Effect of Sediment Manipulation on the Biogeochemistry of Experimental Sediment Systems
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ABSTRACT


Before biological or biogeochemical experimentation, sediments are often manipulated and defaunated with the use of many different approaches and only modest consideration of treatment effects on sediment biogeochemistry and fluxes. Mesocosm experiments require large quantities of sediment and no standard protocol to defaunate and equilibrate muddy sediments before initiation of experiments has been determined.

Using fine-grained sediments, we examined a number of treatments: (1) intact with macroinfauna; (2) intact, macroinfauna individually removed; (3) homogenized surface sediment with macrofauna; (4) homogenized deep sediment without macroinfauna; and (5) intact deep sediment without macroinfauna. In weekly batch core flux incubations, we measured dissolved oxygen, dinitrogen gas, ammonium (NH₄⁺), nitrate plus nitrite (NO₃⁻ + NO₂⁻), silicate (Si), and soluble reactive phosphorus (SRP) fluxes over a 5-week period. In addition, we determined porewater ammonium concentrations over time.

Different sediment preparation techniques, with the same muddy sediment, significantly affected nutrient and gas fluxes, and the amount of nutrient and gas fluxes differed between sediment preparation techniques. Severely manipulated sediments, such as homogenized treatments, had high initial effluxes but eventually equilibrated to lower and more constant nutrient and gas fluxes. Moreover, biogeochemical flux changes for all treatments became similar after about 2 to 3 weeks. Sieved sediments exhibited low fluxes over the entire 5-week period, and flux rates did not change over time. A feasible method for sediment preparation for mesocosm studies is to use homogenized deep sediment equilibrated over an almost 2-week period. Overall, sediment preparation and the time after sediment manipulation affect sediment biogeochemical processes and must be considered before initiating experiments.

ADDITIONAL INDEX WORDS: Sediment equilibration, disturbance, defaunation, sediment homogenization, mesocosm, experimental ecosystem, sediment-water exchange.

INTRODUCTION

Sediments used in micro- or mesocosm studies have been prepared in a variety of ways for use in biogeochemical flux experiments or mesocosm experiments (i.e., Table 1). After collection, sediment has been manipulated for different experimental purposes. Manipulations have included organic matter (Caffrey et al., 1993; Enoksson, 1993; Newell, Cornwell, and Owens, 2002; Sloth et al., 1995), addition of organisms (Andersen and Kristensen, 1988; Kim et al., 2004; Pelegri, Nielsen, and Blackburn, 1994; Porter et al., 2004a), removal of macrofauna (Doering et al., 1987; Martin and Banta, 1992; Oviatt et al., 1995; Sundback et al., 1991), addition of microphytobenthos (Newell, Cornwell, and Owens, 2002), and addition of both microphytobenthos and macrofauna (Rysgaard et al., 1994). Some experiments have used intact sediments and macrofauna (Doering, Oviatt, and Kelley, 1986). Natural sediments have been sieved to remove macroinfauna (Hansen, King, and Kristensen, 1996; Meyers, Fossing, and Powell, 1987; Pelegri, Nielsen, and Blackburn, 1994) and homogenized (Kim et al., 2004; Porter et al., 2004a, 2004b; Ziebis, Huettel, and Forster, 1996). Another defaunation approach is inducing anoxia in the sediment, followed by upward migration of animals (Jorgensen, 1980) and removal at the sediment surface (Andersen and Christensen, 1988; Hansen and Blackburn, 1991). Other studies have used intact cores with macrofauna collected with benthic chamber tubes for small-scale studies of in situ conditions (Cowan and Boynton, 1996; Giblin, Hopkinson, and Tucker, 1997; Nielsen, Nielsen, and Rasmussen, 1995) or with box cores for use in mesocosms (Doering, Oviatt, and Kelley, 1986;
Table 1. Techniques that have been used to defaunate and manipulate sediments and the time scales for sediment equilibration in other studies. The method listed in the Reference column indicates how these treatments relate to the treatments used in this study (for treatments in this study see text and Table 2).

