

## ORIGINAL ARTICLE

# Microbial composition affects the functioning of estuarine sediments

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Although microorganisms largely drive many ecosystem processes, the relationship between microbial composition and their functioning remains unclear. To tease apart the effects of composition and the environment directly, microbial composition must be manipulated and maintained, ideally in a natural ecosystem. In this study, we aimed to test whether variability in microbial composition affects functional processes in a field setting, by reciprocally transplanting riverbed sediments between low- and high-salinity locations along the Nonesuch River (Maine, USA). We placed the sediments into microbial ‘cages’ to prevent the migration of microorganisms, while allowing the sediments to experience the abiotic conditions of the surroundings. We performed two experiments, short- (1 week) and long-term (7 weeks) reciprocal transplants, after which we assayed a variety of functional processes in the cages. In both experiments, we examined the composition of bacteria generally (targeting the 16S rDNA gene) and sulfate-reducing bacteria (SRB) specifically (targeting the *dsrAB* gene) using terminal restriction fragment length polymorphism (T-RFLP). In the short-term experiment, sediment processes (CO<sub>2</sub> production, CH<sub>4</sub> flux, nitrification and enzyme activities) depended on both the sediment’s origin (reflecting differences in microbial composition between salt and freshwater sediments) and the surrounding environment. In the long-term experiment, general bacterial composition (but not SRB composition) shifted in response to their new environment, and this composition was significantly correlated with sediment functioning. Further, sediment origin had a diminished effect, relative to the short-term experiment, on sediment processes. Overall, this study provides direct evidence that microbial composition directly affects functional processes in these sediments.

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## Introduction

Microbial composition—the identity and relative abundance of microbial taxa—varies over space and time (Fuhrman *et al.*, 2006; Martiny *et al.*, 2006; Gilbert *et al.*, 2012; Hanson *et al.*, 2012) and in response to abiotic perturbations (Allison and Martiny, 2008). However, the question remains where and when such variation affects the functioning of ecosystems (Schimel, 1995; Balser and Firestone, 2005; Strickland *et al.*, 2009). To test the effect of microbial composition on process rates, composition must be manipulated and maintained, ideally in a natural ecosystem. In this way, one can directly tease apart the effects of composition, the environment and their interactions (Reed and Martiny, 2007). In larger organisms, composition can often be controlled by seeding or caging (for

example, Power, 1990; Tilman *et al.*, 1996), but such precise manipulations of microorganisms in a field environment are usually untenable.

Instead, microbial ecologists have three main options for explicitly testing the effect of microbial composition on ecosystem functioning, each with their own benefits and limitations. The first strategy is to use laboratory microcosms, then measure one or more aggregate processes of the assemblage. For instance, Bell *et al.* (2005) constructed communities from cultivatable bacteria in sterile microcosms and found that bacterial richness and composition affected respiration rates. Similarly, direct manipulation of mycorrhizal fungal diversity altered plant biomass and soil nutrients in a greenhouse experiment (van der Heijden *et al.*, 1998). To increase the community complexity, other studies have also inoculated microcosms with diverse, intact microbial communities (for example, Langenheder *et al.*, 2005). For instance, Strickland *et al.* (2009) found that respiration varied greatly among leaf litter microcosms inoculated with three different soil communities. In contrast, Langenheder *et al.* (2006) and Dimitriu *et al.* (2010) found weak or no effects of the microbial inoculum on functioning

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in aquatic and peat microcosms. Still other studies have diluted a single intact community to alter taxon richness and composition to test for effects of composition on microcosm functioning. In general, such dilution experiments have found weak or no effects of the inoculum on functioning in a variety of systems (Griffiths *et al.*, 2001; Franklin and Mills, 2006; Wertz *et al.*, 2006; Dimitriu *et al.*, 2010). Of course, a downside to all microcosm experiments is that they are carried out in the laboratory or greenhouse, which may or may not be a good proxy of the natural environment.

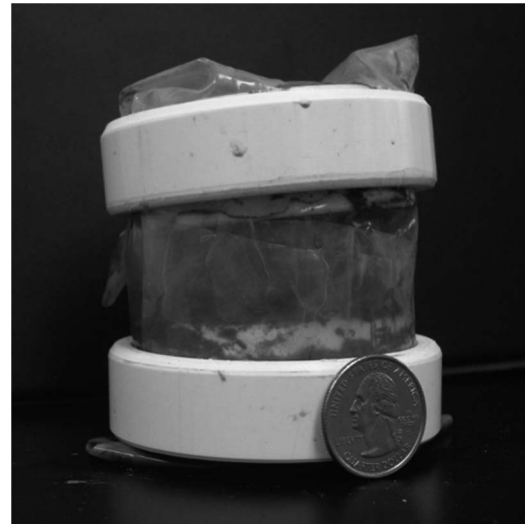
A second approach is to remove or inhibit a target group of microorganisms using chemicals in the field. For instance, studies using a fungicide (benomyl) to suppress arbuscular mycorrhizal fungi demonstrate that fungi can have large effects on plant communities (Hartnett and Wilson, 1999; Daleo *et al.*, 2007). Similarly, nitrapyrin applications that inhibit ammonia-oxidizers suggest that these microorganisms influence not only soil nitrogen cycling, but also plant biomass and litter decomposition (Austin *et al.*, 2007). Although these studies can be carried out in a natural environment, a caveat to these approaches is that they may have unintended consequences for the abiotic environment and other organisms. Indeed, what is toxic to some microorganisms, may stimulate the growth of another (for example, Dantas *et al.*, 2008).

Finally, the influence of microbial composition can be tested by using *in situ* transplants of whole microbial communities (for example, Bottomley *et al.*, 2004; Waldrop and Firestone, 2006). The upside of this approach is that it considers a complex community in a natural setting. For instance, Balser and Firestone (2005) reciprocally transplanted soil cores between a forest and grassland site. Changes in microbial composition were associated with changes in soil functioning and in particular, nitrogen cycling. However, two limitations with such experiments are that the soil matrix differs along with the microbial community and that the transplanted cores may not fully equilibrate with the surrounding environment. Further, such studies do not usually prevent migration of microorganisms after transplantation (but see Gasol *et al.*, 2002), such that the effect of composition on functioning becomes more ambiguous over time; it is then unclear whether changes in microbial composition over time are because of microbial migration or growth within the soil cores.

