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Long-term response of marine benthic fauna to thin-layer capping with powdered activated carbon in the Grenland fjords, Norway



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HIGHLIGHTS

Biological effects were studied nine years after capping with activated carbon (AC).

- Powdered AC reduced benthic species richness, abundance and biomass.
- Nine years after capping, the communities had still not recovered.
- Key species such as *Amphiura filiformis* were persistently eliminated.
- The impaired benthic communities were not captured in ecological status indices.

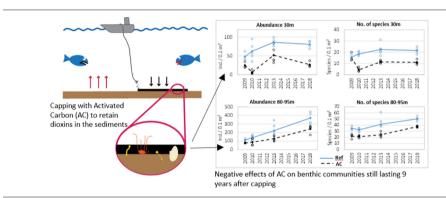
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ABSTRACT

The Grenland fjords in Norway have a long history of contamination by large emissions of dioxins and mercury. As a possible sediment remediation method in situ, thin-layer capping with powdered activated carbon (AC) mixed with clay was applied at two test sites at 30 m and 95 m depth in the Grenland fjords. This study presents long-term effects of the AC treatment on the benthic community structure, i.e. nine years after capping. Capping with AC significantly reduced the number of species, their abundance and biomass at the two test sites, compared to uncapped reference sites. At the more shallow site, the dominant brittle star species *Amphiura filiformis* disappeared shortly after capping and did not re-establish nine years after capping. At the deeper site, the AC treatment also caused long-lasting negative effects on the benthic community, but some recovery was observed after nine years. Ecological indices used to assess environmental status did not capture the impaired benthic communities caused by the capping. The present study is the first documentation of negative effects of powdered AC on marine benthic communities on a decadal scale. Our results show that the benefits of reduced contaminant bioavailability from capping with AC should be carefully weighed against the cost of long-term detrimental effects on the benthic community. More research is needed to develop a thin-layer capping material that is efficient at sequestering contaminants without being harmful to benthic species.

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1. Introduction

Marine sediments are major repositories for contaminants such as metals and persistent organic pollutants (POPs) derived from human activities (Larsson, 1985). The contaminants can be toxic for marine

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life, and pose a risk for transfer to higher trophic levels including humans (Greenfield et al., 2015). Today, new contaminant emissions are often reduced due to more strict discharge regulations; however, high concentrations of toxic substances from historic emissions are still prevailing in the environment and especially in aquatic sediments (Frid and Caswell, 2017). Leakage from contaminated sediments may therefore act as new sources of pollution in areas where the primary sources have been substantially reduced or closed down. Sediment remediation in many lakes and polluted coastal areas has become a necessity in order to decrease ecological and human health risks and to meet the requirements to good chemical and ecological status according EU's Water Framework Directive (WFD) (Council directive, 2000) and EU's Marine Strategy Framework Directive (MSFD) (Council directive, 2008).

Contaminated sediments are generally handled either by relying on natural recovery (Förstner and Apitz, 2007), by mechanical removal of contaminated sediment (dredging), or by sediment isolation through covering with clean materials (conventional capping). Recently, thinlayer capping (<10 cm) with active sorbents, such as activated carbon (AC), has been proposed as a more cost-efficient and less disruptive remediation alternative to dredging or conventional isolation capping (Ghosh et al., 2011). Carbonaceous sorbents such as AC have a strong sorption capacity for hydrophobic organic contaminants (HOCs) thus reducing their release to the water column and their bioavailability (Luthy et al., 1997; Grathwohl and Kleineidam, 2000; Cornelissen et al., 2005). As most persistent contaminants accumulate in sediments, they may pose a toxicity risk to benthic communities, i.e. sedimentliving invertebrates and microorganisms. Benthic organisms would also be most affected by disruptive sediment remediation methods. This can be devastating to the ecosystem since benthic organisms play a key role in several essential ecosystem functions, such as nutrient cycling, carbon turnover and secondary production including nutrient supply for commercial species, as well as transport and fate of pollutants (Harman et al., 2019).

With regard to thin-layer capping with AC, the macrofauna through their sediment reworking activities (bioturbation) will increase the sediment particle mixing and thus facilitate the contact between AC and the contaminants, which is assumed to increase the efficiency of the capping treatment (Ghosh et al., 2011). Although thin-layer capping has been considered less harmful to the benthic fauna than dredging and isolation capping (Ghosh et al., 2011), several studies have reported negative short-term effects of AC such as reduced growth, lower survival and reproduction (Kupryianchyk et al., 2011; Nybom et al., 2012, 2015). However, studies on more long-term effects in the field are sparse, and before thin-layer capping with strong sorbents such as AC can be recommended for more extensive use, it is important to get more knowledge not only on capping efficiency regarding contaminant sequestration, but also on eventual long-term ecological effects on benthic communities.

Sediments in the Grenland fjords, southeast Norway, have elevated concentrations of dioxins and mercury due to historic emissions from a magnesium smelter located at the head of the fjord system. Although new emissions have ceased, the sediments are still a major source of the previously released contaminants (Fagerli et al., 2016; Schaanning et al., 2021). Due to the large size of the contaminated fjords' area, dredging is not a feasible option. In 2009, a large pilot field study was set up in order to test the effects of thin-layer capping with various capping materials, i.e. powdered AC, clay and crushed limestone. The effects on contaminant fluxes and benthic fauna have previously been investigated one month, one year and four years after capping (Cornelissen et al., 2012, 2015; Samuelsson et al., 2017; Raymond et al., 2020). A new investigation was carried out nine years post capping, and effects on contaminant fluxes are reported by Schaanning et al. (2021). The present study addresses the state of the marine benthic macrofaunal communities after nine years, as well as the time trends observed throughout the nine years investigation period. Effects are determined using a combination of community parameters and evaluated using a combination of univariate and multivariate statistical methods. We also evaluate the performance of biodiversity indices currently used in benthic ecological status assessment in Norway, Sweden and the EU to assess the effects of capping with powdered AC.