<table>
<thead>
<tr>
<th>Defaunation/Sediment Manipulation Method</th>
<th>Treatment Added</th>
<th>Type of Sediment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fauna gently removed from cores</td>
<td>After 7 d O₂ and CO₂ were at steady state. The treatment was added at day 20</td>
<td>Polyhaline natural sediment, fine silts and clays, 2–5% organic content</td>
<td>Sundback et al., 1991; IS + m</td>
</tr>
<tr>
<td>24-h anoxia, animals removed. Three more days of anoxia. Additions: suspension of macroalga</td>
<td>As soon as possible</td>
<td>Natural intact, 18% sand, 60% silt, 22% clay</td>
<td>Hansen and Blackburn, 1991; IS + m</td>
</tr>
<tr>
<td>Intact sediment with macroinfauna, collected with 0.25-m² box cores and placed into MERL mesocosms. Additions: infaunal bivalve suspension feeders</td>
<td>Weeks after sediment collection</td>
<td>Natural intact, 18% sand, 60% silt, 22% clay</td>
<td>Doering, Oviatt, and Kelley, 1986; Doering et al., 1987; IS – m</td>
</tr>
<tr>
<td>Intact sediment cores collected, purged with N₂ for 20 min, sealed to make anaerobic, left for 24 h to kill macroinfauna or trigger them to come to the surface; the following day all visible individuals were removed before oxygenated water was added to the cores. Additions: different species of infauna</td>
<td>2 d after re-oxygenation</td>
<td>Mesohaline natural sediment</td>
<td>Oviatt et al., 1995; IS + m</td>
</tr>
<tr>
<td>Uppermost 5–10 cm dredged and sieved (1.5 mm), then homogenized. Additions: yeast, detritus, control (nothing added), additions within hours of collection</td>
<td>Within hours of collection</td>
<td>Organic-poor, sandy</td>
<td>Andersen and Kristensen, 1988; IS + m</td>
</tr>
<tr>
<td>O₂ levels reduced to drive macroinfauna to the surface, upper 2–3 cm sieved (0.3 mm) and the sediment below left anoxic for 17 d; sieved sediment added back to cores, re-oxygenated. Additions: macroinfauna, polychaetes Nephys incisa or M. ambigua</td>
<td>After 2 mo with macroinfauna</td>
<td>Natural, 17% clay, 93% silt/clay</td>
<td>Kristensen and Hansen, 1995; Siev – m</td>
</tr>
<tr>
<td>Top 0–8 cm sieved (1 mm), allowed to settle for 3 d, homogenized</td>
<td>After 3 wk</td>
<td>Natural</td>
<td>Martin and Banta, 1992; Siev – m</td>
</tr>
<tr>
<td>Top 4 cm sieved (0.5 mm), added to remaining sediment</td>
<td>After 0 d</td>
<td>Natural</td>
<td>Hansen, King, and Kristensen, 1996; Siev – m</td>
</tr>
<tr>
<td>Homogenized. Additions: artificial burrows</td>
<td>After 13 d (after oxygen was at steady state)</td>
<td>Silty</td>
<td>Meyers, Fossing, and Powell, 1987; Siev – m</td>
</tr>
<tr>
<td>Sieved (0.5-mm mesh), homogenized. Additions: amphipods</td>
<td>0 d after sieving</td>
<td>Sandy, intertidal</td>
<td>Pelagri, Nielsen, and Blackburn, 1994; Siev – m</td>
</tr>
<tr>
<td>Sieved (0.5 mm), homogenized. Additions: organic matter (yeast)</td>
<td>After 14 d to steady state</td>
<td>Freshwater lake sediment</td>
<td>Caffrey et al., 1993; Siev – m</td>
</tr>
<tr>
<td>Surface sediment sieved (0.5 mm)</td>
<td></td>
<td>Clean silver sand, 210 μm, organic content 0.1%</td>
<td>Rysgaard et al., 1994; Siev – m</td>
</tr>
<tr>
<td>Sediments added to tanks. Tanks each were connected to 16-L benthos chambers with mussels. Additions: bivalve suspension feeders</td>
<td>After 6 wk</td>
<td>Azoic sand, median grain size 210 μm, 0.16% organic matter</td>
<td>Escaravage et al., 1996</td>
</tr>
<tr>
<td>Sediment container placed at bottom of each tank. Tank was connected to 16-L benthos chambers with mussels. Additions: bivalve suspension feeders</td>
<td>0 d after final homogenization</td>
<td>Natural, 70%–95% medium sand (200–630 μm)</td>
<td>Prins et al., 1995</td>
</tr>
<tr>
<td>Homogenized</td>
<td></td>
<td>Natural</td>
<td>Ziebis, Huettel, and Forster, 1996; HD – m, HS + m</td>
</tr>
<tr>
<td>Sieved (2-mm mesh), mixed by hand, packed in cores, radiated by a 1-h ionizing radiation from a cesium-137 gamma source, placed in filtered seawater, re-aeration up to 3 mo after radiation, homogenized</td>
<td>4 d after homogenization cores taken from homogenized sediments and treatments initiated</td>
<td>Natural</td>
<td>Goldo et al., 1994</td>
</tr>
<tr>
<td>Uppermost 5 cm sediment dredged and sieved (1.5 mm) followed for freezing for 48 h at –20°C. After thawing sediments were homogenized. Additions: Nereis virens, aerobic control without N. virens, anaerobic control without N. virens</td>
<td></td>
<td></td>
<td>Kristensen and Blackburn, 1987</td>
</tr>
</tbody>
</table>

MERL = Marine Ecosystem Research Laboratory, Narragansett, Rhode Island.
DOERING et al., 1987; OVIATT et al., 1995). Alternative de-
faunation approaches have included deep-freezing (BRUCK-
NER et al., 1995; KRISTENSEN and BLACKBURN, 1987) or core
irradiation (GOLDE et al., 1994). All techniques have conse-
quences for microbial populations. Ammonium concentra-
tions are enhanced and dissolved oxygen concentrations can be
reduced in disturbed sediments (MARINELLI and WOODIN,
2002, 2004; WOODIN, MARINELLI, and LINDSAY, 1998). A sys-
tematic study to determine the most effective protocol for sed-
iment handling for experimental purposes has not been pub-
lished.

