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Ecosystem effects of materials proposed for thin-layer capping of contaminated sediments

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ABSTRACT: Ecotoxicological effects of 2 carbonaceous and 7 mineral capping materials suggested for *in situ* remediation of contaminated sediments in the Grenland fjords, Norway, were investigated in a mesocosm experiment. The primary objective was to compare the various materials with regard to potentially harmful effects on the benthic ecosystem. The materials assessed were activated carbon, Kraft-lignin, sand and clay materials, and 3 industrial by-products. Using sediment box-core samples with intact benthic communities, effects on structural (bacterial, macro- and meiofauna diversity) and functional (sediment-to-water nutrient fluxes, oxygen fluxes and bacterial production) endpoints were assessed. Significant deviations from the control (no capping) were detected for all of the tested materials for at least one endpoint. Generally, materials similar to the indigenous sediment (clay, sand) had relatively low deviations from the control, whereas industrial products (plaster, 2 types of crushed marble) resulted in deviations for most endpoints and large reductions in community richness and abundance. For example, at the end of the experimental period, the number of macrofauna taxa was <10 in these treatments, compared to >27 in uncapped mesocosm and field control sediments. The results from the study show that reducing harmful ecosystem effects from thin-layer capping by selecting capping materials based on robust, multi-endpoint mesocosm bench-tests is both possible and recommendable. Potential ecosystem impacts are particularly important to consider when large areas and areas with adequate ecological status are considered for thin-layer capping.

KEY WORDS: Remediation *in situ* · Activated carbon · Clay · Structural and functional effects · Benthic organisms · Sediment · Mesocosm

INTRODUCTION

The benthic sediment ecosystem is one of the largest habitats on Earth, and provides essential ecological functions, such as degradation of organic matter and contaminants and recycling of nutrients. These functions are essential for the sustainability and productivity of aquatic ecosystems (Wall 2004). Aquatic sediments are often affected by anthropogenic pollution, and different remediation actions may be required in order to attenuate toxic effects. Traditionally, either dredging or capping with a thick

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(30 to 50 cm) layer of uncontaminated sediment has been used for remediation of contaminated sediments. Dredging, however, is associated with resuspension of large amounts of contaminated particles, which can have negative effects on biota as well as transport and deposition costs of the dredged materials (Yell & Riddell 1995). Capping with thick layers of sand and sediment has been used as an alternative remediation strategy to dredging but this method requires large amounts of capping material and may have severe negative effects on the covered sea floor (Bolam et al. 2006, Ware et al. 2010).

Thin-layer capping *in situ* is a recent technique that uses a thinner layer of capping material (i.e. 2 to 5 cm instead of 50 cm) with a high sorption capacity for the contaminants. A reduction in contaminant bioavailability and sediment-to-water fluxes has been observed in laboratory and small-scale field studies using various carbonaceous materials, mainly activated carbon (AC) (Ghosh et al. 2011). Some laboratory studies have reported moderate negative effects on benthic organisms from thin-layer capping, such as disrupted feeding behavior, reduced growth rate and increased mortality on individual species (Millward et al. 2005, McLeod et al. 2008, Jonker et al. 2009, Paller & Knox 2010). Available field studies show contradicting results; for example Cho et al. (2009) observed no negative impacts and Cornelissen et al. (2011) observed significant impacts on the benthic community by AC capping treatment. In light of these results, concern has been raised about possible unwanted effects on benthic communities. More information on potential negative impacts of the capping materials as well as their positive effects is needed before this remediation technique is applied at larger scales in the field.

To gain more information on the potential negative impacts, a mesocosm experiment was performed with benthic communities from the Grenland fjord system, Norway. This fjord system is contaminated with high concentrations of dioxins released from historic industrial activities (Breedveld et al. 2010). Remediation measures have been discussed, but due to the large area (20 to 40 km^2) traditional remediation measures such as dredging and thick capping are not possible.

The present study investigates the ecological effects of thin-layer capping on a whole benthic ecosystem using both functional (e.g. nutrient fluxes) and structural (bacterial and faunal community composition) endpoints. Nine capping material candidates were tested for potential negative effects. The remediation effectiveness in terms of contaminant retention, i.e. capping efficiency, was not measured in this experiment but is the focus of related companion studies. A conservation assessment approach was used, i.e. any deviation from an uncapped control was considered to be a negative effect.

MATERIALS AND METHODS

Experimental set-up

The experiment was performed using facilities and principles described in Berge et al. (1986), Schaanning et al. (2008) and Trannum et al. (2010). Box cores with undisturbed sediment were collected on October 3–4 2007 at 80 m depth in Langangsfjorden (59° 02′ 30″ N, 9° 47′ 15″ E), one of the outer Grenland fjords in SE Norway, using a 0.1 m^2 box corer (KC Denmark A/S) and transported to the Solbergstrand Marine Research Station. Forty box-core samples were collected using transparent polycarbonate inner liners. On board, bottoms were added to the liners by inserting a 1 mm stainless steel plate at the base of the box corer, and the liners were taken out from the corer, as aquaria or 'boxcosms' with intact sediment fauna. During the 8–36 h storage on deck and transport to the research station, the boxcosms were covered with lids and black plastic to shield them from dust and sunlight, and the overlying water was siphoned off to reduce sediment resuspension. The 40 boxcosms were randomly allocated to 10 treatments (9 capping materials and 1 control without capping), with 4 replicate boxcosms per treatment. In addition, 4 samples were collected adjacent to the box-core collection site for initial field controls (FC) of sediment macrofaunal and bacterial community analyses. In the laboratory the boxcosms were randomly allocated to 3 large water containers that were continuously flushed with clean seawater pumped from 60 m depth, in order to maintain the boxcosms at in situ temperature. The water temperature was 8 to 10°C and salinity 34. The same water was also pumped into a header tank, from which multichannel peristaltic pumps supplied the boxcosms with a continuous flow of 14 to 26 ml min^{-1} of seawater. Submerged aquaria pumps placed under the lid of each boxcosm ensured a well-mixed water layer. Sediments were capped (see 'Capping materials') and then monitored for 137 d. The boxcosms were supplied with microalgae as food on 3 occasions, 8, 12 and 16 wk respectively, after the addition of capping material. On each occasion, 20 ml of a mixture of marine microalgae (Shellfish Diet 1800®, Reed Mariculture) was added, corresponding to 8.8 g

C m^{-2} (in the same range of magnitude as natural organic material deposited during phytoplankton spring bloom pulses). During the experiment water samples were taken for nutrient flux measurements, dissolved O_2 measurements were made and sediment O_2 depth profiles were also measured (see 'Oxygen penetration depth in the sediment', below). At the end of the experiment, samples were taken for meiofauna (animals ranging in size between macrofauna and 40 µm) and bacteria analyses and the remaining sediment was sieved (1 mm) for macrofauna analyses. See Table S1 (in the supplement at www.int-res.com/articles/suppl/m449p027_supp.pdf) for a time schedule of the sampling.