2. Materials and methods

2.1. Study area

The Grenland fjord system is composed of connected fjord branches located on the Norwegian Skagerrak-coast (Fig. 1). Two of the fjord branches are the Eidangerfjord and the Ormerfjord. The sediments in the fjords have been severely contaminated with several hydrophobic organic contaminants, including dioxins and furans, by industry, shipping and other human activities. The main source of contamination was a magnesium process plant on Herøya (1951–2002, 12 kg PCDD/F-TEQ/year at most), situated in the inner part of the fjord system (Frierfjord), close to the outlet of a river (Skien River). Although the factory no longer adds new contamination to the fjord, the previous pollutant discharges are still stored in the sediment due to their persistence and hydrophobic character.

2.2. Sediment treatments and monitoring

In September 2009, two test sites in the Grenland fjords were capped with powdered AC mixed with clay; one site of 100×100 m (10 000 m²) at ca 30 m depth in the Ormerfjord and another site of $200 \times 200 \text{ m} (40,000 \text{ m}^2)$ at ca 95 m depth in the Eidangerfjord (Fig. 1). The AC capping material consisted of suction-dredged clay amended with powdered AC (Jacobi Carbons, BP2 fine powder; average particle size of 20 µm, 80% smaller than 45 µm). The target AC concentration was 10% of dry weight (d.w.) clay, which was reached in the AC mix used in the Ormerfjord, whereas the AC mix used in the Eidangerfjord reached a concentration of 7% of d.w. clay. After one month, cap thicknesses were measured to 11 ± 6 mm in the Ormerfjord and 12 ± 3 mm in the Eidangerfjord (Eek et al., 2011). A more comprehensive description of the capping materials and how they were applied can be found in Cornelissen et al. (2012). In each fjord, a reference site without capping was also established. The levels of PCDD/F and Hg are three to four times higher at the deeper Eidangerfjord compared to the shallower Ormerfjord (Samuelsson et al., 2017), but at the same time the more polluted Eidangerfjord is more diverse, presumably because of the larger depth.

Benthic macrofauna sampling was carried out one month, one year, four years and nine years after capping. Initial effects and effects four years after capping are presented in Samuelsson et al. (2017) and Raymond et al. (2020). As severe negative effects of AC were found after four years in both fjords, the long-term effects were examined in the present study, in order to observe if the benthic communities had recovered 9 years post capping. To our knowledge this is the first study that investigates the long-term ecological effects of thin-layer capping with AC, i.e. almost a decade after treatment. The capping efficiency on reduction of dioxin fluxes and bioavailability are presented in our companion papers Cornelissen et al. (2012, 2015) and Schaanning et al. (2021).

Natural variation of benthic communities between years is common in species diverse systems as in this experiment, depending on variations in environmental conditions and opportunities for e.g. spawning, settling and species competition. It is therefore required to compare the capped fields with an uncapped field, and the differences between the fields are interpreted as a capping impact. Comparisons to precapping conditions would also have been valuable, but was not included in this study because the similarity between test and reference sites before capping could be assessed based on previous long-term monitoring programs using Day-grabs (0.025 m²) for benthic fauna (Samuelsson pers. com.) and sediment profile camera image analyses (SPI) for

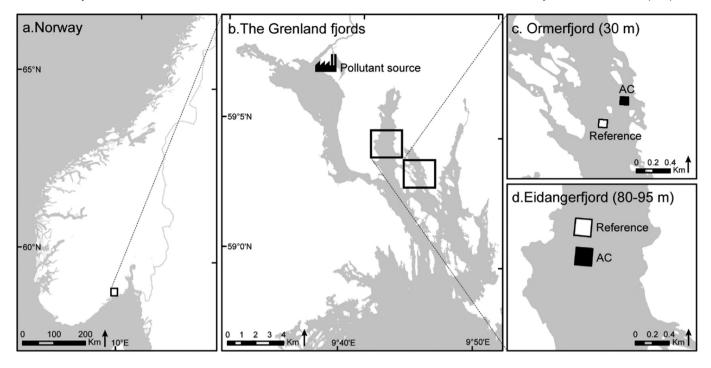


Fig. 1. Map of the Grenland fjords showing the test-sites (AC) and reference sites in Ormerfjord and Eidangerfjord, sampled in 2018.

sediment characteristics (Schaanning et al., 2011). The reference site in the Ormerfjord was at the same depth at the AC site, while in the Eidangerfjord the reference site was located at a slightly more shallow (80 m) depth in order to avoid impact from trawling in the fjord. The trawling ceased after establishment of the experiment and a second reference site at 95 m depth was established in 2010 to be compared with the reference site at 80 m depth. No major differences were found between the two reference sites after four years and thus only the reference site at 80 m depth was included in this study. For a more detailed comparison between the 80 m and 95 m reference sites see our companion paper Raymond et al. (2020).

