 0.552$) on total bacterial densities in sediments were not significant (Fig. 7a). Likewise, active bacterial densities were not related to treatment, time, nor their interaction ($p > 0.412$ for all; Fig. 7b).

The most obvious feature of the DGGE gels was the consistency in banding patterns, between replicates, but also across sample types (lugworm density) and time (Fig. 8). No significant differences were noted between background (i.e., $t = 0$ d and/or 0-worm treatments) and intermediate time or worm density treatments (i.e., 5-worm plots or 15-worm after 2 d; data not shown). Upon closer inspection, however, at least one band (#30) did appear to become relatively more intense in the high-worm plots after 13 d. At least in part due to this band, this treatment (high-worm density, 13 d) was distinguishable from $t = 0$ ($R = 0.741$, $p < 0.10$) and low-worm treatments at 13 d ($R = 0.556$, $p < 0.10$) when weighted comparisons were performed (Fig. 9). This band corresponded with one of the bands (#43 in Fig. 3) shown to be of greater relative intensity in fresh fecal material, as compared to ambient sediments.

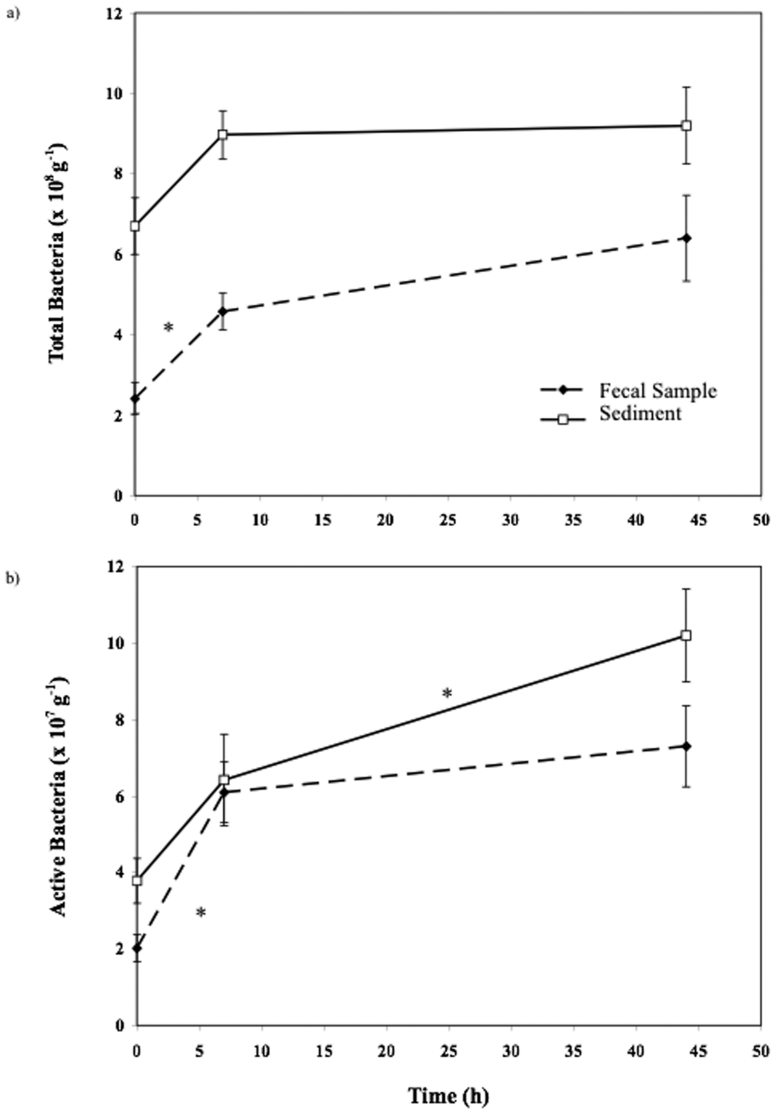


Figure 5. Mean \pm SEM of (a) total, and (b) active bacterial densities in *A. marina* fecal coil and adjacent surficial sediments following tidal immersion. Asterisks indicate significant ($\alpha < 0.05$) temporal change.

d. DNA sequencing

The excised band was determined to be a previously unknown species of *Bacillus*, most similar to *Bacillus hwajinpoensis* (Accession # AM274755, 97% 16 S rRNA gene sequence similarity). *B. hwajinpoensis* is a facultative anaerobe, which has previously been isolated from marine waters (Yoon *et al.*, 2004) and coastal sediments (Kopke *et al.*, 2005).