To build on these previous studies, we aimed to demonstrate an approach that can explicitly test whether variability in microbial composition affects functional processes in a field setting, while controlling for both the abiotic environment and microbial migration. To do this, we reciprocally transplanted riverbed sediments between two different abiotic environments: an oligohaline (salinity ~2) and an euhaline (15–30) location. (For simplicity, we refer to these locations as the

‘freshwater’ and ‘saltwater’ sites.) We placed the transplanted sediments into microbial ‘cages’ (Gasol *et al.*, 2002; Reed and Martiny, 2007) to prevent the movement of microorganisms, while allowing the sediments to experience the abiotic conditions (such as temperature, salinity and nutrients) of the surroundings. We focused on estuarine sediments for three reasons. First, the aquatic environment allows the use of cages made of dialysis tubing (Figure 1). Second, salinity is known to be a key abiotic factor determining microbial composition (Lozupone and Knight, 2005), particularly along estuarine gradients (for example, Freitag *et al.*, 2006; Mohamed and Martiny, 2010); therefore, we predicted that changes in salinity would quickly select for distinct microbial communities. Third, by sampling two locations from the same river, differences in the substrate matrix between locations could be minimized.

We report the results from two experiments at these locations. In the first experiment—hereafter, the ‘short-term’ experiment—we reciprocally transplanted sediment cores in cages from the freshwater and saltwater environments. After 1 week, we removed the cages from the riverbed and immediately assayed a variety of functional processes. This time frame was chosen to allow time for the sediments to equilibrate with the environment, but not enough time for a discernable change in microbial composition because of altered growth rates. In the second, ‘long-term’ experiment, we allowed the sediments to remain in the riverbed for 7 weeks before measuring the functional parameters. In both experiments, we examined the composition of bacteria generally (targeting the 16S rDNA gene) and sulfate-reducing bacteria (SRB) specifically (targeting the *dsrAB* gene) using terminal restriction fragment length polymorphism (T-RFLP). SRB are ubiquitous in freshwater and marine sediments, and



**Figure 1** Dialysis tubing sediment cages with PVC collars. United States quarter dollar (24.26-mm diameter) shown for scale.

their composition appears to vary along estuarine gradients (for example, Hawkins and Purdy, 2007).

If microbial composition directly affects the functional processes of these sediments, we would expect to find support for three hypotheses: (1) In the short-term experiment, sediment processes would depend on both the sediment's origin (reflecting differences in microbial composition between salt and freshwater sediments), as well as the surrounding environment. (2) In the long-term experiment, microbial composition in the transplanted sediments would shift in response to their new environment. (3) As a result of this compositional shift, the sediment's origin would have a diminished effect (relative to the short-term experiment), and the surrounding environment a greater effect, on sediment processes.

## Materials and Methods

The experimental sites were located 2.5 km apart along the riverbed of the Nonesuch River in Maine, USA. The saltwater, euhaline (salinity >25) site bordered the Scarborough Marsh. Plant composition in this marsh is typical of northern New England salt marshes and dominated by *Spartina patens* and *S. alterniflora* (Ewanchuck and Bertness, 2004). The 'freshwater,' oligohaline (salinity <5) site upstream bordered a diverse marsh, similar to other oligohaline marshes in the area (Crain, 2007).

### *Microbial cages and transplant experiments*

To limit the migration of microorganisms while allowing for solutes to permeate, we constructed cages around the 6-cm diameter by 6-cm deep sediment cores out of dialysis tubing. We used large diameter tubing (6.4 cm) with molecular weight cutoff of 8 kD (Spectrum Laboratories, Inc, CA, USA), which should exclude the movement of microbial eukaryotes, bacteria, archaea and most viruses.

To test the potential for bacterial migration, we performed an initial experiment in the lab. Dialysis 'cages' were filled with sterile saline solution (0.87% NaCl) and placed in 1-l beakers filled with saline and inoculated with *Xanthomonas* sp. (average  $2.7 \times 10^7$  CFU ml<sup>-1</sup>). After 5 days, only 247 CFU ml<sup>-1</sup> ( $\pm 87$ ) were detected inside the cages, while an average of  $1.1 \times 10^8$  CFU ml<sup>-1</sup> ( $\pm 2.3 \times 10^6$ ) were detected in external media. Thus, only about 1 per million cells penetrated the cages over 5 days.

For the field experiments, dialysis tubing pieces (32-cm long) were rinsed thoroughly at least 48 hours before use, incubated in sterile water at 4 °C for 24 h and then rinsed again in sterile DI water before use in the field. After sliding the sediment core into the dialysis tubing, each end was folded over a polyvinyl chloride (PVC) cylinder placed just inside the tubing, fitting an o-ring over the folded

end onto the internal cylinder, and slipping an outer PVC cylinder over the o-ring.

For both experiments, 32 sediment cores were taken from exposed riverbed sediment at low tide and placed into microbe cages. The cages were placed in river water from their native site until all cages were transplanted back into the riverbed of the appropriate site. The sediments were placed in the holes made by the original cores in their original vertical orientation. For the short-term experiment, the cores were incubated for 7 days during mid-June.

Within 2 weeks of completing the short-term experiment (the beginning of July), we initiated the long-term experiment. Before placing the cores into the cages, a subset of sediment was sampled and frozen for initial sediment analyses and DNA extraction. These cores were incubated for a total of 61 days. To prevent degradation of the dialysis tubing during this longer incubation period, the tubing was replaced (while preventing contamination of the sediments) every 7–10 days. During this experiment, the integrity of many cages at the freshwater marsh site was compromised, possibly because of runoff from nearby development. This treatment was therefore eliminated from the analyses.

### *Gas sampling*

At the end of both experiments, we removed the cores from the riverbed and opened the dialysis bag to reveal the top of the sediment core. The entire cage was placed into a 6-cm long 5-cm diameter schedule 40 PVC, which acted as collars for vented static chambers. The enclosed cores were placed in two gallon buckets containing ~500 ml of river water. PVC caps fitted with rubber septa were placed over the enclosed cages. Total headspace volume was 150 ml.