2.3. Field work

In October 2018, i.e. nine years after capping, benthic macrofauna samples were collected with a van Veen grab (0.1 m²) from the capped sites and the untreated reference sites in the two fjords. Four replicate were taken at each location in 2018 (3–5 replicates the previous years, see Raymond et al. (2020)). The samples were sieved through 1 mm mesh size and the retained organisms were preserved in 4% buffered formaldehyde stained with Rose Bengal. Sediment cores were sampled with a Gemini-corer for analysis of sediment fine fraction and total organic carbon (TOC) and total nitrogen (TN) (0–1 cm sediment fraction). The field work was carried out in the same season as in the previous benthic investigations (October-November) and with the same equipment, following the same sample procedures. Complementary data such as water temperature and salinity were measured at each sampling campaign using CTD and a bottom water sampler (modified Niskin bottle). The bottom water temperature ranged between 12.1–14.9 and 7.0–12.0 °C in the Ormerfjord and Eidangerfjord, respectively, and the salinity between 33.2-33.6 and 34.0-34.6.

2.4. Laboratory analyses

Sediment fine fraction (% particles < 0.063 mm) was determined by wet sieving. TOC and TN were determined using a CHN (i.e. Carbon,

Hydrogen, and Nitrogen) analyser after removal of inorganic carbon by acidification.

The fauna was identified to lowest possible taxonomic level. As different institutions and persons were responsible for the previous macrofauna identification, the species lists from previous and this investigation were harmonized and checked according to the world register of marine species (WoRMS) matching tool prior to the statistical analyses. Biomass was determined in wet weight (w.w.) for each taxon and obtained by placing the organisms on a filter paper, blotted dry for a few seconds, and then placed on a new, pre-weighed filter paper and weighed with a resolution of 0.1 mg.

2.5. Benthic indices

Based on number of species and total abundance (number of individuals), five benthic indices used in the WFD monitoring system for Norwegian coastal waters (Veileder 02:2018) were compared: 1) the Shannon-Wiener diversity index (H′og²; Shannon and Weaver, 1963), 2) Hurlbert's diversity index (ES₁₀₀; Hurlbert, 1971), 3) the Norwegian Quality Index (NQI1; Rygg, 2006), 4) the Indicator Species Index (ISI₂₀₁₂; Rygg and Norling, 2013), and 5) the Norwegian Sensitivity Index (NSI; Rygg and Norling, 2013). The AZTI Marine Biotic Index (AMBI; Borja et al., 2000) was also calculated as it is a parameter in the NQI1 index. AMBI is widely used to assess ecological status in other European countries. In addition, the Benthic Quality Index (BQI; Leonardsson et al., 2009) used to assess the ecological status in Sweden was calculated.

2.6. Statistical analyses

The 'species × sample' matrix was analyzed with multivariate statistics using the Bray-Curtis similarity index (Bray and Curtis, 1957) calculated from fourth-root transformed data. A non-metric MDS-ordination was performed to visualize the patterns. PERMANOVA (global i.e. overall main test, and pairwise tests) was used as a permutation test (Anderson, 2001) with Euclidian distance to test for effects of capping with AC and time on the univariate parameters and the community

composition. Both 'year' and 'site' were set as fixed factors. Prior to PERMANOVA, the PERMDISP-test was used to check for homogeneity of variances, and data were transformed when needed; square-root for abundance, fourth-root for biomass and community, while number of species were left untransformed. Further, pair-wise comparisons between treatments were performed with the PERMANOVA t-statistics. The significance level for all statistical tests was set at $\alpha=0.05$. All analyses were done using the PRIMER package version 6.1.13 with the PERMANOVA+ version 1.0.3 add-on. The two fjords were kept separate in all analyses as they in addition to the depth differences, also are different in hydrography, sediment and benthic community structure.

3. Results

3.1. Sediment parameters

Sediment fine fraction (<0.06 mm), content of total organic carbon (TOC), total nitrogen (TN) and TOC/TN ratio of the surface sediment (0-1 cm) are presented in Table 1. The sediment was a fine-grained silt-clay sediment type, with a 70-90% range of fine particles in both fjords. The amount of TOC was higher in the Eidangerfjord (23.8 mg/g TOC) compared to the Ormerfjord (9.1 mg/g TOC), which is in agreement with the Eidangerfjord being a deep (95 m) accumulation bottom type compared to the more shallow (30 m) Ormerfjord being more of a transport bottom type. Also, the amount of total nitrogen was twice as high (2.0 mg/g TN) in the Eidangerfjord compared to the Ormerfjord (0.9 mg/g TN). This shows that the availability of organic matter and food is larger in the Eidangerfjord. The TOC content of the AC test sites was higher than in the reference sites in both fiords (3 times higher in the Ormefiord and 1.5 times higher in the Eidangerfiord). Notably. also TN was higher, thus indicating more nutrient availability at the AC sites. The TOC/TN ratio ranged from 10.1 to 12.0, and there was no systematic differences between the fjords or sites.

3.2. Fauna community structure

In total, 74 taxa were identified from 2875 individuals found in the 16 grab samples collected in 2018. The most dominant species in 2018 are listed in Table 2. Average number of species per test site ranged from 11 to 50 and average abundance (number of individuals) from 27 to 370 (per 0.1 m²) (Fig. 2). Both number of species and their abundance were higher in the Eidangerfjord than in the Ormerfjord, in accordance with the higher nutrient level found there (Section 3.1). Average biomass values at the four sites ranged from 1.6 to 13.4 g w.w. (per 0.1 m²), and there was no significant difference in biomass between the two fjords (Fig. 2). Notably, both fjords had lower number of species, abundance and biomass at the AC sites than in the reference sites, and the difference was particularly large in the Ormerfjord. Here, the total biomass was over eight times higher at the reference site than at the AC site. In the Eidangerfjord, the biomass at the reference site was almost four times higher than at the AC site. However, the standard deviations show large variation between the replicates in both sites, mainly because of the presence/absence of large species like sea urchins.