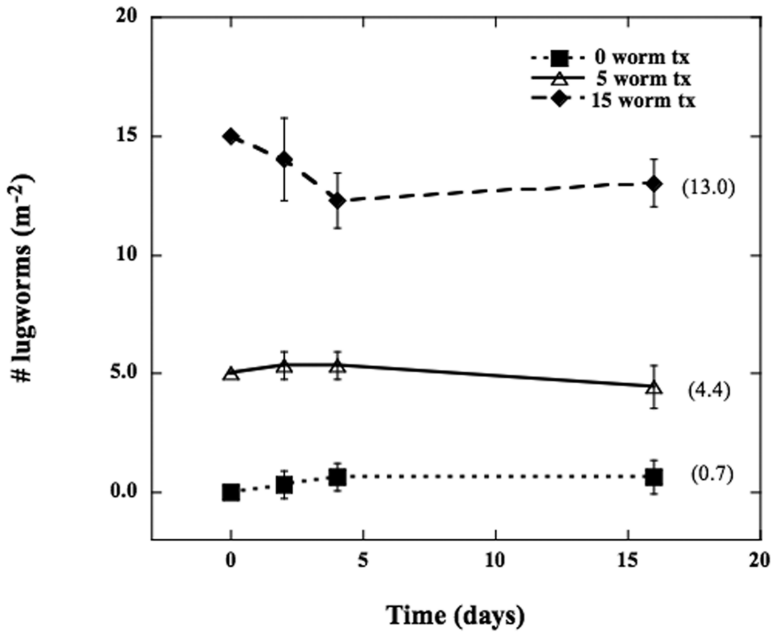


Figure 6. Mean \pm SEM of lugworm densities in low (0 W), medium (5 W), and high (15 W) worm density treatments. Values in parentheses indicate final means (at 16 d).

4. Discussion

a. Numerical patterns of bacteria

Elevated bacterial densities in lugworm feeding funnels relative to surrounding surficial sediments is in accord with prior studies (Retraubun *et al.*, 1996; van Oevelen *et al.*, 2006). Passive deposition of low-density organic matter into feeding depressions (Yager *et al.*, 1993) likely accounts for elevated bacterial biomass. Bacterial removal with passage through the gut of *A. marina* was also in line with previous estimates of digestion (30-90%, Grossmann and Reichardt, 1991) or assimilation efficiencies of bacterial biomass (32-53%, Andresen and Kristensen, 2002) for this animal, and generally in agreement with such estimates for other marine deposit feeders. It is important to note that our estimates, like those cited above, are likely to be conservative estimates of digestive removal. Numerous studies (Plante *et al.*, 1989; Thorsen, 1999) have demonstrated that bacteria can grow in posterior portions of deposit-feeder guts, including *A. marina* (Andresen and Kristensen, 2002), thus it is possible that some removal is masked when egesta are compared to ingesta.

Compositional changes in bacterial assemblages due to gut passage were also observed and have been observed by other researchers in numerous deposit-feeding species (Findlay *et al.*, 1990b; Grossmann and Reichardt, 1991; Plante and Wilde, 2004). More recent studies of this type have employed molecular techniques, and include studies with

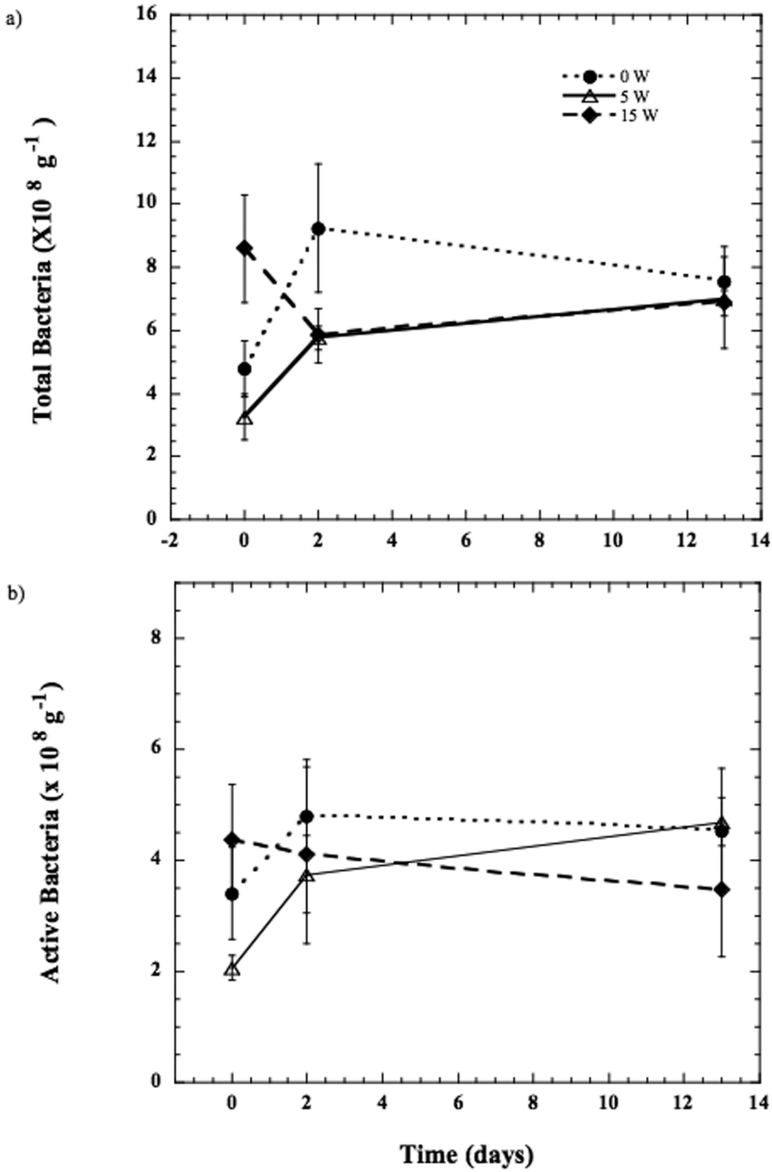


Figure 7. Mean and \pm SEM of (a) total, and (b) active bacterial densities through time in plots of 0, 5, or 15 lugworms m^{-2} . Asterisks indicate significant ($\alpha < 0.05$) temporal change.

lugworms. Grossi *et al.* (2006) employed both fatty acid and rRNA intergenic spacer (RISA) analyses to characterize changes in bacterial communities with gut transit through *A. marina*. Passage through these lugworms fed dead phytoplankton cells in laboratory mesocosms resulted in clear changes in bacterial community structure.