The time elapsed between sediment manipulations and the
start of sediment incubations can vary among experiments
and could affect sediment biogeochemistry and subsequent
nutrient fluxes. Experiments have been started immediately
after the sediment manipulation (DOERING, OVIATT, and
KELLEY, 1986; DOERING et al., 1987; KRISTENSEN and HAN-
SEN, 1995; MEYERS, FOSSING, and POWELL, 1987), 2 months
after the addition of macrofauna (MARTIN and BANTA,
1992), 3 weeks after sediment collection (HANSEN, KING,
and KRISTENSEN, 1996), 7–14 d after the addition of organic
matter (CAFFREY et al., 1993), or “weeks after sediment col-
collection” (OVIATT et al., 1995). Sometimes the steady state of a
variable is used to guide researchers as to when to start the
experiment or add the treatment of interest. The time to
reach steady state varies widely. For example, observations
of the time for oxygen, carbon dioxide, or both to reach steady
state ranged from 7 to 13 days (HANSEN and BLACKBURN,
1991; PELEGRI, NIELSEN, and BLACKBURN, 1994; RYSGAARD
et al., 1994) with variable times in which manipulations were
commenced after apparent steady state.

Experimental studies that use mesocosms require large
amounts of sediment, and techniques such as sieving to re-
move macrofauna to prepare defaunated sediment are logi-
tically difficult. Often the desire is to remove macrofauna in
micro- and mesocosm studies because this allows the inves-
tigator to attain the experimental fauna and faunal popula-
tion density of choice (ANDERSEN and KRISTENSEN, 1988;
KIM et al., 2004; PELEGRI, NIELSEN, and BLACKBURN, 1994;
PIETHOS and RICE, 2003; PORTER et al., 2004a). MARTIN and
BANTA (1992) used sieved unsieved sediment to main-
tain sediment structure. Alternatively, some mesocosm stud-
ies with sediment have used azoic sand (PRINS et al., 1995),
mixtures of sand and silt (PETERSEN, CHEN, and KEMP,
1997; PETERSEN, SANFORD, and KEMP, 1998), or box cores
with intact sediment and organisms (DOERING, OVIATT,
and KELLEY, 1986; DOERING et al., 1987; OVIATT et al., 1995).

This study examines the effect of sediment manipulation
and defaunation on nutrient and gas flux rates over time for
muddy sediments. Although some aspects of sediment pre-
paration have been addressed in previous studies (BRUCKNER
et al., 1995; FLETCHER, REYNOLDS, and TAYLOR, 2001),
we pose three questions: (1) Are biogeochemically disturbed
or defaunated sediments able to equilibrate to similar and
relatively constant nutrient and gas flux rates over time? (2)
If so, how much time elapses before disturbed sediments
equilibrbase to constant gas and nutrient flux rates? (3) What
is a feasible large-scale sediment defaunation technique for
mesocosm studies and studies with sediment cores?

METHODS
Sediment Treatments
Sediments were collected from a shallow water of the
mesohaline Choptank River (Chesapeake Bay) by hand-coring
with 12.7-cm-diameter acrylic chambers. Grain sizes of two
cores in the vicinity of our sediment collection site were 20% sand,
21% silt, 59% clay (CORNWELL, unpublished data). Macrofauna
was abundant at our site, and two cores at about 10 cm sediment depth were collected to determine macrofauna
abundance. One 12.7-cm-diameter sediment core, ren-
dered anaerobic by sealing the core, and animals individually
picked, contained two polychaetes, 46 amphipods, and one
unidentified crustacean. A second 12.7-cm-diameter core with
~10 cm of sediment and an overlying water column of 26 cm
contained 13 polychaetes, 52 amphipods, one snail, and two
bivalves. Sediments were manipulated in six different ways
(Table 2); some treatments were defaunated (−m, minus
macrofauna) by sealing the core, sieving, or covering the sed-
iment with plastic, and others were not (+m, plus macrofa-
una; Table 2). We chose sediment preparation techniques (e.g.,
IS−m, IS+m, HS−m, HD−m, Siev−m; Table 2) that com-
monly have been employed in other studies. The HD−m
treatment was included as a feasible option for large-scale
sediment preparation for mesocosm studies. The ID−m treat-
ment was chosen to represent dredged sediment.

The IS−m cores were driven to anoxia, and macrofauna
were picked from the sediment surface daily until no fauna
was observed after a 4-d period. The top 10-cm section of
cores, with macrofauna, was used for the IS+m treatment,
and the section below 10 cm depth was used for the ID+m
treatment. The HS+m treatment consisted of ho-
mogenized sediment from the top 10 cm of cores and included
macrofauna.

The sediment sections below 10 cm were placed into anoth-
er bucket, thoroughly mixed, placed into core tubes, and
smoothed to form the HD−m sediment treatment; no ma-
acrofauna were below 10 cm depth. For the Siev−m sedi-
tment treatment, sediment was sieved through a 0.5-mm
mesh to remove macrofauna, and sediment was placed into
core tubes with a depth sufficient to compensate for sediment
compaction and provide about 10 cm sediment height.

Failure of the aeration system lead to a lack of oxygenation
of the initial HS+m treatment after 2 weeks, and the HS
was remixed. This treatment was reinitiated at that point
(labeled HS+m'). The flux experiments were run with the
other cores or, in one case, run as the only cores with control
cores. An offset correction in time was applied to the HS+m'
cores.

Setup of Cores
Our incubation system consisted of 12.7-cm-diameter acrylic
cores, with a 10-cm sediment depth and a 26-cm overlying
water column (total water volume was 3300 ml). Cores for
the anaerobic treatments (IS−m, HD−m) were collected 6
days before the first flux experiment and left at room tem-
perature under anaerobic conditions until the start of re-aer-
atation. Organisms were removed daily from IS−m. Cores for
treatment ID-m were collected 2 days before the start of the experiments and re-aerated. Cores for the aerobic treatments IS+m, HS+m, and Siev-m were collected 1 day before the start of the initial flux experiments. All sediment cores were transferred to a dark, aerated (by bubbling) water basin filled with 2 μm of filtered flow-through estuarine water at 22–23°C.