Capping materials

On Day 0 of the experiment, 36 of the boxcosms were treated with capping materials (Table 1). The materials had been chosen as potential capping materials for the remediation of the Grenland fjords. Prerequisites were cost-effectiveness and availability in sufficient amounts for remediation of large areas (several km^2). The amount of capping material (thickness) in each treatment was selected based on previous performance in laboratory studies (activated carbon and lignin) and the amounts of material that could be readily deposited in the field in a large remediation operation. The following materials were used: suspended clay (CS), cut clay (CC), sand (SA), hyperite (HY), activated carbon (AC), Kraft-lignin (LG), coarse marble (MC), fine marble (MF) and plaster (PL).

CS consisted of a 20 mm thick capping layer of preglacial clay originating from road construction excavations. CS was added by suspending clay in seawater before mixing the suspension into the water column of each boxcosm ca. 10 cm above the sediment surface. CC consisted of the same clay as CS but in small (ca. 1 cm^3) lumps of cut clay. A 20 mm thick layer of lumps was produced by pressing clay through a 1 cm stainless steel mesh over the surface of the boxcosm. The lumps of clay sank rapidly and degraded slowly over time. At the end of the experiment, the lumps were partially decomposed. SA consisted of sand collected from a terrestrial sand pit at Drøbak, Norway. A 20 mm thick layer of sand was added using the same methodology as for the CS treatment. HY consisted of a crushed mineral material obtained from a local stamp mill (SECORA AS). A 20 mm thick layer of hyperite was added using the same methodology as for the CS treatment. AC con-

Table 1. Treatments and capping materials used in the experiment, and their respective abbreviations. Grain size classification (except for AC and LG) — sand: 63–2000 µm; silt: 4–63 µm; clay: <4 µm. TOC: total organic content

Treatment	Definition	Material added	Approx. layer thickness (mm)	Grain size distribution (% sand:% silt:% clay)	TOC (% dry wt ± SD)
FC	Field control	–	–	7:48:45	2.7 ± 0.3
CT	Control	No addition	–	7:48:45	2.7 ± 0.3
CS	Clay, suspended	Suspension of pre-glacial clay collected from the Oslo fjord	20	2:50:48	0.8 ± 0.0
CC	Clay, cut	Lumps, ca. 1 cm^3 pre-glacial clay (same as CS)	20	2:50:48	0.8 ± 0.0
SA	Sand	Natural sand (moraine deposits)	20	100:0:0	0.2 ± 0.1
HY	Hyperite	Machine-processed hyperite stone (Gabbro) from Hedmark, Norway	20	87:11:2	0.3 ± 0.1
AC	Activated carbon	Powdered (1 kg m^{-2}) in saturated NaCl solution	1–2	>90%: <180 µm; 80%: <40 µm	84.0 ± 2.0
LG	Kraft-lignin	Processed lignin suspended with clay in seawater (50/50 clay/lignin) (2 kg lignin m^{-2})	10	43%: 40–100 µm 57%: <40 µm (same clay as CS)	63.0 ± 1.0
MC	Marble, coarse	$CaCO_3$ residues from paper production, regularly discharged in suspension to the fjord recipient	20	88:12:0	0.3 ± 0.0
MF	Marble, fine	As MC, but a finer fraction	20	12:60:28	0.0 ± 0.0
PL	Plaster	Gypsum plaster, $CaSO_4$, produced from H_2SO_4 and $CaCO_3$ as an industrial waste product	20	14:69:17	0.5 ± 0.1

sisted of Silcarbon TH90 Extra, a type of powdered activated carbon (Clairs). The used powdered carbon originates from coconut shells and has an average particle size of 20 µm. A 1 to 2 mm thick layer of AC (1 kg C m^{-2}, dry wt) was produced by suspending powdered carbon in a 100 g l^{-1} NaCl solution and thereafter adding it on the sediment surface. LG consisted of coniferous Kraft-lignin obtained from the black liquor of paper-pulping processing (Innventia AB) mixed with clay. The Kraft-lignin used is obtained through a refining procedure called LignoBoost (Öhman 2006) and had an average molecular weight of 4500 g mol^{-1}. The clay used was the same as for the CS treatment and was mixed with Kraft-lignin in a 50:50 weight ratio. The LG mixture was added in the same way as CS in a 10 mm thick capping layer (ca. 2 kg wood lignin m^{-2}, dry wt). MC and MF consisted of 2 marble materials of differing grain size and was supplied by Hustadmarmor AS. A 20 mm thick layer of marble was added using the same methodology as for the CS treatment for both MC and MF treatments. PL consisted of gypsum plaster produced by limestone neutralization of industrial acid waste and was supplied by NOAH AS. A 20 mm thick layer of plaster was added using the same methodology as for the CS treatment.

Following the addition of all capping materials, the water pumps and water flow were stopped for 24 h in order to allow the capping materials to settle.

Structural endpoints

Macrofauna community

At the end of the experiment, the sediment from each boxcosm was passed through a 1 mm sieve. The obtained sample was preserved with 10% buffered formaldehyde and later transferred to 75–80% ethanol. The organisms were identified to the lowest possible taxonomic level. The 4 field control samples were collected using a 0.1 m^2 van Veen grab, sieved and preserved during field collection and analyzed following the same procedures.