With regard to the species numbers, all four sites had almost the same number of molluscs (bivalves and gastropods) (Fig. 2). There

were more species of annelids in the Eidangerfjord than in the Ormerfjord, and there was also a tendency of more species of both free-living and tube-building annelids at the reference sites than at the AC sites. For crustaceans, similar numbers of species were found at both sites in the Eidangerfjord. The Ormerfjord had only a few crustacean species at the reference site and none at the AC site.

In the Ormerfjord, the reference site was numerically dominated by the brittle star *Amphiura filiformis*, which accounted for almost half of the total abundance (48.3%) (Table 2). This species was absent at the AC site. Sea urchins (Echinoidea), brittle stars (Ophiuroidea) and freeliving annelids dominated the biomass in the reference site (Fig. 2). The bivalve *Nucula nitidosa* dominated the AC site, with 26.2% of the total abundance, followed by the annelid *Nephtys incisa* and the gastropod *Hyala vitrea* (Table 2). Bivalves were also dominating the biomass at the AC site, which had very low or even lacked the biomass-dominating species that were found at the reference site (Fig. 2).

In the Eidangerfjord, the reference site was numerically dominated by free living and tube-building annelids, while the biomass was dominated by sea urchins (Echinoidea) followed by free-living and tube-building annelids (Fig. 2). The AC site was also numerically dominated by free-living and tube-building annelids, but tube-building annelids had a lower abundance than in the reference site. The annelid *Spiophanes kroyeri* was the most dominant species in both sites, although with twice as high abundance in the reference site (Table 2). Like in the Ormerfjord, sea urchins and brittle stars (Ophiuroidea) were more or less absent in the AC site.

3.3. Ecological status

The pronounced differences in species numbers and abundance due to the AC treatments were not always evident when comparing the benthic indices. The benthic indices used to assess the ecological status in Norwegian waters showed in general only a small degree of difference between the AC sites and the reference sites in 2018 (Fig. 3). It should be noted that the index ES₁₀₀ could not be calculated for the Ormerfjord due to the very low abundances. The index ISI₂₀₁₂ showed somewhat larger difference between the AC and reference sites, particularly in the Ormerfjord. According to the Norwegian classification, the condition was good based on NQI1 and NSI at all sites, moderate based on H' at both sites in the Ormerfjord, but good at both sites in the Eidangerfjord, good based on ISI₂₀₁₂ at the AC site in the Ormerfjord and very good at the three other sites. The Benthic Quality Index (BQI) was better at revealing differences in ecological status following AC capping, the AC site in the Ormerfjord achieved only poor/unsatisfactory status in 2018, while the reference site had moderate status (Fig. 3). The AC site in the Eidangerfjord, on the other hand, achieved high status, as did the reference site according to BQI, indicating a better recovery in the Eidangerfjord than in the Ormerfjord.

3.4. Trends in benthic communities

The nMDS-ordination of both fjords shows that for most years there was more similarity between the samples within the sites than between the sites (Fig. 4). In the Ormerfjord, the AC site showed more variation over time, and in particular the samples in 2010 were highly spread

Table 1
Sediment fine fraction (% < 0,063 mm), total organic carbon (TOC), total nitrogen (TN) and TOC/TN ratio, determined in top layer (0–1 cm) of sediments at the AC and reference sites in Ormerfjord and Eidangerfjord, 2018.

		Sediment fine fraction (%)	TOC mg/g	TN mg/g	TOC/TN
Ormerfjord	AC (30 m)	91	28.8	2.4	12.0
Ormerfjord	Ref. (30 m)	77	9.1	0.9	10.1
Eidangerfjord	AC (95 m)	74	35.0	3.1	11.3
Eidangerfjord	Ref. (80 m)	80	23.8	2.0	11.9

Table 2Mean abundance (N) of the most dominant species in the test site and reference site in Ormerfjord and Eidangerfjord (per 0.1 m²). The percentage of total abundance of the species is calculated. Taxonomic group in parenthesis, where A = Annelida, M = Mollusca, C = Crustacea, O = Ophiuroidea, N = Nemertea, P = Phoronida.

Ormerfjord AC	N % Ormerfjord Ref		Ormerfjord Ref	N	%	
Nucula nitidosa (M)	7.0	26.2	Amphiura filiformis (O)	39.0	48.3	
Nephtys incisa (A)	4.3	15.9	Hyala vitrea (M)	5.0	6.2	
Hyala vitrea (M)	4.3	15.9	Amphiura sp. juvenile (O)	3.8	4.6	
Varicorbula gibba (M)	2.0	7.5	Abyssoninoe hibernica (A)	3.5	4.3	
Thyasira flexuosa (M)	1.5	5.6	Callianassa subterranea (C)	3.3	4.0	
Prionospio fallax (A)	0.8	2.8	Nephtys incisa (A)	3.0	3.7	
Diplocirrus glaucus (A)	0.8	2.8	Prionospio multibranchiata (A)	2.8	3.4	
Abra nitida (M)	0.8	2.8	Diplocirrus glaucus (A)	1.8	2.2	
Abyssoninoe hibernica (A)	0.5	1.9	Pectinaria belgica (A)	1.3	1.5	
Trichobranchus roseus (A)	0.5	1.9	Cylichna cylindracea (M)	1.3	1.5	
Callianassa subterranea (C)	0.5	1.9	Varicorbula gibba (M)	1.3	1.5	
Amphiura chiajei (O)	0.5	1.9				
Phoronida indet (P)	0.5	1.9				
Eidangerfjord AC	N	%	Eidangerfjord Ref	N	%	
Spiophanes kroyeri (A)	54.5	22.6	Spiophanes kroyeri (A)	120.5	32.6	
Chaetozone setosa (A)	43.5	18.0	Paramphinome jeffreysii (A)	42.0	11.4	
Paramphinome jeffreysii (A)	25.3	10.5	Prionospio dubia (A)	23.8	6.4	
Aphelochaeta marioni (A)	14.0	5.8	Heteromastus filiformis (A)	20.0	5.4	
Heteromastus filiformis (A)	12.3	5.1	Parathyasira equalis (M)	19.8	5.3	
Parathyasira equalis (M)	9.3	3.8	Prionospio cirrifera (A)	16.0	4.3	
Thyasira sp. juvenile (M)	7.0	2.9	Aphelochaeta marioni (A)	12.3	3.3	
Leucon (Leucon) nasica (C)	6.8	2.8	Abyssoninoe hibernica (A)	11.3	3.0	
Nemertea indet (N)	6.0	2.5	Chaetozone setosa (A)	8.3	2.2	
Eudorella emarginata (C)	5.3	2.2	Thyasira sp. juvenil (M)	7.3	2.0	