At the same time in both locations, headspace gas was sampled every 20 min for a period of 60 min. Gas samples were stored in 2-ml gas sampling vials. While in the field, a set of control vials were filled with 100 ppm of CH<sub>4</sub> or 1000 ppm of CO<sub>2</sub> to test for leakages, but none were detected. The gas samples were analyzed by gas chromatography (Shimadzu GC2014 equipped with a thermal conductivity detector to measure CO<sub>2</sub> and a flame ionization detector to measure CH<sub>4</sub>, and fitted with a 1-ml sample loop; Shimadzu Corporation, Kyoto, Japan). We calculated the gas production rates as the slope of the linear regression of gas concentration over time.

### *Sediment biogeochemistry*

Salinity, sediment moisture, inorganic N and potential gross nitrification were analyzed for each replicate after both the short- and long-term experiments. Total C and N and sediment texture were measured during the short-term experiment of the

initial samples. After the short-term experiment, pH and  $\text{SO}_4$  concentrations were also measured.

Salinity was measured by refractometer on pore water obtained after centrifuging 1.5-ml sample of sediment taken from the center of the core. Sediment moisture was measured by determining weight loss after drying 10 g of soil for 24 h (or until constant weight) at 105 °C. pH was measured using a 1:1 DI water sediment mixture and pH meter. Sediment texture was measured on four random samples of native freshwater sediments and salt-water sediments taken at the beginning of the long-term experiment (Elliott *et al.*, 1999).

Inorganic N was measured by extracting 10 g of wet sediment with 75 ml of 2 M KCl. Concentrations of  $\text{NH}_4$  and  $\text{NO}_3$  were measured using a microplate technique (Weatherburn, 1967; Doane and Horwath, 2003). Extractable  $\text{SO}_4$  was extracted from 10 g of wet sediment using 40 ml of 0.016 M  $\text{KH}_2\text{PO}_4$ , and analyzed with a Hach pocket colorimeter (Hach, Loveland, CA, USA). Half the samples were also analyzed by IC (Maine Soil Testing Laboratory, University of Maine) with similar results. Total C and N of sediments were measured via Carla Erba combustion (ANR Analytical Labs, University of California, Davis).

Potential gross nitrification was estimated by the pool dilution technique (Hart *et al.*, 1994). For each core, two equivalents of ~15 g of dry sediment were placed into 50-ml specimen cups. To remove any  $\text{NH}_4$  + limitation, each sample was amended with 3 ml of 100 mM  $\text{NH}_4\text{Cl}$  (~30 mM  $\text{NH}_4^+$  final concentration). Samples then received 5 ml of 0.1 mM  $\text{K}^{15}\text{NO}_3$  solution (50 µg of  $\text{K}^{15}\text{NO}_3$ ), which was slowly injected into the sediment. The initial samples were immediately extracted with 75 ml of 2 M KCl and capped tightly, while the final samples were capped and incubated at 25 °C for 24 h before being extracted with 2 M KCl.

#### Enzyme activities

A 10 g sub-sample of sediment (stored at -20 °C until analyzed) was used for enzyme activity analyses. Activities of three ecto-enzymes, leucine aminopeptidase, phosphatase and sulfatase were measured in both acidic (pH 5) and basic (pH 8) buffers. Leucine aminopeptidase, phosphatase and aryl sulfatase activities were measured with methylumbelliferyl-linked substrates (Saiya-Cork *et al.*, 2002).

#### Microbial community analysis

For community analysis by T-RFLP, sediment cores 1 cm in diameter and 1-cm deep were taken from the top of each sediment cage in the field, placed on ice for transport and stored at -80 °C until analyzed. DNA was extracted from 0.5 g of sediment. To minimize shearing and PCR inhibition, we used a protocol combining the PowerSoil DNA Isolation kit (MO BIO Laboratories, Carlsbad, CA,

USA) and the FastDNA Spin kit for soil (Qbiogene Inc., Montreal, CA, USA). Briefly, samples were placed in MO BIO Power Clean bead tubes and homogenized via the MPBio FastPrep for 15 s at setting 4. Homogenized samples were transferred to FastDNA Spin Kit tubes, and the manufacturer's instructions were followed. Genomic DNA was screened for shearing and quantified. For all samples, four 25-µl PCR reactions were combined. The reaction mixtures contained 2 µl of genomic DNA (~20 ng of DNA), 23-µl Pre-Mix D (Epicentre Biotechnologies, Madison, WI, USA), 2.5 U of Platinum Taq polymerase (Invitrogen Corporation, Carlsbad, CA, USA), and 12.5 pmoles of each primer. The 5' end of the forward primers of each primer pair was labeled with 6-FAM. The *dsrAB* fragment (1.9 kb) was amplified using the forward primer *dsr1fF* and reverse primer *dsr4R* (Wagner *et al.*, 1998). We used the following conditions for PCR amplification of *dsrAB*: initial denaturing step at 94 °C for 6 min, 32 cycles of 94 °C at 90 s, 58 °C annealing for 45 s, 72 °C extension for 90 s and final extension at 72 °C for 10 min. The 16S rRNA gene fragment was amplified using the forward primer 8F (Lane, 1991) and reverse primer 1492R (positions 1492–1509) (GYTACCTTGTTACGACTT) (modified from Lane, 1991). The PCR conditions were a modified touchdown protocol: initial denaturing step at 94 °C for 3 min, 23 cycles of 94 °C for 45 s, 58 °C for 20 s, 72 °C for 90 s, 10 additional cycles with the annealing temperature decreasing by 0.2 °C each cycle and 5 final cycles with the annealing temperature at 56 °C. The *dsrAB* PCR products were purified using the QIAquick PCR purification kit (Qiagen Inc., Valencia, CA, USA) and 16S PCR products were gel extracted using the Gel DNA Recovery kit (Zymo Research Corp., Orange, CA, USA). PCR products were quantified using a NanoDrop spectrophotometer (NanoDrop Products, Wilmington, DE, USA).