Meiofauna community

Two sediment cores were sampled per boxcosm using perspex tubes with a sampling area of 16.7 cm^2 (samples taken before the macrofauna sampling). The top 5 cm of each tube was collected, passed sequentially through 500 and 40 µm sieves and preserved in 40% ethanol. The meiofauna was extracted from the 40 µm sediment fraction using density extraction with Levasil (Starck Sol) at a specific gravity of 1.15 repeated 3 times (Näslund et al. 2010). Extracted meiofauna were sorted, counted and identified to major taxa under a 50× ocular stereomicroscope. Foraminifera were abundant in all samples but were excluded from analyses as the density extraction does not work well for foraminifers, and determining if they were alive or dead at the time of sampling was not possible. Any macrofauna found in the meiofauna samples were added to the macrofauna counts.

Bacterial community

Approximately 1.5 ml of surface sediment (0 to 2 mm) was collected from each boxcosm into Eppendorf tubes using sterilized equipment. The samples were immediately frozen at −20°C and kept there until analysis. The diversity of the sediment bacterial community was assessed using terminal restriction fragment length polymorphism analysis (T-RFLP) of the bacterial 16S rRNA gene. The method is commonly used to acquire a genomic fingerprint of communities, giving estimates of the most dominant community members present and their relative abundances (Schütte et al. 2008). T-RFLP analyses were performed according to Näslund et al. (2008) with the following exceptions. The DNA extractions were done using the Powersoil kit (MO BIO Laboratories) with a FastPrep beadbeater (MP Biomedicals) at a speed of 5.5 m s^{-1} for 1 min, and cutting with a restriction enzyme was performed using MspI (Fermentas). The size determinations of labeled fragments were made using an ABI3730XL DNA analyzer (Applied Biosystems) at Uppsala Genome Center, Sweden.

Functional endpoints

Bacterial production

Bacterial production in the surface sediment was measured by determining the bacterial leucine incorporation. Sediment samples were taken using the same procedure as for the bacterial community samples (see 'Bacterial community'). The procedures of van Duyl & Kop (1994) were followed with the exceptions that Ultima Gold (Perkin Elmer) was used as scintillation fluid and [^{3}H]-counts were performed using a Rackbeta 1214 Liquid Scintillation Counter (LKB Wallac).

Biogeochemical fluxes

Oxygen and nutrient fluxes (J) were calculated from the concentration difference in the water flowing in and out of each box: $J = (C_i - C_0) \times Q / A$, in which C_i is the concentration in the header tank, C_0 is the concentration in the water column in the boxcosm, Q is the flow of water through the box, and A is the sediment area of the boxcosm (Trannum et al. 2010). Flux measurements were integrated over time and divided by the duration of the experiment (no. of days) to calculate mean flux per day. Water samples were collected for analyses of nitrate (NO_3^-), ammonium (NH_4^+), silicate (H_4SiO_4) and phosphate (PO_4^{3-}). Samples for analyses of nitrate, ammonium and phosphate were preserved with sulfuric acid. All samples were stored in darkness at −20°C until analyzed at the Norwegian Institute for Water Research (NIVA) laboratory in Oslo with automated spectrophotometric methods according to Grasshoff et al. (1983). Nitrite was not determined separately; hence the concentrations and fluxes given for the nutrient NO_3^- represent the sum of nitrate (NO_3^-) and nitrite (NO_2^-) ions. An oxygen electrode (Oxi 340i, WTW) was used to measure the concentration difference in O_2 between the header tank and the overlying water in each box. Water samples were collected several times during the experiment (see Table S1 in the supplement for the sampling schedule).

Oxygen penetration depth in the sediment

In the sediment, oxygen was determined *in situ* at 250 µm depth intervals using a microelectrode with a tip diameter of 100 µm (Ox-100, Unisense A/S). The electrode was mounted on a motorized micromanipulator attached to a frame, which was moved from one box to the next. O_2 profiles were recorded starting 4 mm above the sediment surface and into the sediment down to zero O_2 level. O_2 sediment penetration depth was defined as the depth of 5% oxygen saturation. Measurements were only made for 2 to 3 profiles in 1 replicate per treatment at 8 time points due to logistical constraints.

Statistical analyses

Differences among treatments were analyzed using permutational analysis of variance (PERMANOVA; Anderson 2001, McArdle & Anderson 2001) with the program PERMANOVA. Compared to the commonly used analysis of variance (ANOVA) or multivariate analysis of variance (MANOVA) if multiple variables are included, PERMANOVA offers the advantages of the possibility to use other distance measurements than Euclidian (e.g. Bray-Curtis dissimilarity) and of calculating probability values using permutations, instead of relying on tabled p-values (which requires that data are normally distributed). If data are normally distributed and Euclidian distance measurement is used, the resulting p-values are identical to those obtained in a traditional ANOVA. For the PERMANOVA analyses, Monte-Carlo sampling was used due to the low number of replicates. Bray-Curtis dissimilarity was used for community data (macrofauna, meiofauna and bacteria) and Euclidian distance for other endpoints (abundance, species richness, bacterial production and biogeochemical fluxes). The Bray-Curtis dissimilarity integrates both taxa and their respective abundances to calculate dissimilarity between samples. As post hoc analysis for the PERMANOVA tests, treatments with capping materials were compared pair-wise to the control treatment (CT) using the same procedures (equivalent to Dunnet's post hoc test in a traditional ANOVA). Macro- and meiofauna community data were $\log(x + 1)$ transformed, and the statistical significance level was set to 0.05 for all analyses. Non-metric multidimensional scaling (nMDS) using Bray-Curtis dissimilarity indices was performed in PAST 2.02 in order to visualize differences between communities simplified into a 2-dimensional space. In general, 2 samples located far from each other in the nMDS plot are more dissimilar than 2 samples located close to each other.