out in the plot. In Eidangerfjord, the benthic communities showed a more parallel development through time at both fields,

The statistical test PERMANOVA was used to analyse differences between sites and over time for the benthic fauna (Table 3). According to the overall main test, there was a significant effect of time and site on all parameters; i.e. number of species, abundance, biomass and community composition. The difference between AC and reference was also significant in both fjords for all parameters, independently of time. The interaction between time and site was significant for community composition in both fjords, but for the other parameters not significant in the Eidangerfjord. This means that the effect of AC did not differ over time, in contrast to the Ormerfjord.

The pairwise PERMANOVA-test is the most interesting test revealing trends for each parameter. In 2009, one month after capping, there was no significant difference between the AC site and the reference neither in the Ormerfjord nor in the Eidangerfjord. During all the following years, there was a significant difference in number of species and the community composition. For abundance and biomass there was often,

but not always, significant differences between the AC and reference sites. It was also interesting that most of the p-values in both fjords were higher in 2018 than in 2013, which showed that the differences between AC and reference sites had been reduced between 2013 and 2018, indicating a gradual recovery after the AC treatments.

The actual trends of abundance, number of species and biomass are shown in Fig. 5. Except for number of species and biomass in 2009, both reference fields showed higher parameter values than the AC sites throughout the time period. In the Ormerfjord, the abundance and number of species at the reference site increased from 2009 to 2013, and was relatively unchanged from 2013 to 2018 (Fig. 5). The AC site in the Ormerfjord showed a decline in these parameters from 2009 to 2010, followed by an increase in 2013, and the values were then stable until 2018, although with a slight reduction in the abundance. For the biomass, there was a large variation between the replicates, mainly driven by the presence/absence of larger species; the high biomass at the reference site in 2013 is for example a result of the presence of more sea urchins (*Brissopsis lyrifera* and *Echinocardium flavescens*). At

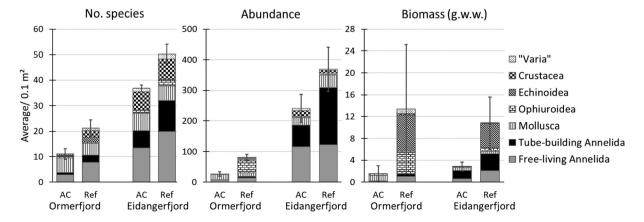


Fig. 2. No. of species, abundance and biomass (mean values, with standard deviation for the total number) for the various sites in the Grenland fjords, 2018, split into different faunal groups. AC = AC site, ref. = reference site.

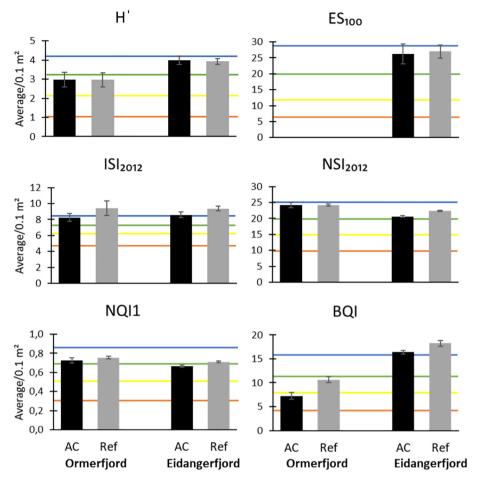


Fig. 3. Benthic status indices (mean values, with standard deviation) for the various sites in 2018 (Grenland fjords). H' = Shannon-Wiener diversity index, ES_{100} = Hurlbert's diversity index, ES_{1012} = Indicator Species Index, ES_{1012} = Norwegian Sensitivity Index, ES_{1012} = Norwegian Quality Index, ES_{1012} = Norwegian Sensitivity Index, ES_{1012} = Norwegian Quality Index, ES_{1012} = Norwegian Sensitivity Index, ES_{1012} = Norwegian Quality Index, ES_{1012} = Norwegian Sensitivity Index, ES_{1012} =

the AC site the biomass decreased from 2009 to 2010, and remained very low also in the following years. The number of species, abundance and biomass were still lower at the AC site than at the reference site in 2018, i.e. nine years after capping.

In the Eidangerfjord, the reference site and the AC sites showed the same trend in abundance and number of species, with an increase after 2010, even though the AC site always had lower values than the reference (Fig. 5). As for the Ormerfjord, the biomass was varying mainly due to the presence of larger species like sea urchins. At the AC site, the biomass remained low over the nine years. The slightly higher value in 2010 was due to one large sea urchin only (*Brissopsis lyrifera*) in one of the samples. The more even response in these parameters through time is in accordance with the lack of a significant interaction between time and site in the Eidangerfjord in the PERMANOVA-test (Table 3).