Flux Experiments

Sediment-water flux experiments were performed in the dark with triplicate cores and bubbled 0.2-μm filtered estuarine water that had been exchanged into the cores before incubation. Tubes filled with 0.2-μm filtered, aerated replacement water were attached to the lids, the cores were sealed without bubbles and arranged around central magnetic turntables that turned magnetic stirrers in each flux chamber. Stirring rates were below the threshold of sediment resuspension but sufficient to ensure a homogeneous water column. During one flux run, filtered water blanks were run in parallel with the sediment incubations to confirm that water column activity was negligible.

Sediment incubations were carried out at the start of the experiment (week 0) and repeated after weeks 1, 2, 3, and 5. Flux incubations for treatment ID-m were carried out in weeks 0, 1, 2, 4, and 5. Each incubation was about 18 hours long. During all incubations, oxygen levels were not depleted below half of the starting oxygen concentration so that responses would be linear and macrofaunal stress would be minimal. A minimum of three time points was measured. Dissolved oxygen, N₂ gas, and nutrients were sampled. Each sample point removed only ~1.5% of the core water volume, and no correction for the replacement water addition was required.

During sampling, one 5-ml water sample was collected by gravity flow through valves in the chamber lid into a small glass cylinder for immediate analysis of dissolved oxygen by a Clark-type electrode. The electrode response was checked with Winkler titrations before the experiments. Three 7-ml water samples per core were collected by gravity flow into glass tubes and sealed with a plastic sleeve and a second glass tube (KANA et al., 1998) for N₂:Ar gas ratio analysis to determine N₂ gas fluxes. A 25-ml water sample was withdrawn by syringe from the water overlying each core, filtered through a 0.45-μm filter, and frozen at −20°C (PARSONS, MAITTA, and LALLI, 1984) in 5-ml vials for nutrient (NH₄, NO₃ + NO₂, SRP, Si) analyses after the experiment.

Time Between Flux Experiments

Between flux experiments, open sediment cores were aerated by bubbling in the dark in a common flow-through seawater basin held at 22–23°C. Water was continuously exchanged at a turnover time of once per day with the use of 2 μm of filtered ambient Choptank estuary water at a salinity of 11–12 psu. After the week 1 flux measurements, the oxygen supply was interrupted in the seawater basin affecting treatments with high sediment oxygen demand (treatments HS+m, IS+m). After the hypoxic event, we discarded all cores from treatment HS+m (Table 2) used for the weekly flux run and mixed an additional set of three replicate cores for treatment HS+m.

Evaluating Fluxes Over Time

Three approaches were used to determine the changes in fluxes over time: (1) the results of the fluxes of solutes and gases were visually compared over weekly core incubations over a 5-week period (except week 4); (2) the fluxes of weeks 0, 1, 2, and 3 were each compared with week 5, using week 5 as a benchmark; and (3) the flux changes from week to week were compared statistically. To reduce the volume of data, for (2) and (3), we focused on ammonium and dissolved oxygen because these are biogeochemically and ecologically particularly important; moreover, low fluxes of soluble reactive phosphorus made further analysis trivial.

<table>
<thead>
<tr>
<th>Treatment ID</th>
<th>Preparation Procedures for Sediment Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>IS-m</td>
<td>Intact surface sediment, macrofauna individually removed</td>
</tr>
<tr>
<td>HD-m</td>
<td>Homogenized deep, no macrofauna</td>
</tr>
<tr>
<td>IS+m</td>
<td>Intact sediment with macrofauna</td>
</tr>
<tr>
<td>HS+m, HS+m'</td>
<td>Homogenized surface sediment, macrofauna</td>
</tr>
<tr>
<td>Siev-m</td>
<td>Sieved (500 μm), macrofauna removed</td>
</tr>
<tr>
<td>ID-m</td>
<td>A deep sediment section of a core (section between 10 and 20 cm).</td>
</tr>
</tbody>
</table>
Pore Water Extractions

We prepared 7.6-cm-diameter acrylic cores corresponding to each treatment, held them in the same flow-through water bath as the flux cores, and sectioned them for porewater analyses on weeks 0, 2, and 5 of the experiment. For homogenized treatments (HD-m, HS+m) porewater from one sediment sample from the homogenized mix was used to represent the sediment core at all depths for week 0. Sediment cores were sectioned under N2 atmosphere in a glove bag to minimize oxidation artifacts. Sediments from 0 to 2 cm were sectioned in 0.5-cm intervals, with 1-cm intervals from 2 to 4 cm, and sections of 2-cm intervals for sediment below 4 cm depth. Pore waters were centrifuged at 3600 rpm for 20 minutes, and the supernatant was sieved through a 0.45-μm filter and frozen for subsequent porewater ammonium analyses.

Analytical Methods and Analysis

Ammonium and soluble reactive phosphorus were analyzed colorimetrically (PARSONS, MAITA, and LALLI, 1984), and both nitrite + nitrate and silicate were determined on a Technicon autoanalyzer (ZIMMERMANN, PRICE, and MONTGOMERY, 1977). Immediately after sampling, the water in the sealed glass tubes was analyzed for N2:Ar gas ratios with a modified membrane inlet mass spectrometer described in detail in KANA et al. (1994). The N2 gases results were expressed as N2-N. Rates of N2 gas flux were determined during all flux experiments except in week 0 because of equipment malfunction. Data for N2-N flux rates for treatment HS+m in week 5 were lost.