RESULTS

Structural endpoints

Macrofauna

At the end of the experiment there was a significant treatment effect (global $p < 0.05$) on the macrofauna community composition, abundance and richness (Figs. 1a & 2a, Table 2). Significant differences in macrofauna community composition compared to the control treatment were found for plaster ($p = 0.004$), fine marble ($p = 0.014$), coarse marble ($p = 0.022$) and lignin ($p = 0.041$) (Table 2). Comparisons of macrofauna richness revealed large reductions in number of taxa in all of the capping treatments, except for sand, which did not differ significantly from the control (Table 2). Plaster, fine

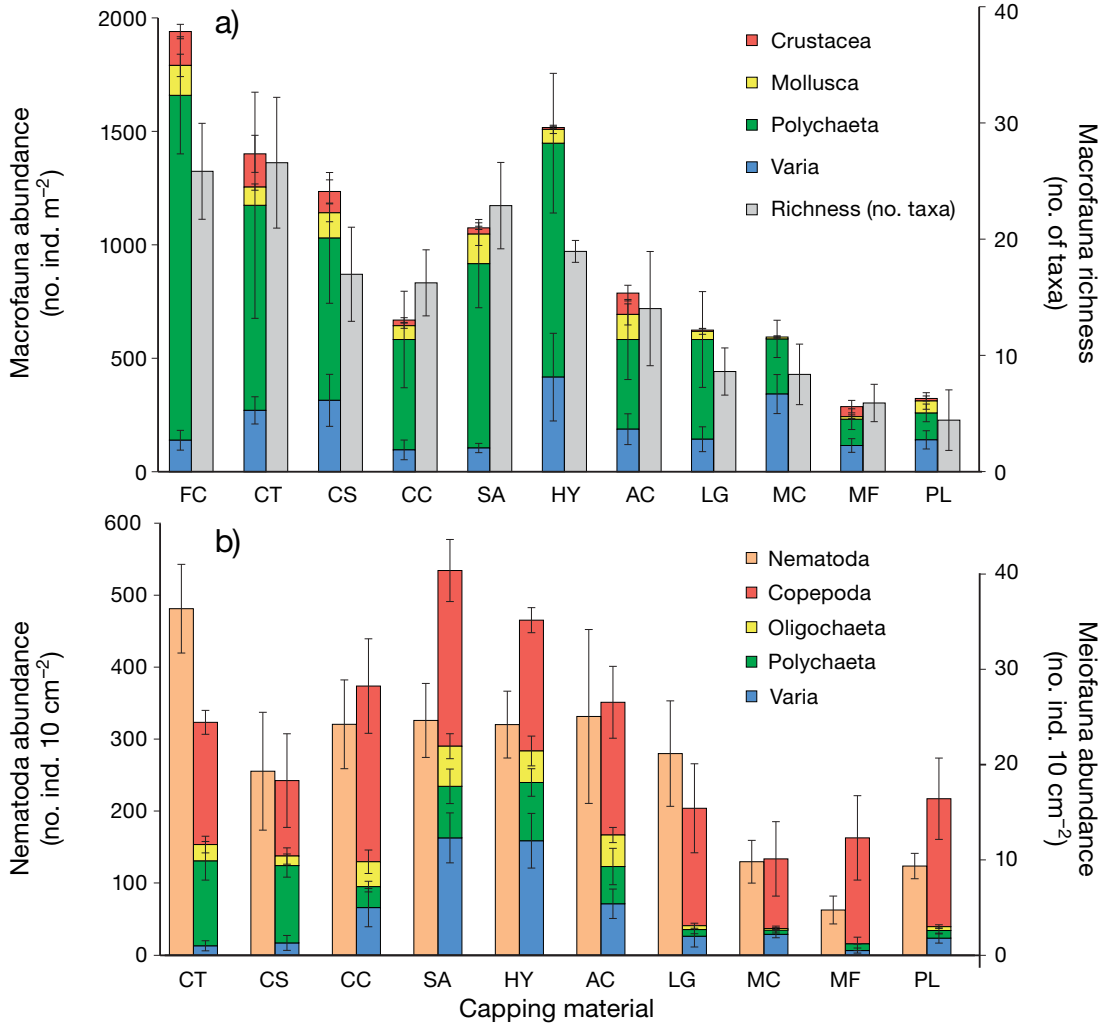

Fig. 1. (a) Macrofauna communities (coloured bars: abundance; grey bars: richness) and (b) meiofauna abundances in the different capping material treatments at the end of the experiment. Note the different scale used for Nematoda in (b). Data: mean ± SE. FC: field control, CT: control, CS: suspended clay, CC: cut clay, SA: sand, HY: hyperite, AC: activated carbon, LG: Kraft-lignin, MC: coarse marble, MF: fine marble, PL: plaster

marble, coarse marble and lignin showed the largest reductions in species richness compared to the control. Macrofauna abundance was lower than in the control treatment for the fine marble ($p = 0.03$) and plaster ($p = 0.03$) treatments. The control treatment was not significantly different from the field control samples for any of the macrofauna endpoints (Fig. 1a). This indicates that the experimental system was successful in maintaining the macrobenthic communities in conditions that approximated those of the field for more than 20 wk. See Table S2 (in the supplement at www.int-res.com/articles/suppl/m449p027_supp.pdf) for a complete list of taxa observed in the various treatments at the end of the experiment.

Meiofauna

A significant treatment effect was found on meiofauna abundance (PERMANOVA, $p = 0.0046$) (Fig. 1b, Table 2). Meiofauna abundance was lower in the plaster ($p = 0.005$), fine marble ($p = 0.002$) and coarse marble ($p = 0.006$) treatments than in the control treatment. The greatest reductions in abundance were observed for nematodes and polychaetes (Fig. 1b). A few treatments generated large increases in specific groups of fauna compared to the control, e.g. sand and hyperite, where rotifers were more abundant than in the control (although not leading to significant changes at the community level). Meiofauna community composition also differed signifi-