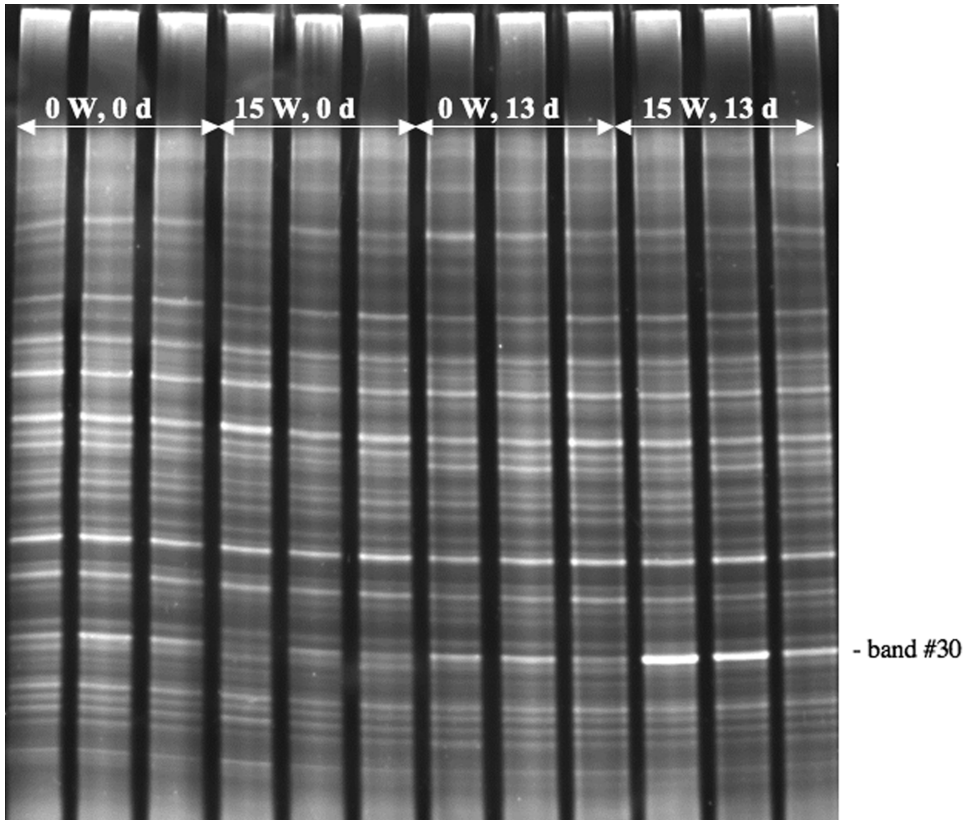


Figure 8. Representative DGGE gel profiles (unretouched) of sediment samples at 0 and 13 d incubation, in plots of 0 or 15 lugworms m^{-2} (designated 0 W or 15 W). Indicated bands are unique or are more intense in particular sample types, and are discussed in text.

b. Recolonization of patches during tidal emersion

Recolonization of fecal patches was not observed over the ~ 4.5 h of intertidal exposure in this study. This is in contrast to earlier work, which showed significant quantitative and qualitative recovery of bacterial assemblages in deposit-feeder egesta over time intervals (2-3 h) similar to those examined here (Plante and Wilde, 2001; 2004). These studies, although quite similar in design, were conducted with different invertebrate species, and at lower latitude (South Carolina, USA). Water temperatures were significantly warmer at the South Carolina locale, which may have contributed to higher rates of bacterial growth or movement. In addition, inhibitory chemicals within lugworm egesta have been shown to slow recolonization. Plante and Stinson (2003) found that bacterial recolonization into artificial fecal coils was inhibited when fluid from the egesta of *Arenicola cristata* was added. However, because any such inhibitors would have been absent from peroxide-