Statistical Analyses

An analysis of variance (ANOVA) was performed for the fluxes of each week after checking the data for normality of the error term with the Shapiro Wilks test and after checking for homogeneity of variance with Levene's test. Data were natural log-transformed as necessary, with ln notations on Figures 1–5 when transformation was necessary. When transformations did not correct for homogeneity and normality, the nonparametric Wilcoxon rank test comparison (WR notation in Figures 1–5) was used. With the use of least squares means tables (SAS Institute, Cary, North Carolina), we compared the significances between treatments within each flux experiment as indicated by letters in Figures 1–5.

In addition, we performed repeated measures analyses of variance (JOHNSON and Wichern, 1982; SCHEINER and GUREVITCH, 1993) on the nutrient and gas flux results from treatments IS+m, HD—m, IS+m, HS+m, Siev—m, and ID—m to test whether flux rates were influenced by the type of sediment manipulation and whether differences varied over time. The repeated measures ANOVA is a two-way ANOVA that properly accounts for the dependence over time. The repeated measures ANOVA included a test for time effects, treatment effects, and a time and treatment interaction. Week 5 was used as a benchmark for stabilization in the nutrient and gas concentrations. For comparison, we included treatment IS+m in the analysis. Flux measurements on IS+m had been performed on a different core after week 1. In addition, we used repeated measures ANOVA (JOHNSON and Wichern, 1982; SCHEINER and GUREVITCH, 1993) on treatments IS—m, HD—m, IS + m, HS+m, Siev—m, and ID—m to statistically determine whether the nutrient and gas flux rates among the different treatments would become similar between treatments over time. Treatment ID—m was run in week 4, instead of in week 3 as noted in the figures and tables, and was excluded from the statistical analyses of week 3. For HS+m, all statistical analyses were performed on the remixed HS+m cores. To determine the mean difference between week 3 and 5 for each individual treatment, we performed a t test with the null hypothesis that the mean difference was zero between weeks 3 and 5. All analyses were performed in SAS 8.0 (SAS Institute).

RESULTS

Porewaters

Profiles of ammonium concentrations of the homogenized treatments (HD—m, HS+m, Siev—m) changed dramatically over time, whereas all other treatments had a sigmoid shape at the onset (Figure 6). High initial ammonium concentrations at the surface of homogenized sediment treatments changed over 2 weeks to sigmoid-shaped profiles with lower ammonium concentrations near the surface and higher concentrations deeper into the core (Figure 6, top). Ammonium profiles at weeks 2 and 5 were similar in shape. Initial porewater ammonium concentrations of the homogenized sediment treatments were highest in HD—m (~810 μmol L⁻¹), followed by HS+m (~450 μmol L⁻¹), and lowest in Siev—m (~180 μmol L⁻¹), as shown in Figure 6 (top). The intact deep and intact surface sediment had high ammonium concentrations at the surface (Figure 6, bottom). High initial ammonium porewater concentrations near the surface of the homogenized deep and the homogenized surface sediment resulted in high initial ammonium effluxes (Figure 1). Initially, the intact deep and intact surface sediment also had high ammonium concentrations at the sediment surface (Figure 6, bottom).

Fluxes

Fluxes of Solutes and Gases over a 5-week Period

Soluble reactive phosphorus fluxes were not detectable throughout these experiments; however, the nitrogen and gases were dynamic over time. In week 0, ammonium effluxes were close to zero in treatments Siev—m and ID—m; ~400 mol m⁻² h⁻¹ in treatments IS—m, HD—m, IS+m, and HS+m; and ~700 μmol m⁻² h⁻¹ in treatment HS+m (Figure 1). The pattern of ammonium efflux rates in week 1 were similar to week 0, with somewhat lower rates. Ammonium fluxes decreased over time and differences between treatments also decreased over time (Figure 1). ANOVA and least squares mean comparisons indicated significant differences between the treatments. Oxygen uptake rates were ~1500 μmol m⁻² h⁻¹ at the start of the experiment (week 0), except for treatments IS+m and

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Figure 1. Ammonium fluxes measured approximately weekly over a 5-wk period. The large open circle represents the mean flux rate and the bar the standard deviation. The small closed circles represent the flux rate of the individual cores that constitute the mean flux rate and contribute to the standard deviation from the mean. Results of an ANOVA run for each week are reported on the left of each panel. WR = data analyzed with the Wilcoxon Rank test, Ln = log-transformed data. Probability is indicated as ns = nonsignificant (p > 0.05) and significant (p < 0.05, p < 0.01, and p < 0.001). For sediment preparation treatments, see Table 2.

Figure 2. Dissolved oxygen fluxes measured approximately weekly over a 5-wk period. The large open circle represents the mean flux rate and the bar the standard deviation. The symbols and statistics are the same as in Figure 2. For sediment preparation treatments, see Table 2.

The week 0 N₂-N gas fluxes from the remixed treatment HS-m averaged ~300 μmol m⁻² h⁻¹ (Figure 4). Then week 1 rates of N₂-N production ranged from about 50 to 200 μmol m⁻² h⁻¹ in all treatments and were highest in treatments with macroinfauna (IS+m, HS+m; Figure 4). On the basis of only one core, treatment IS+m had very high N₂-N fluxes in week 5.