For T-RFLP analysis, 200 ng of products were digested with 20 U of enzyme for 4 h, according to the manufacturer's protocol. The *dsrAB* products were digested with *NdeII* (Promega Corporation, Madison, WI, USA) and 16S rDNA products were digested with *MspI* (New England BioLabs, Ipswich, MA, USA). After digestion, samples were desalinated using a QIAquick Nucleotide Removal kit (Qiagen). Digested products were submitted to Laragen, Inc. (Los Angeles, CA, USA) for electrophoresis. Peak areas were quantified using the GeneMarker software (SoftGenetics, LLC, State College, PA, USA), and peak areas were standardized and 'true peaks' identified using the methods of Abdo and coworkers (Abdo *et al.*, 2006).

To confirm the repeatability of the fragment patterns, 7% of the samples were processed in duplicate with independent PCR reactions and enzyme digestion. The T-RFLP patterns were highly correlated between replicates; the average  $R^2$  between band lengths of replicated samples was  $0.96 \pm 0.06$  s.d.

### Statistical analyses

For the short-term experiment, we used a two-way ANOVA (JMP software, v. 5.1, SAS, Cary, NC, USA) to test for the effects of sediment origin (a proxy for microbial composition), the incubating environment and their interaction on sediment characteristics and functional processes. For the long-term experiment where we lost one of the treatments, we used a one-way ANOVA to test for differences between the treatments and a Tukey's multiple means comparison for *post-hoc* comparison of means. We used *t*-tests to test whether sediment characteristics differed by origin in the saltwater environment after the 2-month incubation. To correct for non-normality and heteroscedacity, in the short-term experiment  $\text{NH}_4^+$  concentrations was log transformed, methane production rates were rank transformed and gross nitrification data was arcsin-square root transformed; in addition, appropriate outliers were removed (<5%). In the long-term experiment, basic leucine aminopeptidase activity was also log transformed, and one outlier was removed.

To test whether community composition differed among cores types, we calculated Bray–Curtis similarities between the T-RFLP samples and tested for differences using an analysis of similarity (ANOSIM) (Primer 6 software, PRIMER-E Ltd, Ivybridge, UK) (Clarke and Warwick, 2001). To examine overall functional properties, we calculated a standardized, Euclidean distance of all nine functional parameters between each pairwise combination of the sediment cores. We then used ANOSIM to test whether sediment origin or environment affected this overall functional metric. Finally, to compare sediment composition and functioning directly, we used RELATE (Primer 6) to test for a correlation between compositional and functional similarity among sediment cages.

## Results

A main goal of our experimental design was to minimize differences in the sediment matrix contained within the cages, while maximizing differences in the abiotic environment that could permeate the cages. Sediment texture was not significantly different between the sites; average percent sand, clay and silt across all samples were 28.4%, 13.4% and 58.1%, respectively. The carbon to nitrogen ratio (C:N) was also similar in the sediments at both locations. Total C and N differed slightly, however, with the freshwater sediments containing a higher percentage of both C and N than the saltwater sediments (Table 1).

### Short-term experiment

**Abiotic conditions.** After 1 week, abiotic conditions within the transplanted sediments changed

**Table 1** Characteristics of the sediment cores from freshwater and saltwater environments. Freshwater and saltwater values are means with 1 s.e. in parentheses

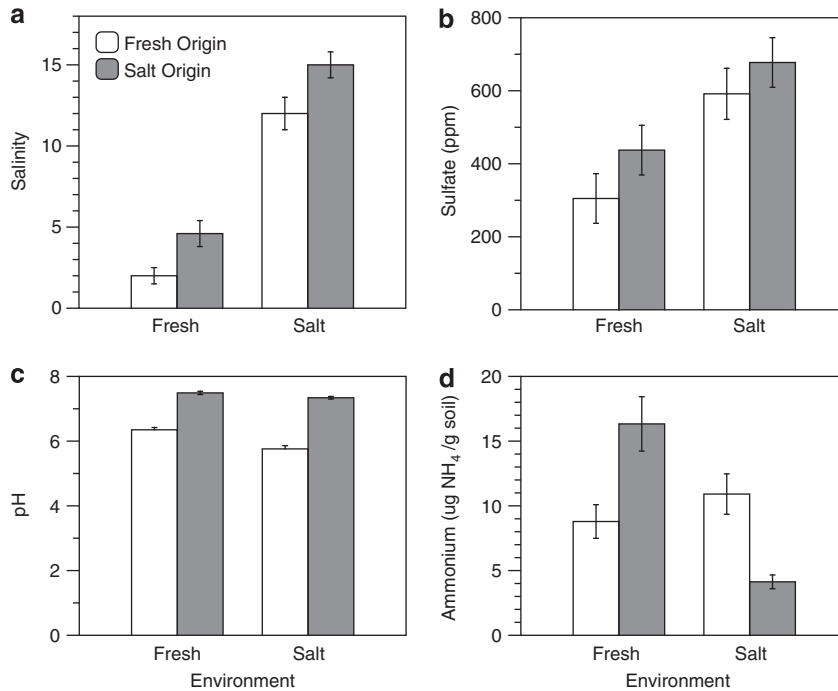
	Freshwater	Saltwater	t (d.f.)	P
% Sand	27.7 (2.0)	29.1 (1.8)	−0.047 (8)	0.65
% Silt	60.2 (3.2)	56.3 (3.0)	0.86 (10)	0.40
% Clay	12.1 (1.9)	14.6 (1.8)	−1.01 (10)	0.33
C:N	13.2 (0.4)	13 (0.3)	0.45 (527)	0.65
% C	4.3 (0.1)	2.3 (0.1)	12.00 (27)	<0.0001
% N	0.4 (0.04)	0.2 (0.009)	5.6 (16)	<0.0001

A *t*-test assuming unequal variances was used to test for differences between the two locations.

dramatically. Salinity in the saltwater sediments that were transplanted to the freshwater environment dropped to from 15 to 4.6, but remained significantly >2, the salinity of the native freshwater sediments (Figure 2a; Table 2). Sulfate concentrations equilibrated even more quickly, as they depended only on the surrounding environment and not on the sediments' origin (Figure 2b; Table 2).