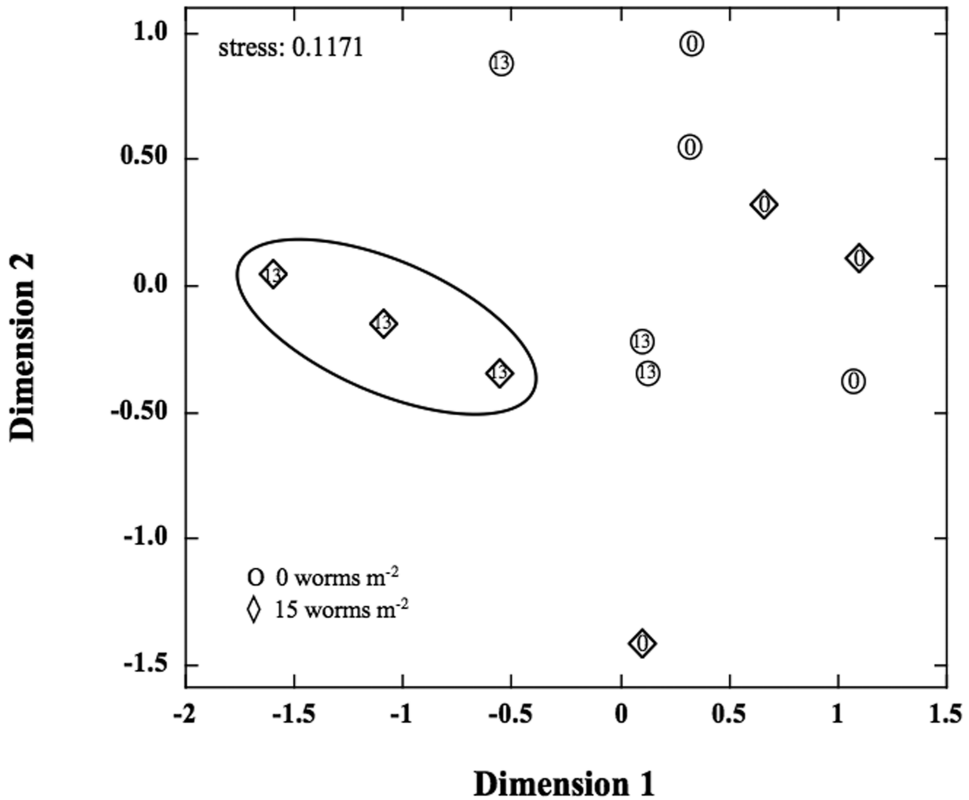


Figure 9. NMDS map showing the differences in the structure of bacterial assemblages in low (0 worms m⁻²) and high lugworm (15 worms m⁻²) treatments through time. The numbers inside the symbols refer to days of experiment. The oval shows clustering of the 15 worm, 13 d replicates relative to other treatments and times.

treated sediment in the present study, lack of recovery in mock coils suggests that factors other than negative cues in natural coils accounted for negligible recolonization.

c. Landscape-scale effects of lugworm feeding

Disturbances can generate diversity at landscape (or 'regional') scales. Disturbances are patchy both in space and time, thus create heterogeneity in abundance and composition, and tend to increase species diversity at spatial scales greater than that of individual patches. However, quantitative differences among treatments of varying levels of biotic disturbance were not detected in the present study, either in average bacterial densities or in variability (patchiness). There are a number of hypotheses that might explain the lack of a quantitative effect in bulk sediments despite the numerical reduction observed between ingesta and fresh egesta. The most obvious is that lugworms in this experiment were not

numerous enough, fed too slowly, or both, which limited the magnitude of effect. As predicted, our re-located lugworms were relatively sedentary. Although *A. marina* frequently moves its feeding pocket and point of defecation over the scale of decimeters (Farke *et al.*, 1979), it rarely makes larger-scale migrations (Beukema, 1995). Thus, worm densities were maintained close to target densities throughout the two weeks of study and treatments, therefore, spanned the natural densities of *A. marina* at this site. However, densities in many locales, especially in the eastern Atlantic, are much greater than observed in this study (up to 80 adults m⁻²; Cadée, 1976; Beukema, 1995; Andresen and Kristensen, 2002), and therefore the effects of lugworm feeding on landscape-scale microbial attributes observed in the present study are rather conservative.

Another, not unrelated, possibility is that recovery was so rapid that changes were not detected when bulk sediments were randomly sampled after two or thirteen days. Although our results showed no significant recovery over the intertidal period (~4.5 h), other studies have shown rapid recovery of the egesta of other deposit-feeding species by bacterial migration (Plante and Wilde, 2004). Grossi *et al.* (2006) documented recovery in *A. marina* egesta, but over much longer time scales (19 days). Bioturbation (Hylleberg, 1975; Retraubun *et al.*, 1996; Banta *et al.*, 1999) and gut passage (Plante *et al.*, 1989, but see Grossmann and Reichardt, 1991) can stimulate microbial growth, countering digestive removal in the gut. Thus, despite lack of apparent recovery during the period of low-tide exposure, numerical recovery by migration may have been complete over longer intervals (e.g., by the 2 d sampling).

Alternatively, resuspension and homogenization during tidal immersion could obliterate any numerical signal from deposit feeding. Combined, biological and physical observations provide robust support for this hypothesis. Both active and total bacterial densities in disturbed fecal patches increased significantly after one and/or four periods of tidal immersion. Both quantities were significantly lower in fresh egesta as compared to surrounding sediments, but were no longer distinct after two days of tidal flood and ebb. Concomitant increases in bacterial densities within surficial sediment may have resulted from temporal changes in external factors (e.g., organic supply) or could reflect normal cyclical changes within the period of exposure (e.g., related to diatom migrations; van Duyl *et al.*, 1999) because initial samples were taken just prior to immersion, while subsequent samples after one and four tidal cycles were taken soon after emersion. These bacterial increases in (control) sediments do not negate the recovery in egesta because the *difference* between abundances in fecal matter and sediment clearly diminished over time. Observation that fecal patches were no longer recognizable after a single tidal immersion also supports the notion that physical disturbance was significant. Clearly, small-scale patchiness due to *A. marina* feeding could be erased by this physical disturbance. Whether the homogenization of disturbed fecal patches with unperturbed sediments would result in an overall reduction of bacterial abundances, or qualitative effects, over larger spatial scales (e.g., our meter-scale experimental landscapes) is less clear.