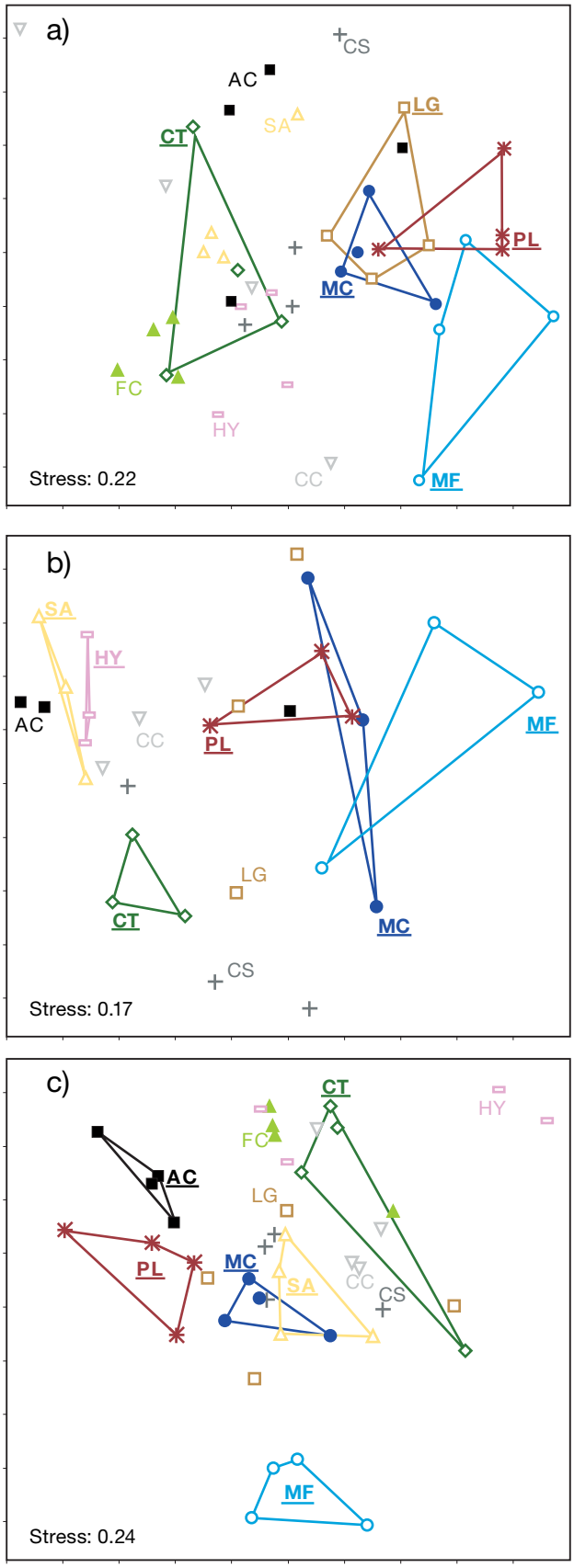

Fig. 2. Non-metric multidimensional scaling (nMDS) plots of (a) macrofauna, (b) meiofauna and (c) bacterial communities. The figures show community dissimilarities among samples, where 2 samples close to each other are more similar than 2 samples located far from each other. Untreated controls (CT) and capping treatments with significantly different communities from the CT treatment (PERMANOVA, $p < 0.05$) are depicted with polygons. See Fig. 1 legend for treatment abbreviations

cantly among treatments (PERMANOVA, p = 0.0002) (Fig. 2b, Table 2). Compared to the control, differences in meiofauna community composition were found for sand (p = 0.046), hyperite (p = 0.015), plaster (p = 0.030), fine marble (p = 0.022) and coarse marble (p = 0.045). Note that no meiofauna samples were taken for the field control samples. See Table S2 in the Supplement for a complete list of taxa observed at the end of the experiment.

Bacterial community

The bacterial communities differed among treatments (PERMANOVA, p = 0.0001). Activated carbon (p = 0.005), plaster (p = 0.019), fine marble (p = 0.008), coarse marble (p = 0.013) and sand (p = 0.049) had altered bacterial communities compared to the control (Fig. 2c). The control treatment was not significantly different from the field control samples. See Table S2 in the supplement for a complete list of observed taxonomical units (restriction fragments) in the various treatments at the end of the experiment.

Functional endpoints

Bacterial production

Significant differences among treatments were also found in bacterial production (PERMANOVA, p = 0.0001) (Fig. 3a). Activated carbon (p = 0.004), plaster (p = 0.018), fine marble (p = 0.001) and coarse marble (p = 0.001) had an increased bacterial production compared to the control.

Biogeochemical fluxes

There were significant differences in phosphate fluxes among treatments (PERMANOVA, p = 0.028) (Fig. 3b). Two treatments, plaster (p = 0.013) and fine

Table 2. Post hoc comparisons between uncapped control (CT) and other treatments after permutational analysis of variance (PERMANOVA), showing p-values from global tests (difference among all treatments) and pair-wise comparisons with the control treatment (cf. Dunnett's test). Significant differences ($p < 0.05$) from control in **bold**. Comm.: community, Abun.: abundance, Rich.: richness. C-Prod.: carbon production, na: not analyzed, (–): not applicable. See Table 1 for treatment abbreviations

Treatment	p-values												O_2 depth (mm)[a]	No. of signif.[b]
	Macrofauna			Meiofauna		Bacteria		Nutrient Flux						
	Comm.	Abun.	Rich.	Comm.	Abun.	Comm.	C-Prod.	PO_4^{3-}	H_4SiO_4	NH_4^+	NO_3^-	O_2		
FC	0.126	0.484	0.89	na	na	0.127	na	na	na	na	na	na	na	0
CS	0.510	0.729	**0.017**	0.370	0.097	0.082	0.104	0.432	**0.005**	0.070	0.678	0.743	5.3	2
CC	0.464	0.338	**0.031**	0.094	0.144	0.062	0.875	0.560	0.057	0.415	0.494	0.248	5.3	1
SA	0.334	0.885	0.313	**0.046**	0.154	**0.049**	0.148	0.277	0.213	0.744	0.487	0.816	16.3	2
HY	0.302	0.913	**0.040**	**0.015**	0.106	0.191	0.685	0.863	0.108	0.259	0.897	0.875	7.0	2
AC	0.216	0.423	**0.013**	0.061	0.356	**0.005**	**0.003**	0.556	0.056	0.950	0.213	0.903	4.6	3
LG	**0.041**	0.225	**0.001**	0.198	0.077	0.063	0.633	0.157	**0.039**	0.575	**0.002**	**0.024**	1.7	5
MC	**0.022**	0.200	**0.001**	**0.045**	**0.004**	**0.013**	**0.001**	0.349	**0.001**	0.848	0.435	0.707	4.0	7
MF	**0.014**	**0.030**	**0.001**	**0.022**	**0.002**	**0.008**	**0.001**	**0.009**	**0.001**	0.573	**0.009**	0.714	2.6	10
PL	**0.004**	**0.027**	**0.001**	**0.030**	**0.005**	**0.019**	**0.017**	**0.013**	**0.001**	**0.023**	**0.039**	0.377	3.3	11
Global	0.0002	0.008	0.0002	0.0002	0.005	0.0002	0.0002	0.027	0.0002	0.026	0.003	0.362	–	–
Replicates	4	4	4	3	3	4	4	4	4	4	4	4	1	–