In contrast to salinity and sulfate, pH was relatively static over 1 week and was primarily determined by the origin of the sediment. However, pH decreased slightly, but significantly, when freshwater sediments were transplanted to saltwater (Tukey *post-hoc* test:  $P < 0.05$ ), the opposite direction to that of the native saltwater sediments (Figure 2c; Table 2). Ammonium concentrations did not differ by sediment origin directly, but were affected by a significant origin by environment interaction (Figure 2d; Table 2). These results might indicate that the abiotic environment in the cages had not yet come to equilibrium with the new environment. Alternatively, the interactive effect of origin and environment on ammonium and the unexpected shift in pH in the saltwater environment could mean that abiotic conditions are also influenced by the outcome of sediment functioning, including the influence of microbial composition on these functions.

**Bacterial composition.** As expected, bacterial composition differed greatly between the saltwater and freshwater sediment cages and changed little after 1 week in the opposite environment. General bacterial composition, as determined by 16S rDNA T-RFLP profiling, differed greatly by sediment origin (two-way ANOSIM:  $R = 0.995$ ,  $P = 0.001$ ). In contrast, the surrounding environment (hereafter, environment) had a much weaker influence on bacterial composition. Bacterial composition in sediments from the same origin differed only slightly by environment ( $R = 0.052$ ,  $P = 0.044$ ; Figure 3a). The composition of SRB, as determined by T-RFLP of the *dsrAB* gene, showed a similar pattern as general bacterial composition. Composition differed significantly by the sediment origin (ANOSIM;  $R = 0.518$  and

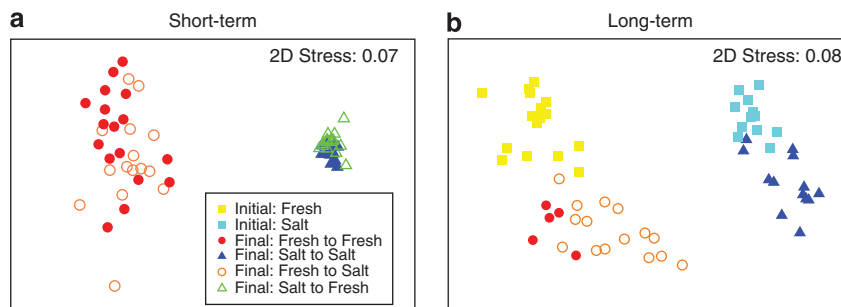


**Figure 2** Means (± 1 s.e.) of (a) salinity, (b) sulfate, (c) pH and (d) ammonium at the end of the short-term experiment.

**Table 2** Effects of sediment origin and environment on sediment characteristic at the end of the short-term experiment

	Origin	Environment	Origin x environment
Salinity	$F_{1,60} = 191, P < 0.0001$	$F_{1,60} = 2278, P < 0.0001$	$F_{1,60} = 1.44, P = 0.2$
Sulfate	$F_{1,59} = 2.5, P = 0.1$	$F_{1,59} = 15, P < 0.0003$	$F_{1,59} = 0.12, P = 0.7$
pH	$F_{1,60} = 428, P < 0.0001$	$F_{1,60} = 31, P < 0.0001$	$F_{1,60} = 11.0, P = 0.001$
Ammonium	$F_{1,56} = 0.20, P = 0.7$	$F_{1,56} = 11, P < 0.002$	$F_{1,56f} = P < 0.0001$

*F* statistics, degrees of freedom, and significance values are reported for the two-way ANOVA tests.



**Figure 3** NMDS (nonmetric multidimensional scaling) plots representing the Bray–Curtis similarity of general bacterial communities (on the basis of 16S rDNA T-RFLP profiles) in the sediments (a) at the end of the short-term experiment and (b) at the beginning (initial) and end (final) of the long-term experiment. The x-axis in both panels largely differentiates the communities by sediment origin. The y-axis in panel (b) differentiates the communities by the time of sampling (initial versus final samples).

$P = 0.001$ ), but not by the environment ( $R = -0.031$  and  $P = 0.85$ ; Supplementary Figure S1a).

**Functional assays.** To test for the effect of these compositional differences on sediment functioning, we measured nine functional parameters on the sediments in each cage after the week-long experiment. A variety of the parameters depended on the origin of the sediment, even when controlling for the

environment. Sediment origin had a significant main effect on nitrification rates and four of the six enzyme activities (Table 3; Figure 4c; Figure 5a-d). Further, sediment origin and environment interacted to affect methane production. The saltwater sediment produced significantly less methane in the saltwater than freshwater environment (Figure 4b), as expected from prior studies of methanogenesis in saltmarsh sediments (for example, Senior *et al.*,

**Table 3** Effect of sediment origin and environment on sediment processes for the short- and long-term experiments

	Short-term			Long-term	
	Origin	Environment	Origin x Environment	Origin	Environment
CO <sub>2</sub> production	$F_{1,60} = 2$	$F_{1,60} = 17^{***}$	$F_{1,60} = 0.9$	$F_{1,32} = 0.07$	$F_{1,32} = 2$
CH <sub>4</sub> flux	$F_{1,58} = 2$	$F_{1,58} = 0.1$	$F_{1,58} = 4^*$	$F_{1,31} = 2$	$F_{1,31} = 28^{***}$
Nitrification	$F_{1,48} = 81^{***}$	$F_{1,48} = 9^{**}$	$F_{1,48} = 0.09$	$F_{1,29} = 4$	$F_{1,29} = 6^*$
<i>Acidic</i>					
Aminopeptidase	$F_{1,59} = 0.7$	$F_{1,59} = 2$	$F_{1,59} = 2$	$F_{1,32} = 0.7$	$F_{1,32} = 0.002$
Phosphatase	$F_{1,60} = 36^{***}$	$F_{1,60} = 2$	$F_{1,60} = 3$	$F_{1,30} = 25^{***}$	$F_{1,30} = 0.03$
Sulfatase	$F_{1,60} = 35^{***}$	$F_{1,60} = 8^{**}$	$F_{1,60} = 2$	$F_{1,32} = 2$	$F_{1,32} = 14^{**}$
<i>Alkaline</i>					
Aminopeptidase	$F_{1,54} = 36^{***}$	$F_{1,54} = 6^{**}$	$F_{1,54} = 0.1$	$F_{1,30} = 18^{**}$	$F_{1,30} = 4^*$
Phosphatase	$F_{1,60} = 11^{**}$	$F_{1,60} = 0.2$	$F_{1,60} = 1$	$F_{1,32} = 1$	$F_{1,32} = 6^*$
Sulfatase	$F_{1,56} = 0.02$	$F_{1,56} = 0.3$	$F_{1,56} = 0.5$	$F_{1,32} = 0.02$	$F_{1,32} = 1.5$