d. *Compositional effects at the landscape scale*

The intermediate disturbance hypothesis (IDH) predicts highest species richness and diversity at intermediate levels (frequency, magnitude or intensity) of disturbance (Connell, 1978). We observed no clear differences in species richness (i.e., number of DGGE bands) of the bacterial assemblages among different disturbance regimes. DGGE may underestimate such differences, however, as it is possible that bacteria could be killed by digestive processes within the animal gut, yet DNA would persist long enough to be extracted, and subsequently produce DGGE bands (Josephsen *et al.*, 1993; Nocker *et al.*, 2007). Despite these possible limitations, the relative intensity of bands (and presumably population densities) did change to a degree that was distinguishable by ANOSIM.

Bias during PCR amplification has been demonstrated (Suzuki and Giovannoni, 1996), causing some to question the use of DGGE band intensity in microbial community analysis. However, Murray *et al.* (1996) showed a strong relationship between band intensity and relative DNA abundance in a complex template mixture, and numerous studies have shown that variation in relative band intensity can be related to differences among bacterial assemblages (McCaig *et al.*, 1999; Portillo and Gonzalez, 2008). Therefore, both banding pattern and relative intensities are informative for the analysis of complex microbial assemblages, provided that all samples are treated using identical techniques (Fromin *et al.*, 2002). 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).

In particular, one phylotype, identified as a *Bacillus* species, consistently increased in representation in the high-worm treatment. This increase in relative abundance could be due to resistance to digestion, or this bacterium could be a symbiont that was seeded into ambient sediments as ingested material passed through the gut. The former seems more plausible for two reasons. First, although the corresponding DGGE band is notably stronger in fecal samples and in high-density worm sediment samples, weak bands indicate that this bacterium was present in lower abundance even in sediment without lugworms. Second, previous studies have shown that gram-positive bacteria generally are more resistant to digestion by deposit feeders than are gram-negative types (Plante and Mayer, 1994; Plante and Shriver, 1998b). Plante and Shriver (1998a) tested lytic susceptibility of a number of sedimentary isolates to digestive fluids of *A. marina*. Whereas approximately one-third of gram-negative strains were susceptible, all tested gram-positive isolates (11 of 11) were resistant.

Resistant bacteria may come to dominance under high grazing pressure simply because their relative densities increase as other species are removed. Alternatively, deposit feeding might represent a frequent disturbance, which favors those species that can recolonize quickly via immigration or rapid growth. That the *Bacillus* sp. in this study was present in relatively high numbers in fresh egesta favors the former explanation.

e. Concluding remarks

Bacteria are key players in sedimentary geochemical reactions (Riemann *et al.*, 2000; Pinhassi *et al.*, 2005) and benthic food web dynamics (Jurgens and Gude, 1994). The present study demonstrates that lugworm feeding disturbs bacterial assemblages and creates patches of altered density and composition. Patchiness in bacterial community structure can have significant implications for varied biotic interactions, e.g., invertebrate recruitment and feeding rate (Snelgrove and Butman, 1994). Effects over larger spatial and temporal scales were also examined by manipulating lugworm densities. Even under maximal deposit feeder abundances, no differences in bacterial density could be detected at landscape scales. However, shifts in dominance patterns among phylotypes were noted in highest lugworm density plots, illustrating that deposit feeding can influence bacterial composition (and potentially community function) at large spatial and temporal scales. When the size of individual disturbance events is small relative to the size of the landscape, and when rate of recovery is faster than return time of disturbance, the landscape will reflect a steady-state mosaic. In such a shifting mosaic, communities continuously change through disturbance and succession, but the proportion of landscape in various stages of succession remains relatively constant (Turner, 1998). Thus, the landscape appears stable, exhibiting little change through time, and has low variability (spatially consistent at any given time), as was observed for bacterial density and species richness in the present study. Alternatively, the homogenizing effect of the twice daily tidal immersion could reduce patchiness of bacterial biomass and composition. Although some degree of sediment resuspension occurs with tidal change, it is clear that homogenization effects must be localized since the lugworm “signal,” i.e. DGGE pattern and predominance of specific bacterial types, consistently persisted over time in high worm-density plots. Future studies of this nature should explore both higher grazing intensities and a range of hydrodynamic regimes to distinguish the relative influences of biotic and physical processes.

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REFERENCES

- Aller, R. C. and J. Y. Yingst. 1985. Effects of the marine deposit feeders *Heteromastus filiformis* (Polychaeta), *Macoma balthica* (Bivalvia), and *Tellina texana* (Bivalvia) on averaged sedimentary solute transport, reaction rates, and microbial distributions. *J. Mar. Res.*, 43, 615-645.
- Andresen, M. and E. Kristensen. 2002. The importance of bacteria and microalgae in the diet of the deposit-feeding polychaete *Arenicola marina*. *Ophelia*, 56, 179-196.
- Banta, G. T., M. Holmer, M. H. Jensen and E. Kristensen. 1999. Effects of two polychaete worms, *Nereis diversicolor* and *Arenicola marina*, on aerobic and anaerobic decomposition in a sandy marine sediment. *Aquat. Microb. Ecol.*, 19, 189-204.
- Beukema, J. J. 1995. Long-term effects of mechanical harvesting of lugworms *Arenicola marina* on the zoobenthic community of a tidal flat in the Wadden Sea. *Neth. J. Sea Res.*, 33, 219-227.