[a]Average O_2 penetration depth; a statistical test was not possible for O_2 penetration depth in sediment as measurements were made in single replicates repeated throughout the experiment. The uncapped control had an O_2 penetration depth of 6.3 mm

[b]Total number of significant differences for the 12 endpoints in pair-wise comparisons with uncapped control

marble ($p = 0.009$), had lower releases of phosphate from sediment to water than the control. Lignin and sand had high phosphate fluxes compared to the control, but this was not statistically significant ($p = 0.157$ and $p = 0.277$ respectively).

Differences were also found in fluxes of silicate (PERMANOVA, $p = 0.0002$) (Fig. 3c). All capping treatments had low releases of silicate compared to the control. The largest differences occurred for plaster ($p = 0.001$) and for the 2 marble treatments (both $p = 0.001$).

There were also differences in ammonium fluxes among treatments (PERMANOVA, $p = 0.0264$). Compared to the control, a small increase in the release of ammonium was observed in most treatments, but only the plaster treatment caused a significant increase ($p = 0.023$) with more than double the flux of the control (Fig. 3d).

There were also significant differences in nitrate fluxes among the treatments (PERMANOVA, $p = 0.0026$). Compared to the control, an elevated negative flux of nitrate was found for plaster ($p = 0.039$), fine marble ($p = 0.009$) and lignin ($p = 0.002$) (Fig. 3e).

The fluxes of oxygen showed no significant difference among treatments (PERMANOVA, $p = 0.362$) (Fig. 3f). In the pair-wise comparison to the control, lignin had a higher oxygen flux than the control ($p = 0.024$)

Oxygen penetration depth in the sediment

Sediment oxygen profiles showed a lower penetration depth for oxygen in the lignin treatment (Table 2), although this could not be tested with inferential statistics due to low replication. Oxygen penetration depth was lower than the control for plaster and both marble treatments also (Table 2). The relatively coarse grained materials hyperite and sand had the 2 largest oxygen penetration depths (Table 2). Generally, the oxygen penetration depth decreased with time and after additions of algae (data not shown).

Pair-wise comparisons of capping treatments versus control

The control treatment was not significantly different from the field control for the endpoints where this comparison was possible (Table 2). Among the capping treatments, suspended clay, cut clay, sand and hyperite differed from the control for 1 to 2 out of 12 measured endpoints. Activated carbon and lignin differed from the control for 3 to 5 endpoints. Coarse marble differed from the control for 7 endpoints, and fine marble and plaster for 10 and 11 endpoints, respectively.