The  $F$  statistic and degrees of freedom are reported for the two-way ANOVA tests. Statistical significance is indicated by asterisks: \* $P \leq 0.05$ , \*\* $P \leq 0.005$ , and \*\*\* $P \leq 0.0001$ .

1982). Overall, sediment origin significantly affected six of the nine functional parameters, either directly or through an interactive effect with the environment. Given that bacterial composition differed greatly between the freshwater and saltwater sediments, these results are consistent with the hypothesis that sediment functioning is determined in large part by bacterial composition (Hypothesis 1).

Not surprisingly, the environment also had a strong, direct influence on sediment functioning (Table 3). In particular, carbon dioxide (CO<sub>2</sub>) production was higher in saltwater than in freshwater (Figure 4a) and was not affected by sediment origin, either directly or through an interactive effect with the environment. The environment also directly affected nitrification rates and two of the enzyme activities (acidic sulfatase and alkaline aminopeptidase; Figure 4b and 4c).

To examine overall sediment functioning, we also calculated a standardized, Euclidean distance of all nine functional parameters between each pairwise combination of the sediment cores. This analysis upholds the results of the individual functional parameters that both origin and environment affect on sediment functioning (two-way ANOSIM; Origin:  $R = 0.198$ ,  $P = 0.001$ ; Env:  $R = 0.224$ ,  $P = 0.001$ ) (Supplementary Figure S2a).

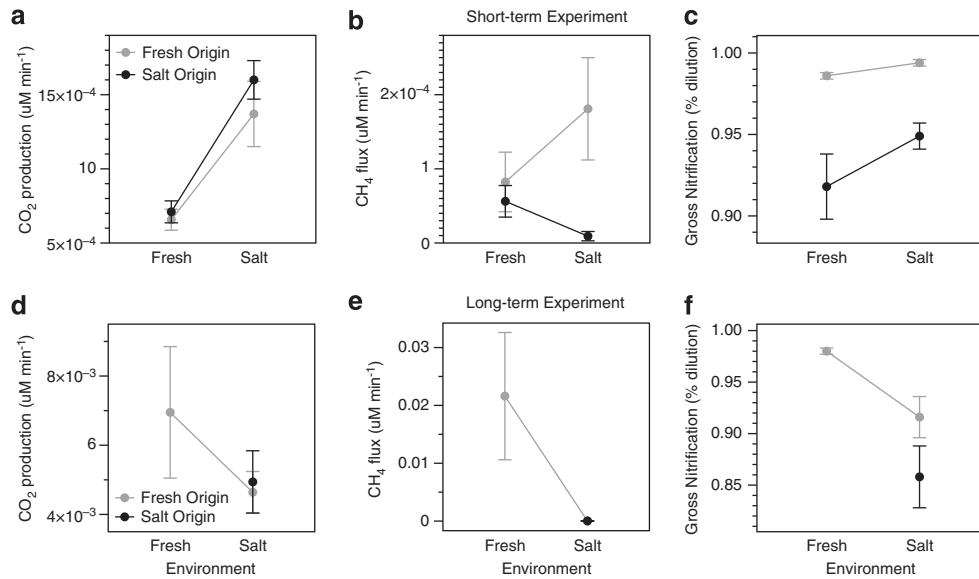
#### Long-term experiment

In the second experiment, we assayed initial bacterial composition (just as the sediments were placed into the cages) and bacterial composition and sediment functioning after 2 months (61 days). Because of an unknown disturbance at the freshwater site, most of the saltwater sediment cores transplanted to this location were destroyed. However, we were still able to test our hypotheses with the sediment cores transplanted from the freshwater to the saltwater environment.

*Abiotic conditions.* After 2 months, salinity of the sediment cores equilibrated with the surrounding environment and did not depend on the core origin. Salinity of the sediments was 27 in saltwater environment, regardless of core origin ( $t_{df=24} = -0.56$ ;  $P = 0.58$ ; Supplementary Figure S3a). In contrast, salinity in the cores from the freshwater sites was only 2.7. As in the short-term experiment, ammonium concentrations did not respond to the surrounding environment. Ammonium differed between the sediments of different origins incubating in the same saltwater site ( $t_{df=22} = -3.5$ ,  $P = 0.002$ ) and was similar in the freshwater sediments regardless of the incubating site ( $t_{df=6} = 0.37$ ,  $P = 0.72$ ; Supplementary Figure S3b).

*Bacterial composition.* Bacterial composition changed over the course of the 2-month experiment. Composition in the sediment cores incubated in their native environment differed significantly from their initial composition (one-way ANOSIM:  $R = 0.233$ ;  $P = 0.001$ ; Figure 3b). These differences in initial and final bacterial composition may reflect an effect of the cages on sediment composition over time, temporal changes in the surrounding environment or a combination of both.

Incubating freshwater sediment cores in a saltwater environment also shifted bacterial composition significantly after 2 months, consistent with Hypothesis 2. At the end of the experiment, the environment had a much stronger affect on bacterial composition than it did in the short-term experiment ( $R = 0.474$ ,  $P = 0.001$  versus  $R = 0.052$ ,  $P = 0.044$ ), although sediment origin continued to have a significant effect on bacterial composition (Origin:  $R = 0.988$ ,  $P = 0.001$ ). Furthermore, bacterial composition in the transplanted freshwater sediments changed in the manner expected; they became more similar to the composition of the native saltwater cores. Although sediment origin continues to have a strong effect on bacterial



**Figure 4** Means ( $\pm 1$  s.e.) of net respiration, net methane production, and gross nitrification by sediment origin and incubating environment for the (a–c) short- and (d–f) long-term experiments. The gray lines connect the sediments originating from the freshwater environment, and the black lines connect the sediments originating from the saltwater environment.

composition, the transplanted freshwater sediments (open orange circles) are more similar to the native saltwater communities (blue triangles) than are the native freshwater sediments (solid red circles) (Figure 3b).