Silicate fluxes were low across all treatments over the entire measurement period from weeks 0 to 5 (Figure 5) and were not significantly different between treatments, with the exception of silicate fluxes between treatments in weeks 1, 2, and 5. Silicate fluxes ranged from a slight uptake of about -70 μmol m⁻² h⁻¹ in treatments IS+m, HS+m, and Siev-m in week 0 and treatments IS+m and HD-m in week 1 to an efflux of about 150 μmol m⁻² h⁻¹ in all other treatments. Variability in silicate fluxes between treatments was slightly larger in weeks 0 and 1.

Overall, for the biogeochemical fluxes, the treatments were...
significantly different as determined by ANOVA and least squares means comparisons, and the differences between treatments decreased with time. Fluxes became similar between treatments for nitrate + nitrite (Figure 3), $N_2$-N (Figure 4), and silicate (Figure 5) after about week 2. Moreover, the F values decreased from 65 to 9 (Figure 1), 141 to 13 (Figure 2), and 22 to 2 (Figure 3) from week 0 to week 5 for ammonium, dissolved oxygen, and nitrate + nitrite fluxes, respectively, also indicating that the differences between treatments became smaller over time.

**Flux Changes of Weeks 0, 1, 2, and 3 Compared with Week 5**

The weekly flux rates were compared with week 5 (Figure 7) because at that time sediment fluxes had generally stabilized. For ammonium, we found the largest flux differences between weeks 0 and 5 and between weeks 1 and 5, in particular in HS+m and IS+m. The flux differences significantly decreased over time. In addition, the fluxes changed similarly for all treatments in weeks 2 to 5 and weeks 3 to 5, respectively.

For dissolved oxygen, we found the largest flux differences between weeks 0 and 5 and between weeks 1 and 5, in particular in HD+m, HS+m, and IS+m. The flux differences significantly decreased over time. In addition, the fluxes changed similarly for all treatments in weeks 2 to 5 and weeks 3 to 5, respectively.

**Flux Changes from Week to Week**

In our comparison of all treatments together from week to week, ammonium and dissolved oxygen fluxes changed significantly from week 0 to 1, week 1 to 2, week 2 to 3 (Table
Figure 5. Silicate fluxes measured approximately weekly over a 5-wk period. The large open circle represents the mean flux rate and the bar the standard deviation. The symbols and statistics are the same as in Figure 2. For sediment preparation treatments, see Table 2.

Thus, sediment preparation techniques significantly affected nutrient and gas fluxes, with some large temporal changes in gas and solute fluxes over time. Changes occurred until about week 2 or 3, when fluxes became similar and lower among all treatments. However, nitrogen gas flux rates remained variable and silicate fluxes fairly high at the end.

The homogenized treatments and the treatments with macroinfauna had high initial fluxes and changed the most over time. These fluxes decreased over the 5-week period. Flux changes became smaller over time in the homogenized treatment and in the intact sediment with macroinfauna; fluxes changed similarly for all sediment preparation techniques after about 2 to 3 weeks. After ~2 weeks, even severely manipulated sediments, such as our homogenized treatments,

Table 3. Statistical differences in dissolved oxygen and ammonium flux rates from week to week as determined by a repeated measures analysis of variance testing for the interaction of the treatment × time effect. For both variables, we found significant trends over time that were not the same for all treatments. Probability is indicated as nonsignificant (p > 0.05), and significant (p < 0.05, p < 0.01, and p < 0.001). Significant values are indicated in bold print.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dissolved Oxygen</th>
<th>Ammonium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p Value</td>
<td>F value for treatment</td>
</tr>
<tr>
<td>Week 0 to week 1</td>
<td>0.0012</td>
<td>12.75</td>
</tr>
<tr>
<td>Week 1 to week 2</td>
<td>0.0003</td>
<td>19.26</td>
</tr>
<tr>
<td>Week 2 to week 3</td>
<td>0.0074</td>
<td>7.33</td>
</tr>
<tr>
<td>Week 3 to week 5</td>
<td>0.1140</td>
<td>2.56</td>
</tr>
</tbody>
</table>
eventually equilibrated to low and constant flux rates for all
nutrients and gas species. A deep sediment core, chosen to
mimic dredged sediment, initially exhibited enhanced fluxes
that also decreased over time. Unlike the other treatments,
sieved muddy sediment exhibited low fluxes over the entire
5-week period, likely a result of "washing" ammonium out of
the sediment during sieving. Overall, the variability in most
treatments was high initially, and with time, the variability
within treatments and between treatments decreased. By the
end of the experiment, the fluxes tended to converge on an
average value.

**DISCUSSION**

It has been assumed in previous studies, but not tested,
that manipulated sediments equilibrate before any treatment
additions, although the time period before treatment addi-

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**Table 4.** Mean difference of dissolved oxygen and ammonium between
weeks 3 and 5 and the p value for the t test of the null hypothesis that the
mean difference is zero between weeks 3 and 5. Probability is indicated as
nonsignificant (p > 0.05) and significant (p < 0.05, p < 0.01, and p <
0.001). Probabilities of 0.1 > p > 0.05 are nonsignificant but are indicative
of a trend. Significant values are indicated in bold print.