As several of the indices performed similarly (Fig. 3), Shannon-Wiener (H'), Norwegian Quality Index (NQI1) as well as the Benthic Quality Index (BQI) were selected to represent the time-trends in ecological status (see Fig. 6). In the Ormerfjord, both H' and BQI at the AC site declined steeply from 2009 to 2010. Then there was an increase to 2013, and from 2013 to 2018 only a very minor increase. Notably, the BQI remained far lower for the AC site than for the reference site throughout the monitoring period, while H' was similar between the sites in 2018. NQI1 was lower at the AC site than at the reference site in 2010 and 2013, but not in 2018. In the Eidangerfjord, the differences between the sites were in general smaller than in the Ormerfjord, which

is in line with the measured biometrics (number of species, abundance and biomass) presented in Fig. 5. The impact of the AC treatment is less evident when comparing the indices than when comparing individual metrics of number of species, abundance and biomass. Nevertheless, there is a tendency towards lower values at the AC sites than at the reference sites over the entire period, especially for the BQI index.

4. Discussion

4.1. Faunal pattern

Throughout the nine years of capping, the AC-exposed communities in both fjords had fewer species with lower abundances and lower biomass compared to the reference sites, although the response was stronger in the Ormerfjord (30 m) than in the Eidangerfjord (95 m) (Table 2, Figs. 2, 5). This stronger effect in the Ormerfjord was also reported in the previous studies conducted one and four years after capping (Samuelsson et al., 2017; Raymond et al., 2020). The stronger impact of AC observed in the Ormerfjord has mainly been attributed to lower resilience as a result of its less diverse benthic community compared to in the Eidangerfjord. Communities with a higher diversity are suggested to increase ecosystem resilience (Douglas et al., 2017). In the present case, the elimination of important bioturbators, such as brittle stars and sea urchins, may have contributed to the lack of recruitment of other species (Widdicombe et al., 2004) and to the slow recovery of the benthic communities observed here 9 years post capping. In line

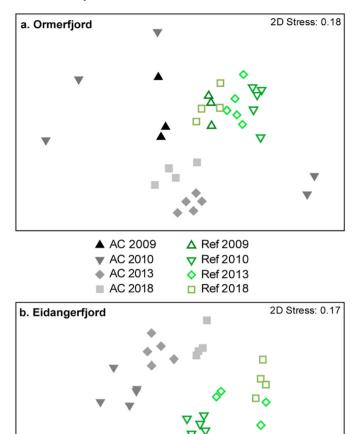


Fig. 4. nMDS-ordination of benthic communities over time in a) Ormerfjord, and b) Eidangerfjord. Fourth-root transformed data, Bray-Curtis similarity.

with this, the AC sites had considerably reduced reworking activity expressed as potential bioturbation and bioirrigation up to four years after capping, probably leading to altered ecosystem functions such as regeneration and ciculation of nutrients, remineralization, and oxygen and water regulation (Raymond et al., 2020). Species-specific traits of particularly important species may override both species richness and functional redundancy in terms of influencing benthic functioning (Lohrer et al., 2004; Norkko et al., 2013). Thus, the prevailing depleted fauna in the Ormerfjord in particular may have led to a long-lasting drop in benthic diversity, as well as an impaired benthic functioning.

The multivariate analyses indicated that there were significant differences in the community composition following AC treatment, which persisted for almost one decade after capping (Table 3). The multivariate analyses also revealed that the largest variation between replicates was found in the AC site of the Ormerfjord. Increased variability often occurs in response to stress (Warwick and Clarke, 1993), and this finding suggests that the benthic community in the Ormerfjord may be more exposed to disturbance pressures compared to the Eidangerfjord. This was especially obvious after one year, when the variability in the Ormerfjord was spread all over the nMDS plot (Fig. 4). The variability then decreased over time i.e. was lower after four and nine years, when the samples were more clustered together. Notably, in the Eidangerfjord, the AC site showed a more similar development with the reference site over time. Also, there were more significant interactions between time and site in the Ormerfjord than in the Eidangerfjord (Table 3), which shows that the effect varied most over time in the Ormerfjord, in accordance with the assumption of a more disturbed community there.

The reference fields in both fjords showed some variance in the univariate metrics over time. This is expected in species diverse systems, and is interpreted as natural variation. The study design therefore mainly compares state of the capped fields to uncapped fields rather than to a state before capping. However, there are some drawbacks with this design. For example, it is assumed that the capped fields would have the same development in community structure as their respective reference field without capping. Natural variation can also be spatial in such system having up to 200 species in total, but, nevertheless, the impaired benthic community in the capped AC field in

Table 3 Summary of PERMANOVA for the benthic communities in the AC and reference sites in the Grenland fjords, 2009–2018, O=Ormerfjord, E = Eidangerfjord. a) PERMANOVA Global test (i.e. overall main test), b) PERMANOVA pairwise post-hoc tests between treatments (degree of freedom, df = 1). Values in bold indicate significant differences, p-values calculated by permutations of residuals under a reduced model, P(perm), $\alpha = 0.05$. PSF = pseudo-F value, t = t-value.