In contrast, we did not detect a significant change in the composition of the SRB. SRB composition differed by sediment origin, but not by the incubating environment (Origin:  $R = 0.788$ ,  $P = 0.001$ ; Env.:  $R = 0.068$ ,  $P = 0.339$ ; Supplementary Figure S1b). Thus, the freshwater sulfate reducing community appears resistant to change even after 2 months of incubation in the saltwater environment.

**Functional assays.** In support of our final hypothesis (Hypothesis 3), sediment origin had less of an influence on the functional assays in the long-term experiment than in the short-term experiment. Sediment origin directly affected only two of the nine functional assays (acidic phosphatase and alkaline aminopeptidase), compared with five in the short-term experiment (Table 3; Figures 4 and 5). Also as hypothesized, the surrounding environment had less of an influence in the long-term experiment; environment had a direct effect on five of the nine assays, compared with two in the short-term experiment (Table 3; Figures 4 and 5). Because of the loss of the sediment cores transplanted from the saltwater to freshwater environment, we were not able to test for the interactive effects of sediment origin and the environment on the functional assays.

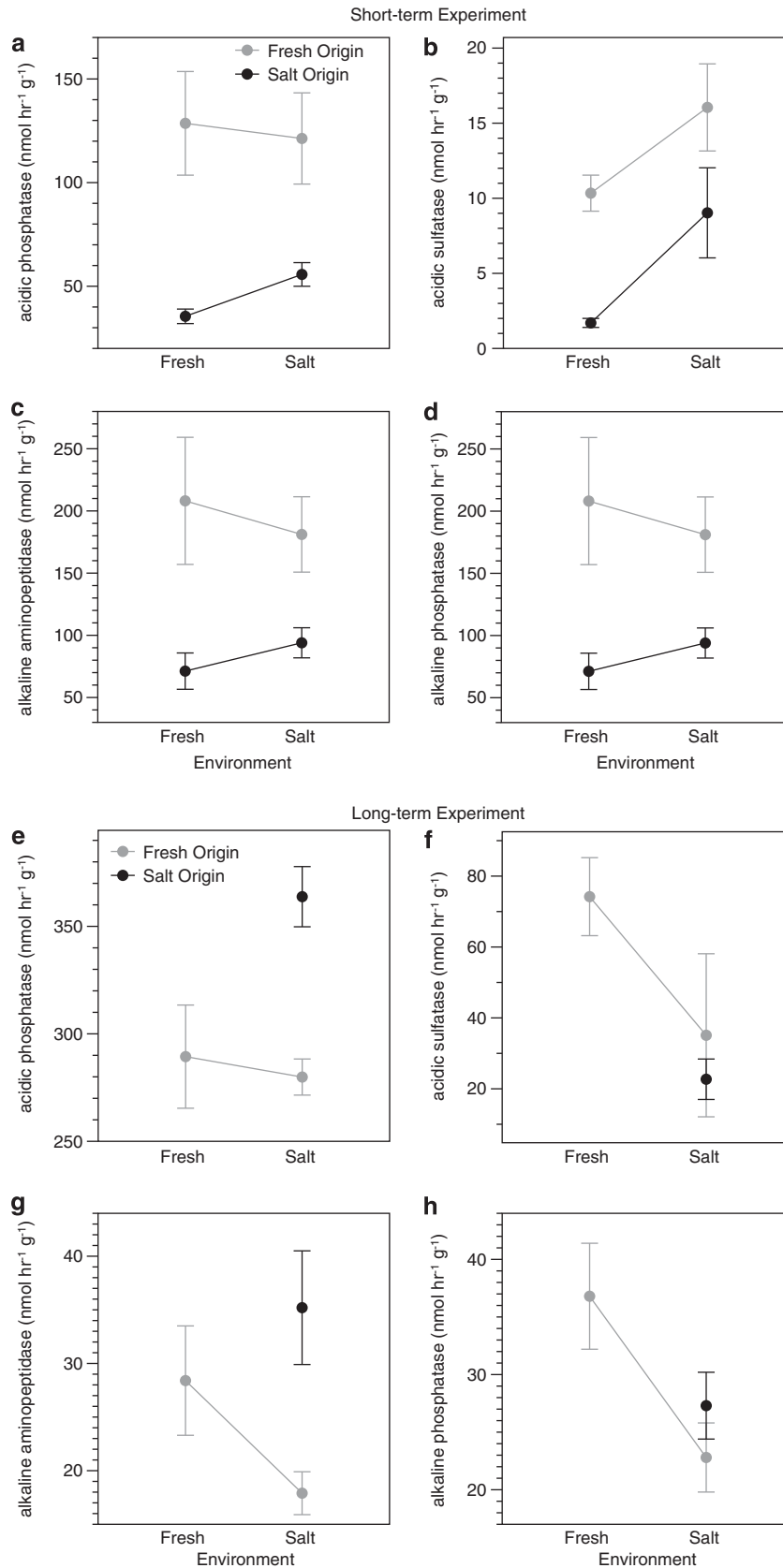
Both sediment origin and environment influenced overall functioning in the sediment cages (Origin:  $R = 0.201$ ,  $P = 0.002$ ; Env.:  $R = 0.442$ ,  $P = 0.016$ ). Similar to the shift observed in bacterial composition, the functional profile of the sediment cores transplanted from freshwater to saltwater was

intermediate to those native to either environment (Supplementary Figure S2b), such that the transplanted freshwater sediments were on average more similar to those of the native saltwater sediments than of the native freshwater sediments. Further, overall function was correlated to bacterial composition. Within the saltwater environment, pairwise functional distance and 16S compositional similarity were correlated, suggesting that more similar bacterial communities led to more similar sediment functional processes (RELATE test; Spearman's  $\rho = 0.285$ ,  $P = 0.001$ ).