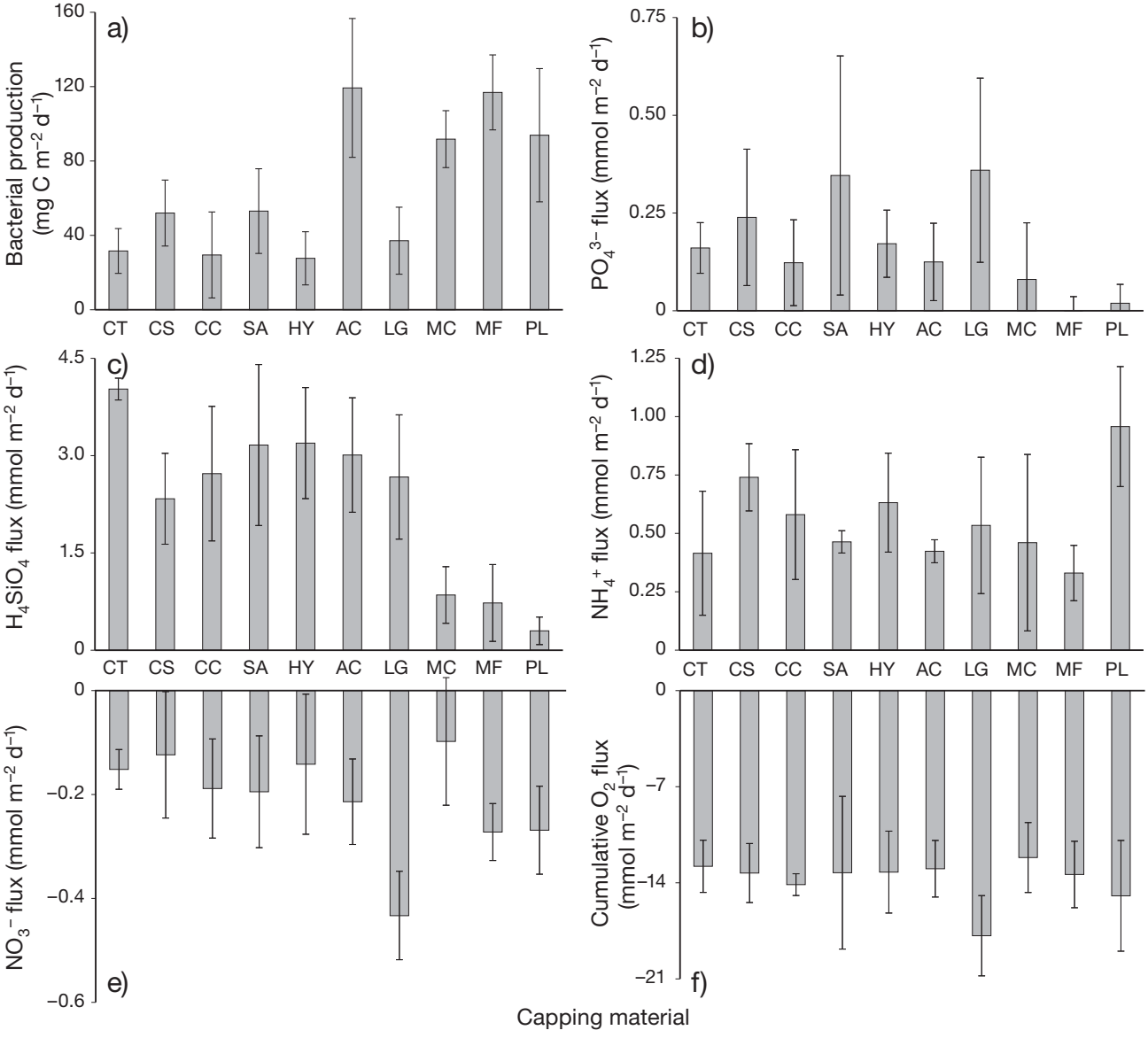

Fig. 3. Functional endpoints: (a) bacterial production at the end of the experiment, (b) phosphate (PO_4^{3-}) flux, (c) silicate (H_4SiO_4) flux, (d) ammonium (NH_4^+) flux, (e) nitrate (NO_3^-) flux, and (f) oxygen (O_2) flux. Data: mean ± SD. See Fig. 1 for treatment abbreviations

DISCUSSION

The results from this study clearly demonstrate that thin-layer capping with various capping materials can cause negative effects on benthic ecosystems and significantly change both structural (e.g. community composition) and functional endpoints (e.g. nutrient fluxes). The general reduction in macrofauna abundance and richness is the most obvious effect of the capping treatments. Even the addition of a thin layer of clean CS, seemingly a moderate disturbance, decreased the biodiversity (i.e. macrofauna and meiofauna richness) during the experimental period of 137 d. The 3 industrial processed mineral products (MC, MF and PL) had the most severe effects on macrofauna richness (number of taxa) and abundance (number of individuals) compared to the control. The toxicity of MC and MF was recently confirmed in a standard growth test on *Skeletonema costatum* (Källqvist 2008), and the toxicity was concluded to have likely been caused by residues of surface-active processing chemicals (i.e. tensides). These toxic residues are therefore likely to have been partly responsible for effects observed in the

marble treatments. The toxicity of PL may have resulted from an initial oxidation of $FeSO_4$ ($FeSO_4$ + ¼O_2 + $H_2O \rightarrow Fe^{3+} + OH^- + SO4^{2-}$), which on Day 1, during a few hours between cap addition and initialization of the water exchange, produced a large reduction of the concentration of O_2 in the overlying water and a simultaneous increase of pH (data not shown). Yellow precipitates (observed on the sediment surface on Day 1) indicated that the initial ferrous iron oxidation was succeeded by extensive formation of ferric iron oxyhydroxides ($Fe_3^+ + 2H_2O \rightarrow FeOOH + 3H^+$). The water circulation had by then been re-initiated, and the predicted pH-decline in the overlying water could not be confirmed.

Charcoal has been shown to influence soil bacterial communities (Pietikäinen et al. 2000), likely through considerable increase in surface area and presence of large amounts of pores and crevices serving as colonizing areas for microorganisms. A similar mechanism may have been responsible for the observed effects on bacterial community structure in the AC treatment. Another interesting result for the structural endpoints was that community responses to the different capping materials were largely similar at all 3 levels of organization studied (i.e. macrofauna, meiofauna and microorganisms). The general deviation from the control seen for MC, MF and PL in all 3 measured community endpoints highlights a considerable risk for long-term perturbations, as immigration of lost species is needed to restore disturbed communities.

The possible long-term effects on benthic communities were not studied in this experiment but should also be considered. This should be made by evaluating possible effects on population level, as well as estimations of how long it will take for benthic communities to recover after placement of a capping layer. Our work was thus limited to the initial effects of the capping layers used in the experiment. Mixed capping layers, as well as capping layers with a natural sediment layer on top, could lead to less impact on the benthic ecosystem.

The observed effects of the capping materials on infauna (e.g. both macrofauna and meiofauna) with reductions in abundance and richness are likely to have influenced other functional endpoints such as bacterial production, oxygen and nutrient fluxes. For example, the presence of benthic fauna (Aller 1982) as well as species diversity (Karlson et al. 2010) is important for oxygen penetration in sediments, and conversely, low oxygen levels usually affect benthic communities negatively (Diaz & Rosenberg 1995, 2008). Moreover, the elevated bacterial production in PL, MC and MF coincides with decreased meiofauna and macrofauna abundances and richness. Benthic fauna is also known to affect bacterial production and microbial community structure (Wieltschnig et al. 2008, Laverock et al. 2010) and functions (e.g. degradation of organic matter and organic pollutants; van Duyl et al. 1992, Näslund et al. 2010). Low abundances of fauna, leading to unused food resources in the form of algae, may have caused the large bacterial production in MF, MC and PL, as algae remains are known to support a high bacterial production (Malm et al. 2004). The increase in bacterial production in AC, however, did not seem to be correlated to lower meio- and macrofauna abundances but could of course be related to the observed changes in bacterial community structure.

Effects caused by thin-layer capping were also found on the benthic nutrient fluxes. The decreased release of phosphate in MC, MF and PL was likely caused by reduced pore water concentrations due to active adsorption of phosphate on precipitated ferric oxide in PL (Krom & Berner 1980) and calcium containing materials in MF and MC (Eek et al. 2007). The low oxygen levels in the sediment may explain the increased release of phosphate in the LG treatment, as phosphate is released from the sediment at low oxygen conditions (Sundby et al. 1986). The low oxygen levels in LG may also explain the elevated nitrate uptake, as an increased activity from nitrate reducing microorganisms is expected at lower oxygen levels.