- Bianchi, T. S. and J. S. Levinton. 1981. Nutrition and food limitation of deposit feeders: II. Differential effects of *Hydrobia totteni* and *Ilyanassa obsoleta* on the microbial community. J. Mar. Res., 39, 547-556.
- Cadee, G. C. 1976. Sediment reworking by *Arenicola marina* on tidal flats in the Dutch Wadden Sea. Neth. J. Sea Res., 10, 440-460.
- Clarke, K. R. and R. N. Gorley. 2006. Primer5: User's manual/tutorial. Primer-E Ltd.
- Clarke, K. R. and R. H. Green. 1988. Statistical design and analysis for a 'biological effects' study. Mar. Ecol. Prog. Ser., 46, 213-226.
- Connell, J. H. 1978. Diversity in tropical rain forests and coral reefs. Science, 199, 1302-1310.
- Dale, N. G. 1974. Bacteria in intertidal sediments: factors related to their distribution. Limnol. Oceanogr. 19, 509-518.
- DeFlaun, M. F. and L. M. Mayer. 1983. Relationships between bacteria and grain surfaces in intertidal sediments. Limnol. Oceanogr., 28, 873-881.
- Deming, J. W. and J. A. Baross. 1993. The early diagenesis of organic matter: Bacterial activity, in Organic Chemistry, M. H. Engel and S. A. Macko, eds., Plenum, 119-144.
- Deming, J. W. and R. R. Colwell. 1982. Barophilic growth of bacteria from intestinal tracts of deep-sea invertebrates. Microb. Ecol., 7, 85-94.
- Dobbs, F. C. and J. B. Guckert. 1988. Microbial food resources of the macrofaunal-deposit feeder *Ptychodera bahamensis* (Hemichordata: Enteropneusta). Mar. Ecol. Prog. Ser., 45, 127-136.
- Dodson, S. I. 1998. Ecology, Oxford University Press, 464 pp.
- Epstein, S. S. 1997. Microbial food webs in marine sediments. I. Trophic interactions and grazing rates in two tidal flat communities. Microb. Ecol., 34, 188-198.
- Epstein, S. S. and M. P. Shiaris. 1992. The rates of microbenthos- and meiobenthos bacterivory in a temperate muddy tidal-flat community. Appl. Environ. Microbiol., 58, 2426-2431.
- Farke, H., P. A. W. de Wilde and E. M. Berghuis 1979. Distribution of juvenile and adult *Arenicola marina* on tidal mud flat and the importance of nearshore areas for recruitment. Neth. J. Sea Res., 13, 354-361.
- Findlay, R. H., M. B. Trexler, J. B. Guckert and D. C. White. 1990a. Laboratory study of disturbance in marine sediments: response of a microbial community. Mar. Ecol. Prog. Ser., 62, 121-133.
- Findlay, R. H., M. B. Trexler and D. C. White. 1990b. Response of a benthic microbial community to biotic disturbance. Mar. Ecol. Prog. Ser., 62, 135-148.
- Fromin, N., J. Hamelin, S. Tarnawski, D. Roesti, K. Jourdain-Miserez, N. Forestier, S. Teyssier-Cuvelle, F. Gillet, M. Aragno and P. Rossi. 2002. Statistical analysis of denaturing gel electrophoresis (DGE) fingerprinting patterns. Environ. Microbiol., 4, 634-643.
- Gray, J. P. and R. P. Herwig. 1996. Phylogenetic analysis of bacterial communities in marine sediments. Appl. Environ. Microbiol., 62, 4049-4059.
- Grime, J. P. 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. Am. Nat., 111, 1169-1194.
- Grossi, V., P. Cuny, S. Caradec, D. Nerini, R. Pancost and F. Gilbert. 2006. Impact of feeding by *Arenicola marina* (L.) and ageing of faecal material on fatty acid distribution and bacterial community structure in marine sediments: An experimental approach. J. Exp. Mar. Biol. Ecol., 336, 54-64.
- Grossmann, S. and W. Reichardt. 1991. Impact of *Arenicola marina* on bacteria in intertidal sediments. Mar. Ecol. Prog. Ser., 77, 85-93.
- Hylleberg, J. 1975. Selective feeding by *Abarenicola pacifica* with notes on *Abarenicola vagabunda* and a concept of gardening in lugworms. Ophelia, 14, 113-117.
- Hymel, S. and C. Plante. 1998. Improved method of bacterial enumeration in sandy and deposit-feeder gut sediments using the fluorescent stain 4,6-diamidino-2-phenylindole (DAPI). Mar. Ecol. Prog. Ser., 17, 299-304.