## Discussion

The results of the two transplant experiments support all three of our hypotheses, indicating that microbial composition affects functioning in these estuarine sediments. Several lines of evidence are particularly revealing. First, we observed significant effects of sediment origin in both the short- and long-term experiments. By itself, the origin effect in the short-term experiment is not definitive evidence that the two communities are functionally different. Salinity changed quickly, but did not completely equilibrate in the transplanted sediments after 1 week; thus, the origin effect might have reflected abiotic differences. In addition, the short-term origin effect might be ambiguous, if the transplanted microorganisms did not have enough time to acclimate to the new conditions. If this was the case, then with more time, the communities might have converged functionally despite their compositional differences (Comte and del Giorgio, 2011). However, the results of the long-term experiment suggest that these possibilities cannot entirely





**Figure 5** Means ( $\pm 1$  s.e.) of the activities of four enzymes by sediment origin and incubating environment for the (a–d) short- and (e–h) long-term experiments. Acidic aminopeptidase and alkaline sulfatase were not affected by sediment origin in either experiment and so are not shown. For ease of viewing, the gray lines connect the sediments originating from the freshwater environment, and the black lines connect those originating from the saltwater environment.

explain the origin effect in the short-term experiment. Even after 6 more weeks for the environment to permeate the cages and the microorganisms to acclimate, an origin effect persisted, providing additional evidence that the remaining differences in microbial composition were responsible for the differences in sediment functioning.

Second, as the transplanted sediment composition shifted in the long-term experiments, we observed a significant correlation between overall bacterial composition and sediment functioning. Such a correlation also supports the idea that bacterial composition has a direct influence on sediment processes (Strickland *et al.*, 2009). At the same time, we did not observe a shift in SRB composition. This result was unexpected, given that sulfate reduction is generally greater in salt marshes than in tidal freshwater habitats (Odum, 1988). At least three reasons may account for this result. First, SRB may be more plastic to salinity changes than expected, such that SRB taxa can function equally well in both these locations. Second, SRB abundance (which we did not assay) may have increased in the saltier environment, even though the relative abundance among taxa (their composition) did not change. Finally, the genetic assay used (the particular primers and/or the fingerprinting method) may have missed finer-scale changes in SRB composition (for example, Santillano *et al.*, 2010).

Third, although salinity in the transplanted cages equilibrated with the surrounding environment after 7 weeks, ammonium concentration did not. These counter results indicate that although the surrounding waters had permeated through the cages, sediment processes internal to the cages directly influenced ammonium concentrations. Microorganisms drive most processes in the nitrogen cycle, and previous studies provide evidence that their particular composition influences these processes rates (Cavigelli and Robertson, 2000; Avrahami *et al.*, 2002; Balser and Firestone, 2005; Bernhard *et al.*, 2007a). A related result is that sediment origin (either directly or interactively with environment) affected nitrification and methane flux, but not CO<sub>2</sub> production. This outcome is consistent with Schimel's (1995) the hypothesis that microbial composition is more likely to influence 'narrow' rather than 'broad' processes. Narrow processes are defined as those carried out by relatively phylogenetically restricted groups of microorganisms. Taxa within these narrow groups may respond similarly to a new environment and therefore as their abundance changes, the processes these organisms carry out will likely change as well. In contrast, broad processes like respiration are performed by many taxa among a variety of phylogenetic groups and thus, even dramatic shifts in microbial composition may maintain similar respiration rates. For example, Balser and Firestone (2005) observed that microbial composition was correlated with soil nitrification rates but not soil respiration. Similarly,

Dimitriu *et al.* (2010) found that the microbial community had a stronger effect on the activity of phenol-oxidase than that of more widely produced enzymes.

Of course, the main challenge of field transplant experiments is to prevent migration and control for all environmental parameters that might affect functional processes; only then can an origin effect be attributed entirely to differences in microbial composition. The cages probably prevented most migration of bacterial cells, but not all, as revealed by initial lab tests. Given the high cell densities in estuarine sediments, it seems unlikely that a few immigrants would grow to dominance in the cages after only 7 weeks, but we cannot be sure. Further, while we purposely compared communities along the same river to try to control for substrate, we were not entirely successful; most metrics were similar, but at least at the beginning of the experiment, carbon and nitrogen content differed slightly in sediments from the two locations. In fact, even if we did not detect any differences in the sediments, we could not exclude the possibility that an unmeasured abiotic variable was in part responsible for the effect of sediment origin. Thus, while every effort should be made to minimize this limitation, whole-community transplant studies will always require a caveat about potentially uncontrolled, abiotic variables.

In sum, this work supports previous correlative studies (for example, Bertilsson *et al.*, 2007; Bernhard *et al.*, 2007b; Comte and del Giorgio, 2010) and lab experiments (for example, Bell *et al.*, 2005; Strickland *et al.*, 2009), suggesting that microbial composition influences some ecosystem processes. To our knowledge, however, this study is only one of a few that directly demonstrates the effect of microbial composition on functioning in the field while controlling for microbial migration (Allison *et al.*, in press; Gasol *et al.*, 2002). Such experiments would also be useful to investigate further details of the relationship between microbial composition and ecosystem functioning. Indeed, using other approaches, many studies have already started to address when, where and why microbial composition is related to ecosystem functioning. For instance, a key to predicting the response of ecosystems to environmental change is to disentangle the importance of shifts in microbial composition versus the physiological plasticity of that community (Schimel *et al.*, 2007; Singh *et al.*, 2010; Comte and del Giorgio, 2011). Generally, ecosystem models assume that process rates—even when directly carried out by microorganisms—are determined by environmental conditions (Schimel and Gullledge, 1998), implicitly assuming that microbial communities are so physiological plastic that any differences in their composition are unimportant. Future transplant experiments might also consider more detailed temporal dynamics of microbial composition and functioning over time (for example, Berga *et al.*, 2012). Such a design could test

whether microbial composition influences the resilience of ecosystem processes to environmental change under field conditions.

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