The considerably reduced silicate fluxes in MF, MC and PL most likely ocurred because these materials consist mainly of terrestrial calcium carbonates or sulfates, thus lacking silicate minerals and diatom skeletons that can dissolve into the water. The 20 mm cap layer of these materials also appears to represent an efficient barrier for the release of silicate from deeper sediment layers over the time span of this experiment. In previous experiments nutrient fluxes through thin cap layers have been found to decrease when cap thickness exceeded 3 to 6 mm (Schaanning et al. 2008, Trannum et al. 2010). The significantly increased ammonium flux in PL compared to that in CT may have been the result of decaying dead fauna, as mortality was high for both meio- and macrofauna in PL and dead animals were observed on the sediments surface the day after cap addition. The added material in PL had a high content of reduced chemical species (Fe_2^+, Mn_2^+) and possibly also ammonium. The release of ammonium in PL was higher at the beginning of the experiment than towards the end (data not shown), which suggests a high initial release of ammonium.

Of the 12 measured endpoints in this study, the oxygen flux was the least sensitive (although greater discrimination of oxygen levels may have been discerned with full replication throughout the experiment), with LG being the only treatment resulting in a significantly lower O_2 flux compared to CT. Lignin biodegradation is known to be related to available oxygen (Fustec et al. 1988). Although the lignin used in this experiment was an industrially processed material, which is refractory, the higher oxygen demand throughout the experiment as well as the low oxygen sediment penetration depth in LG (Table 2) suggested significant biodegradation of the lignin monomers. The other capping treatments had oxygen fluxes similar to those of the control treatment. Oxygen sediment penetration depth was lower in the PL, MF and especially the MC treatment than in CT. In PL, this may have been due to chemical oxidation, but in MF small grain size and conglutination were likely the reasons. The deep oxygen penetration depth for SA and HY showed the importance of differences in grain size yielding greater advective water movements in the interstitial space between the coarser particles. Besides direct effects from capping layers on nutrient fluxes and oxygen levels through physical and chemical mechanisms, reductions in benthic fauna may have caused additional indirect effects. Macro- and meiofaunal bioturbation (i.e. sediment reworking and bioirrigation) can have a profound effect on nutrient fluxes (Aller 1982, Aller & Aller 1992). Thus, reduced faunal abundance, such as the effects observed in LG, MC, MF and PL, may have contributed to the lower nutrient and O_2 fluxes.

Deviations from the control treatment were detected for all the tested capping materials for at least one endpoint. Generally, the more similar the capping material was to *in situ* substrate, the fewer deviations were observed with regards to the various endpoints. Hence, the almost unmodified mineral materials, CS, CC, SA and HY, caused relatively small ecological effects, compared to the industrially processed materials MC, MF and PL. The carbonaceous capping materials, AC and LG, had 3 and 5 endpoints, respectively, that differed significantly from the control. LG also had significant negative effects on the macrofauna community. A 2 mm thin AC layer significantly affected both bacterial endpoints but had less impact on the meio- and macrofauna endpoints, where only a significant decrease in macrofauna richness was found. Negative effects from AC have been described in other studies on macrofauna such as polychaetes (Millward et al. 2005), bivalves (McLeod et al. 2008) and amphipods (Jonker et al. 2009, Kupryianchyk et al. 2011). Mainly, AC seems to affect feeding behavior or other parameters related to feeding and growth. However, these effects could possibly be species-specific or depend on the type of AC used. Studies with lipid content as the only ecotoxicological endpoint did not find significant negative effects from AC (Cornelissen et al. 2006, Fagervold et al. 2010).

Based on the results of this study we consider the capping materials used in the MC, MF, PL and LG treatments to have unacceptably large effects on ecological processes and communities, and we recommend that they are not to be used in field applications unless they are significantly refined and thoroughly re-evaluated in terms of possible ecological effects on the benthic ecosystem. The ecological effects observed from activated carbon in the AC treatments are also rather alarming; there is clearly a need for further research on the long-term ecological effects of activated carbon capping and how to possibly mitigate these effects, for example by mixing AC with clay (Cornelissen et al. 2011) or performing capping operations during optimal periods of the year (e.g. directly before phytoplankton spring blooms, thus allowing natural deposition on top of the capping layer).

We have not investigated capping efficiency (i.e. decrease in sediment to water contaminant fluxes or decreases in bioaccumulation by fauna) in this experiment. Capping efficiency is naturally also of major interest as it is the positive effect that is weighed against ecological impacts. For the mineral, non-carbonaceous materials used in the experiment, a cap thickness of 20 mm is not likely to provide sufficient lowering of organic and inorganic contaminant fluxes, especially where bioturbating fauna is present (Simpson et al. 2002). The physical barrier of thicker silt and clay capping layers (5 to 50 cm) are known to reduce contaminant transfer to overlying water and biota (Brannon et al. 1987), but the negative effects will increase with increasing cap thickness (Smit et al. 2008, Trannum et al. 2010). Furthermore, the large amount of material required to yield a physical barrier in the large area considered for remediation action in the Grenland fjord area would be extremely challenging logistically. The carbonaceous capping materials (AC and LG) primarily reduce the release of organic contaminants by chemical sorption. AC has a high sorption capacity of PAHs (Cornelissen et al. 2006) and PCBs (McLeod et al. 2007), and the sorption efficiency increases with time (Werner et al. 2005), while lignin has a lower expected sorption based on the organic carbon parti-

tion coefficient K_{oc} (MacKay & Gschwend 2000). This suggests that AC likely is a preferable material to LG for capping operations, considering its fewer negative ecological effects and higher sorption capacity for hydrophobic organic contaminants. A recent laboratory study by Kupryianchyk et al. (2011) also concluded that AC addition can lead to substantial improvement of habitat quality for amphipods in severely contaminated sediment.

The primary objective of thin-layer capping operations is to reduce release of contaminants from the sediments. Results from this mesocosm experiment have shown that regardless of the cap material selected, such remediation is likely to be accompanied by harmful effects on benthic fauna, microorganisms or nutrient cycling. When considering thin-layer capping as a remediation alternative for large contaminated areas, managers must therefore consider possible mitigation measures as well as weigh expected negative effects on the ecology of the treated area against expected improvements of contaminant levels and effects. This is particularly important when areas with acceptable pre-remediation ecological status and unacceptable contaminant level status are to be treated.

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