- Josephson, K. L., C. P. Gerba and I. I. Pepper. 1993. Polymerase chain reaction detection of nonviable bacterial pathogens. *Appl. Environ. Microbiol.*, 59, 3513-3515.
- Jurgens, K. and H. Gude. 1994. The potential importance of grazing-resistant bacteria in planktonic systems. *Mar. Ecol. Prog. Ser.*, 112, 169-188.
- Katrak, G. and F. L. Bird. 2003. Comparative effects of the large bioturbators, *Trypaea australiensis* and *Heloccius cordiformis*, on intertidal sediments of Western Port, Victoria, Australia. *Mar. FW Res.*, 54, 701-708.
- Kemp, P. F. 1987. Potential impact on bacteria of grazing by a marine deposit feeder, and the fate of bacterial production. *Mar. Ecol. Prog. Ser.*, 36, 151-161.
- _____ 1990. The fate of benthic bacterial production. *Rev. Aquat. Sci.*, 2, 109-124.
- Kopke, B., R. Wilms, B. Engelen, H. Cypionka and H. Sass. 2005. Microbial diversity in coastal subsurface sediments: a cultivation approach using various electron acceptors and substrate gradients. *Appl. Environ. Microbiol.*, 71, 7819-7830.
- Lucas, F. S., G. Bertru and M. G. Hofle. 2003. Characterization of free-living and attached bacteria in sediments colonized by *Hediste diversicolor*. *Mar. Ecol. Prog. Ser.*, 32, 165-174.
- McCaig, A. E., C. J. Phillips, J. R. Stephen, G. A. Kowalchuk, S. M. Harvey, R. A. Herbert, T. M. Embley and J. I. Prosser. 1999. Nitrogen cycling and community structure of proteobacterial beta-subgroup ammonia-oxidizing bacteria within polluted marine fish farm sediments. *Appl. Environ. Microbiol.*, 65, 213-220.
- Milliken, G. A. and D. E. Johnson. 1984. *Analysis of Messy Data, Volume I: Designed Experiments*, Van Nostrand Reinhold.
- More, M. I., J. B. Herrick, M. C. Silva, W. C. Ghiorse and E. L. Madsen. 1994. Quantitative cell lysis of indigenous microorganisms and rapid extraction of microbial DNA from sediment. *Appl. Environ. Microbiol.*, 60, 1572-1580.
- Moriarty D. J. W., P. C. Pollard, W. G. Hunt, C. M. Moriarty and T. J. Wassenberg. 1985. Productivity of bacteria and microalgae and the effect of grazing by holothurians in sediments on a coral reef flat. *Mar. Biol.*, 85, 293-300.
- Murray, A. E., J. T. Hollibaugh and C. Orrego. 1996. Phylogenetic compositions of bacterioplankton from two California estuaries compared by denaturing gradient gel electrophoresis of 16S rDNA fragments. *Appl. Environ. Microbiol.*, 62, 2676-2680.
- Nocker, A., P. Sossa-Fernandez, M. D. Burr and A. K. Camper. 2007. Use of propidium monoazide for live/dead distinction in microbial ecology. *Appl. Environ. Microbiol.*, 73, 5111-5117.
- Pinhassi, J., R. Simo, J. M. Gonzalez, M. Vila, L. Alonso-Saez, R. P. Kiene, M. A. Moran and C. Pedros-Alio. 2005. Dimethylsulfoniopropionate turnover is linked to the composition and dynamics of the bacterioplankton assemblage during a microcosm phytoplankton bloom. *Appl. Environ. Microbiol.*, 71, 7650-7660.
- Plante, C. J. and P. A. Jumars. 1992. The microbial environment of marine deposit-feeder guts characterized via microelectrodes. *Microb. Ecol.*, 23, 257-277.
- Plante, C. J., P. A. Jumars and J. A. Baross. 1989. Rapid bacterial growth in the hindgut of a marine deposit feeder. *Microb. Ecol.*, 18, 29-44.
- Plante, C. J. and L. M. Mayer. 1994. Distribution and efficiency of bacteriolysis in the gut of *Arenicola marina* and three additional deposit feeders. *Mar. Ecol. Prog. Ser.*, 109, 183-194.
- Plante, C. J. and A. Shriver. 1998a. Differential lysis of sedimentary bacteria by *Arenicola marina* L.: examination of cell wall structure and exopolymeric capsules as correlates. *J. Exp. Mar. Biol. Ecol.*, 229, 35-52.
- _____ 1998b. Patterns of differential digestion of bacteria in deposit feeders: a test of resource partitioning. *Mar. Ecol. Prog. Ser.*, 163, 253-258.
- Plante, C. J. and S. Y. Stinson. 2003. Recolonization and cues for bacterial migration into "mock" deposit-feeder fecal casts. *Aquatic Microb. Ecol.*, 33, 107-115.