a) Permanova global test		No. of specie	es	Abundance		Biomass		Communit	У
	Df	PsF	P(perm)	PsF	P(perm)	PsF	P(perm)	PsF	P(perm)
Year	3	15.86	0.0001	34.50	0.0001	3.40	0.0240	4.47	0.0001
Site	3	104.54	0.0001	119.09	0.0001	21.86	0.0001	18.55	0.0001
O: AC vs REF	1	75.05	0.0001	91.38	0.0001	25.33	0.0001	10.41	0.0001
E: AC vs REF	1	38.62	0.0001	19.53	0.0004	50.19	0.0001	6.77	0.0001
Year × Site	9	3.39	0.0028	6.00	0.0001	3.00	0.0068	2.83	0.0001
O: AC vs REF	3	7.09	0.0016	5.64	0.0043	2.94	0.0534	3.00	0.0001
E: AC vs REF	3	0.46	0.7457	0.44	0.7316	1.28	0.3032	1.90	0.0001
Residual	52								
Total	67								
b) Permanova Pairwise tests		No. of spec	ies	Abundano	re	Biomass		Communi	ty
Site	Year	t	P(perm)	t	P(perm)	t	P(perm)	t	P(perm)
O: AC vs REF	2009	0.13	1.0000	2.78	0.0994	0.06	0.9034	1.68	0.1009
O: AC vs REF	2010	12.00	0.0075	7.19	0.0072	2.31	0.0721	2.36	0.0073
O: AC vs REF	2013	4.56	0.0079	3.18	0.0292	7.58	0.0077	2.94	0.0066
O: AC vs REF	2018	5.55	0.0326	9.08	0.0293	2.91	0.0551	1.94	0.0291
E:AC vs REF	2009	3.98	0.0984	5.46	0.0961	4.97	0.0984	1.34	0.1024
E: AC vs REF	2010	6.85	0.0101	2.12	0.0730	1.82	0.0864	2.03	0.0081
E: AC vs REF	2013	2.69	0.0290	2.28	0.0469	6.10	0.0088	1.92	0.0067
E:AC vs REF	2018	5.92	0.0307	3.02	0.0278	4.36	0.0280	1.85	0.0299

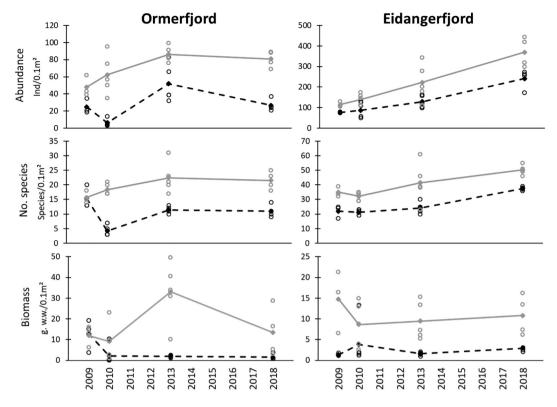


Fig. 5. Abundance, no. of species, and biomass for the various fields over time (2009–2018) in the Grenland fjords. AC site = black, reference site = grey. Filled symbols show the mean value, while the empty symbols show the values for each replicate. Note different scales on the y-axes. 2009 = capping year (sampling 1 month after capping), 2010 = one year after capping etc. The univariate metrics between 2009 and 2013 are also presented in Samuelsson et al. (2017) and Raymond et al. (2020).

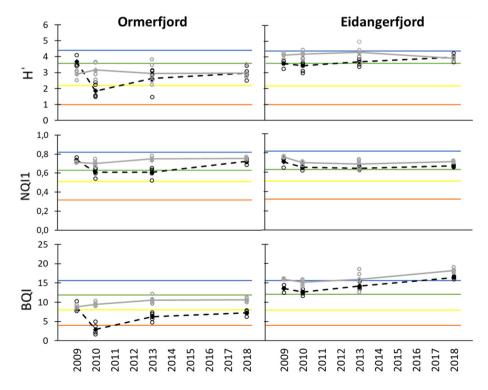


Fig. 6. Benthic indices for the various fields over time (2009–2018) (Grenland fjords). AC site = black, reference site = grey. Filled symbols show the mean value, while the empty symbols show the values for each replicate. H' = Shannon-Wiener diversity index, NQI1 = Norwegian Quality Index, BQI (Benthic Quality Index). The coloured lines show upper range of ecological classes; orange = poor, yellow = moderate, green = good, and blue = high.

especially the Ormerfjord must be linked to the AC capping. Initially, a capped field with only clay was studied to assure that possible effects were due to AC and not capping itself, and this field had no major differences to the reference field (Samuelsson et al., 2017; Raymond et al., 2020). The brittle star Amphiura filiformis dominated the reference field as well as the capping control field with only clay, and Day grab samples (0.025 m²) taken before capping confirm that A. filiformis was present in the AC field before capping (Samuelsson pers. com.). Thus, there are no reasons to believe that the negative effects observed in the AC field on the benthic community would be caused by other than capping with AC. Besides, the multivariate statistics discussed above confirm a disturbed community in the AC field. A study design with samples only as a starting point would have other drawbacks. For example, the development in the AC field in the Eidangerfjord would have been interpreted as a positive development, although compared to the reference field there are 25-73% lower abundance, biomass and number of species compared to the reference field nine years after capping. The concordant increase in both fields indicate a similar recruitment, although in reduced levels in the AC field. The multivariate statistics also confirm a concordant development between the fields in the Eidangerfjord.

The reference field in the Eidangerfjord is not ideal since it is situated with 15 m depth difference compared to the capped field. During the time of the set-up of the experiment, trawling was still ongoing in the area, and it was difficult to find a reference field at the same depth as the capped field that was not affected by trawling, as well as situated in a safe distance from the capped field so no AC could be transported into the field. The depth difference has been investigated in the previous publications by Samuelsson et al. (2017) and Raymond et al. (2020), where it was concluded that no major differences could be observed between the 80 m reference field and an additional reference field at 95 m depth. However, the number of species were in general higher at 80 m depth, although only significantly higher after one year and not after four years, but it could give an impression of higher differences between the reference and the capped field in the Eidangerfjord. Further, the reference site at 80 m hosted more sea urchins Brissopsis lyrifera, which, due to their large size, make up a dominant proportion of the biomass, although the total biomass was not significantly different between the two reference fields after one and four years. The low biomass in the AC capped field in the Eidangerfjord is mainly due to the absence of large organisms such as the sea urchins. The low biomass, in combination with lower abundance, thus suggests that the AC also in the Eidangerfjord has a negative impact on the benthic community.