| Treatment | Dissolved Oxygen | | Ammonium |
|-----------|------------------|------------------|
|           | Difference (μmol m⁻² h⁻¹) | p Value | Difference (μmol m⁻² h⁻¹) | p Value |
| IS⁻m     | -315.75          | 0.0975          | 43.81              | 0.0328  |
| HD⁺m     | 232.54           | 0.2064          | 45.45              | 0.0281  |
| IS⁺m     | 174.15           | 0.5706          | 5.9                | 0.8489  |
| HS⁺m     | -558.52          | 0.0097          | 102.32             | 0.0002  |
| Siev⁻m   | -293.77          | 0.2632          | 5.88               | 0.7388  |
| ID⁻m     | -363.26          | 0.1664          | 12.6               | 0.5684  |
| ID⁺m     | 204.46           | 0.0002          | 10.47              | 0.0015  |
tions has been extremely variable (Table 1). Sometimes oxygen or carbon dioxide fluxes have been used as an indicator for sediment equilibration in which treatments were added after oxygen or carbon dioxide fluxes had stabilized (Hansen and Blackburn, 1991). With different types of sediment, it took between 7 and 14 days for gas fluxes to stabilize (Hansen and Blackburn, 1991; Pedersen, Nielsen, and Blackburn, 1994; Rysgaard et al., 1994). In our comparative study with differently manipulated muddy sediment, it took between 0 and 3 weeks for oxygen fluxes to stabilize. Moreover, at the same time, gas and nutrient fluxes stabilized to low rates and fluxes did not change much after 2 weeks.

Sieving of sediments with a 0.5-mm mesh, a technique commonly used to remove macrofauna (e.g., Caffrey et al., 1993; Pedersen, Nielsen, and Blackburn, 1994; Rysgaard et al., 1994), removes particles larger than the sieve size. Sieving can also break apart aggregates, increase pore spaces, increase the water content of sediment, and disturb pore-water gradients. In addition, macrofauna can be damaged or killed in the sieving process, adding organic matter to the sediment. Initial sieved ammonium fluxes were ~20 μmol m−2 h−1, significantly lower than the other two homogenized sediment treatments, with differences between treatments reflected in the initial porewater concentrations. Low ammonium concentrations and fluxes might result from handling losses and ammonium desorption. Contrary to our expectation, initial ammonium effluxes were very low from the deep sediment section (ID-m treatment).

Sediment homogenization or other sediment defaunation could disrupt natural porewater gradients; alter microbial populations in the sediment; redistribute, bury, or break up labile particulate organic matter; and redistribute iron sulfide minerals to the surface where they would be oxidized. Sediment homogenization has been shown to dramatically affect solute reaction rates (i.e., slurry; Reeburgh, 1983) and sulfate reduction rates measured from porewaters (Liebert, 1997).

Because macrofauna can profoundly affect geochemical fluxes, they are often removed from experiments to simplify experimental treatments and reduce variability. Sediment flux core incubations have been initiated over a broad range of times after the removal of the invertebrates (Table 1). We observed high initial uptake rates of dissolved oxygen and high initial ammonium effluxes in the cores with macrofauna, and it is well known that macrofauna can alter bio-geochemical fluxes through bioirrigation and bioturbation (Aller, 1980, 1982; Forster et al., 1995; Kristensen and Blackburn, 1987; Pedersen, Nielsen, and Blackburn, 1994). However, fluxes decreased over time, possibly because the activity of the infauna decreased or because organic matter was not added to the cores during the experiment to "feed" the fauna and the sediment. It has, for example, been demonstrated that the addition of particulate organic matter increases nutrient flux rates and alters nutrient transformations (e.g., Caffrey et al., 1993; Enoksson, 1993; Newell, Cornwell, and Owens, 2002).

Many of our sediment treatments represented a high sediment disturbance. In nature, the use of fishing gear for clamming, boat moorings, boat propellers, or dredging activities can severely scar and disturb sediments (Dawes et al., 1997; Ruffin, 1998; Walker et al., 1989), affect ammonium concentrations (Falko et al., 2003), and alter sediment biogeochemical fluxes. Similarly, the nutrient and contaminant dynamics in coastal ecosystems could be altered through sediment resuspension (Kim et al., 2004, 2006; Porter et al., unpublished data). Large-scale removal or mixing of sediment by dredging activities (Dawes et al., 1997) can exchange porewater with the water column and alter benthic fluxes. The removal of the top sediment layer will also remove the labile organic matter and the aerobic microbial populations such as the nitrifiers.

We were surprised to find only slightly enhanced initial effluxes of ammonium from the intact deep cores. The cores had been collected 2 days before the initial flux experiments and had been re-erated before the start of the flux experiment, and it appeared that the initial oxygenation of the sediment and establishment of microbial population might have been relatively fast. Subsequent incubations of Chesapeake mainstem sediment sections from 1 m beneath the surface (much deeper than those used in this study) and incubated less than 2 days after collection, resulted in initial ammonium effluxes of more than 2000 μmol L−1 m−2 (Cornwell and Owens, 1999; Cornwell, Owens, and Pride, 2000).

The artificially large enhancement of initial ammonium effluxes can mask ammonium effluxes from the treatment of interest and could be misleading. In general, high ammonium effluxes could obscure the experimental effects of mesocosm experiments (Breitbart et al., 1999) and possibly affect water column primary production (Cowan and Boynton, 1996). In addition, the initial conditions of "artificial" ecosystems can affect experimental responses (Giddings and Edlemon, 1979; Pilson, Oviatt, and Nixon, 1978). Ammonium effluxes and low oxygen conditions can act as adverse chemical cues for larval settlement and recruitment (Marinelli and Woodin, 2002, 2004; Woodin, Marinelli, and Lindsay, 1998). Sediment disturbance affects responses at the population and community level differently and at different time scales (Rhoads, McCall, and Yingst, 1978; Zajac and Whitlatch, 1982a, 1982b, 2003). Sediment disturbance and the degree of disturbance have also been found to affect recovery time of biota to sites (Dernie et al., 2003; Newell, Seiderer, and Hitchcock, 1998; Rhoads, McCall, and Yingst, 1978) with recovery times of >200 days (Dernie et al., 2003).