- Plante, C. J. and S. Wilde. 2001. Bacterial recovery in deposit-feeder egesta: *in situ* growth or immigration? *Limnol. Oceanogr.*, *46*, 1171-1181.
- _____. 2004. Biotic disturbance, recolonization, and early succession of bacterial assemblages in intertidal sediments. *Microb. Ecol.*, *48*, 154-166.
- Portillo, M. C. and J. M. Gonzalez. 2008. Statistical differences between relative quantitative molecular fingerprints from microbial communities. *Antonie van Leeuwenhoek*, *94*, 157-163.
- Powell, S. M., M. J. Riddle, I. Snape and J. S. Stark. 2005. Location and DGGE methodology can influence interpretation of field experimental studies on the response to hydrocarbons by Antarctic benthic microbial community. *Antarct. Sci.*, *17*, 353-360.
- Rees, G. N., D. S. Baldwin, G. O. Watson, S. Perryman and D. L. Nielsen. 2004. Ordination and significance testing of microbial community composition derived from terminal restriction fragment length polymorphisms: application of multivariate statistics. *Antonie van Leeuwenhoek*, *86*, 339-347.
- Reice, S. R. 1994. Nonequilibrium determinants of biological community structure. *Am. Sci.*, *82*, 424-435.
- Retraubun, A. S. W., M. Dawson and S. M. Evans. 1996. The role of the burrow funnel in feeding processes in the lugworm *Arenicola marina* (L.). *J. Exp. Mar. Biol. Ecol.*, *202*, 107-118.
- Rhoads, D. C. and L. F. Boyer. 1982. The effects of marine benthos on physical properties of sediment: A successional perspective, *in* *Animal-Sediment Relations*, P. L. McCall and M. J. S. Tevesz, eds., Plenum, 3-52.
- Riemann, L., G. F. Steward and F. Azam. 2000. Dynamics of bacterial community composition and activity during a mesocosm diatom bloom. *Appl. Environ. Microbiol.*, *66*, 578-587.
- Roberts, D., H. M. Moore, J. Berges, J. W. Patching, M. W. Carton and D. F. Eardly. 2001. Sediment distribution, hydrolytic enzyme profiles and bacterial activities in the guts of *Oneirophanta mutabilis*, *Psychropotes longicauda* and *Pseudostichopus villosus*: what do they tell us about digestive strategies of abyssal holothurians? *Prog. Oceanogr.*, *50*, 443-458.
- Rodriguez, G. G., D. Phipps, K. Ishiguro and J. F. Ridgway. 1992. Use of a fluorescent redox probe for direct visualization of actively respiring bacteria. *Appl. Environ. Microbiol.*, *58*, 1801-1808.
- Rooney-Varga, J. N., R. Devereux, R. S. Evans and M. E. Hines. 1997. Seasonal changes in the relative abundance of uncultivated sulfate-reducing bacteria in a salt marsh sediment and in the rhizosphere of *Spartina alterniflora*. *Appl. Environ. Microbiol.*, *63*, 3895-3901.
- Scala, D. J. and L. J. Kerkhof. 2000. Horizontal heterogeneity of denitrifying bacterial communities in marine sediments by terminal restriction fragment length polymorphism analysis. *Appl. Environ. Microbiol.*, *66*, 1980-1986.
- Schmidt, J. L., J. W. Deming, P. A. Jumars and R. G. Keil. 1998. Constancy of bacterial abundance in surficial marine sediments. *Limnol. Oceanogr.*, *43*, 976-982.
- Snelgrove, P. V. R. and C. A. Butman. 1994. Animal-sediment relationships revisited: cause versus effect. *Oceanogr. Mar. Biol. Ann. Rev.* *32*, 111-177
- Suzuki, M. T. and S. J. Giovannoni. 1996. Bias caused by template annealing in the amplification of mixtures of 16S rRNA genes by PCR. *Appl. Environ. Microbiol.*, *62*, 625-630.
- Thorsen, M. S. 1999. Abundance and biomass of the gut-living microorganisms (bacteria, protozoa and fungi) in the irregular sea urchin *Echinocardium cordatum* (Spatangoida: Echinodermata). *Mar. Biol.*, *133*, 423-433.
- Turner, M. G. 1998. Landscape ecology, *in* *Ecology*, S. I. Dodson, ed., Oxford University Press, 77-122.
- van Duyl, F. C., B. de Winder, A. J. Kop and U. Wollenzien. 1999. Tidal coupling between carbohydrate concentrations and bacterial activities in diatom-inhabited intertidal mudflats. *Mar. Ecol. Prog. Ser.*, *191*, 301-309.

- van Hannen, E. J., M. Veninga, J. Bloem, H. J. Gons and H. J. Laanbroek. 1999. Genetic changes in the bacterial community structure associated with protistan grazers. *Arch. Hydrobiol.*, 145, 25-38.
- van Oevelen, D., L. Moodley, K. Soetaert and J. J. Middelburg. 2006. The trophic significance of bacterial carbon in a marine intertidal sediment: Results of an in situ isotope labeling study. *Limnol. Oceanogr.*, 51, 2349-2359.
- White, P. S. and S. T. A. Pickett. 1985. Natural disturbance and patch dynamics: An introduction, in *The Ecology of Natural Disturbances and Patch Dynamics*, S. T. A. Pickett and P. S. White, eds., Academic, 3-13.
- Yager, P. L., A. R. M. Nowell and P. A. Jumars. 1993. Enhanced deposition to pits: A local food source for benthos. *J. Mar. Res.*, 51, 209-236.
- Yoon, J.-H., I.-G. Kim, K. H. Kang, T.-K. Oh and Y.-H. Park. 2004. *Bacillus hwajinpoensis* sp. nov. and an unnamed *Bacillus* genomospecies, novel members of *Bacillus* rRNA group 6 isolated from sea water of the East Sea and the Yellow Sea in Korea. *Int. J. Syst. Evol. Microbiol.*, 54, 803-808.

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