Interestingly, we did not find any detectable SRP fluxes throughout the entire experiment. Initially, we had expected SRP to desorb from particles under anaerobic conditions and appear as a measurable flux rate. Subsequent incubations of deep sediment cores that had been removed and manipulated under anaerobic conditions until the very start of the flux experiments similarly showed low SRP effluxes (Cornwell and Owens, 1999). It is possible that the surface layer of sediment could rapidly re-oxidize, thereby quickly trapping any upward-moving porewater SRP as it is adsorbed in oxic sediments (Krom and Berner, 1980). Bray, Bricker, and Troup (1973) also found iron oxidation effects of porewater SRP concentrations that removed inorganic phosphate from solution by precipitation as iron phosphate. Understanding
the effects of sediment manipulations on SRP cycling requires further study.

In many land-based mesocosm facilities, such as the Marine Ecosystem Research Laboratory (e.g., Pilson, Oviatt, and Nixon, 1978), the Multiscale Experimental Ecosystem Research Center (e.g., Petersen, Chen, and Kemp, 1997; Porter, 1999), and the mesocosm facility in The Netherlands of Prins et al. (1995), large quantities of sediment are required for the study of realistic ecosystem processes, and it is not always feasible to sieve the sediments. To reduce sediment reactivity and remove macrofauna, azoic sediments have been used (Prins et al., 1995), or natural sediments have been mixed with sand (Petersen, Chen, and Kemp, 1997; Petersen, Sanford, and Kemp, 1998) to keep the organic content small, which, however, has produced cement-like hardened sediments and resulted in alternating patches of coarse and fine sediment (Petersen, Chen, and Kemp, 1997; Petersen, Sanford, and Kemp, 1998).

One useful technique for large-scale sediment defaunation and "equilibration" for mesocosm experiments was illustrated in our treatment with HD-m sediment. Natural sediments are collected, moved into a large dark tank, and rendered anaerobic for 3 to 4 days to trigger macroinfauna to come to the surface; then, the top 10 cm of sediments with macroinfauna are removed. Any macroinfauna that does not reach the sediment surface will be killed and decompose at depths that should not affect the sediment nutrient and gas flux rates into the water column of the system. These defaunated sediments in flux cores can then be equilibrated for a 2-week period in a water basin with filtered water. For experiments in enclosed mesocosms in which no flow-through water stream, such as in our holding tank, can be established, an overlying water column of about 20 cm depth should be added carefully to the tanks, and half of the water should be replaced daily for about 14 days to flush out any ammonium released from the sediments into the water. The sediments in the tanks must be kept in the dark at all times. After the equilibration period, a treatment of choice can be added to the sediments and the mesocosm experiments begun. This homogenized deep sediment treatment has subsequently been used for all our ecosystem experiments that included sediment (e.g., Kim et al., 2004; Porter et al., 2004a, 2004b).

We tested a number of important and commonly used sediment manipulations and defaunation techniques but did not include sediment manipulation techniques such as freezing or irradiation. Freezing of sediments (Bruckner et al., 1995; Smith and Brumsickle, 1989) or sediment irradiation (Golde et al., 1994) does not cause the kind of major disturbance to porewaters, sulfides, and labile organic matter observed in the homogenized and sieved sediments (Bruckner et al., 1995). However, freezing or irradiation treatments can introduce further artifacts because of expansion of porewater spaces in frozen sediments and because of the killing of not only macroinfauna but also meiofauna and all microbial populations in the irradiated sediments. We chose natural sediments for our sediment manipulations because they included natural initial inoculations of microbial populations that, presumably, should lead to a faster re-establishment of natural microbial populations and gradients. Further comparative experiments should be done to compare the effect of freezing, irradiation, and the sediment preparation techniques described here on nutrient and gas fluxes over time.

Conclusions and Recommendations for Sediment Preparation

After about 2 weeks, different defaunation approaches yielded relatively constant nutrient and gas flux rates, presumably after the re-establishment of microbial communities and biogeochemical gradients. Additions of macroinvertebrates and subsequent reactions in defaunated sediments occurred on the surface portion of the sediments; thus, it is important to represent biogeochemical processes at the surface of the sediment adequately. High initial effluxes of ammonium from homogenized sediments must be a concern in experimental ecosystem studies because they could represent a temporal artifact. High initial fluxes might mask the treatment additions or could be misinterpreted as the effect of the treatment of interest. Sieved sediments displayed the lowest overall effluxes and changes in flux rates over time and are a good choice for defaunating sediments if only small amounts of sediments are needed. If large amounts of defaunated and equilibrated sediments are needed, natural surface sediments should be collected and the macrofauna driven to the surface by anoxia and removed. A 2-week sediment equilibration period in the experimental ecosystems accompanied by a daily partial flushing of the overlying water leads to a removal of the initial nutrients released from the sediments. Overall, sediment manipulations result in disruption of biogeochemical processes; a period of equilibration is required before pertinent biogeochemical processes can be studied.

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LITERATURE CITED