4.2. Benthic responses and possible impact mechanisms

The AC-capping itself acted as a disturbance for the benthos, although thin-layer capping with AC mixed with clay has been considered a less harmful capping method than the traditional capping with thick layers (Ghosh et al., 2011). In the present setup it is not possible to distinguish between pre- or post-settlement processes for the prevailing effect of the AC treatment, and several responses may have been involved. If the sediment surface is physically or chemically altered or constantly unstable, it may affect the settling of new recolonizing larval species, which may either actively avoid to settle in the capped area, or may not survive post-settlement if environmental conditions are poor, such as reduced food and nutrient availability (Menzie, 1984; Hyland et al., 1994; Shin et al., 2008; Lam et al., 2010). Avoidance of ACcontaining sediment in laboratory experiment has been documented for some species suggesting that organisms may avoid to settle or actively move away from AC-treated areas (Hellou et al., 2005; Jonker et al., 2009). However, after capping the sediments have received new natural sediment and settling organic matter on top; approximately 2 cm in the Ormerfjord and 4.6 cm in the Eidangerfjord (Schaanning et al., 2021). Thus, such avoidance mechanism to AC would be expected to decrease over time. Bioturbation will, however, redistribute the AC-particles towards the surface sediment to some degree. Particularly in the Ormerfjord, the surface sediment at the capped site was considerable different from the reference site regarding both grain size and content of TOC and TN (Table 1), which also may have affected other sediment properties like sediment heterogeneity and compaction. This may in turn have affected the recruitment patterns and the substrate's suitability as a habitat. Avoidance due to AC presence is thus a possible explanation to the altered community composition, but at the same time other factors were probably also involved.

Post-settlement mortality due to intolerable conditions can probably explain some of the lowered abundances at the AC sites. AC can sorb essential nutrients (Jonker et al., 2004), and sequestration of natural, sedimentary organic matter by AC-particles has been suggested for explananing similar reduction of diversity and abundance of meiofaunal organisms (invertebrates <1 mm) (Bonaglia et al., 2019). Powdered activated carbon may also be more detrimental to benthic fauna than more coarse, i.e. granular AC, since the powdered AC as used here (20 µm), is ingestible by the benthic fauna and may decrease the food assimilation capacity of the organisms as suggested by Nybom et al. (2015, 2016), or by decreasing uptake of nutrients from the gut due to the strong sorption of nutrients to AC. In line with this, it has been reported lower growth of fish after capping with powdered AC, but not for granular AC (Kupryianchyk et al., 2013). As negative effects were observed on filter-feeding and surface-deposit feeding species such as the brittle star Amphiura filiformis and the annelid Spiophanes kroyeri (Table 2), more mechanisms than a low nutrient supply were probably acting as well. The amount of organic carbon and total nitrogen in fact tended to be higher in the capped than uncapped sites, thus there is no indication of a prevailing lack of nutrients. Indeed, previous studies have reported a range of effects of activated carbon; such as altered feeding behavior, reduced growth and reproduction, lower lipid content, and physiological changes and attachment to the skin (Rust et al., 2004; Millward et al., 2005; McLeod et al., 2008; Jonker et al., 2009; Nybom et al., 2012, 2015, 2016; Lillicrap et al., 2015; Samuelsson et al., 2015). More experimental work is needed to disentangle these factors from each other.

The brittle star Amphiura filformis was particulary negatively affected by the AC capping. This species dominated the benthic community at the reference site in Ormerfjord, and was still depleted nine years after treatment at the AC site. The brittle star is a common species in the northeast Atlantic (Rosenberg and Lundberg, 2004). It can be found at depths down to 200 m where it lives buried in the sediment with its disk at about 4-8 cm depth, and one or two arms stretched up above the sediment to collect food (Rosenberg et al., 1997; Rosenberg and Lundberg, 2004), and they have a life span of 20 years or more (O'Connor et al., 1983). A. filiformis is known to be mainly a suspension feeder, but can switch to deposit feeding depending on the hydrodynamic conditions and organic matter availability at the sediment surface (Buchanan, 1964; Solan and Kennedy, 2002). The species is considered an important ecosystem engineer because of its role in sediment-water exchange processes and bioturbation (O'Connor et al., 1983; Loo et al., 1996; Solan and Kennedy, 2002; O'Reilly et al., 2006). Its absence may thus substantially reduce the overall ecosystem funtioning. For example, for the overall bioturbation potential of a community, it has been modelled that the absence of A. filiformis could cause a collaps of the entire community (Solan et al., 2004). While bioturbation in general may increase the release of contaminants from the sediment to the water column (e.g. Josefsson et al., 2010; Thibodeaux and Bierman, 2003; van der Meer et al., 2017), in the case of capping, it can promote mixing of the AC-particles. This can increase the surface available for contaminant sequestration, which results in a decrease in contaminant concentrations in pore water (Lin et al., 2014). Thus, the absence of A. filiformis at the AC site in Ormerfjord may potentially reduce the efficiency of the capping treatment with AC.

The strong response of *Amphiura filiformis* was a bit surprising. In both the AZTI Marine Biotic Index (AMBI- European coast) and the

Norwegian Species Index (NSI-Norwegian coast), this species is classified as relatively tolerant towards disturbance (Rygg and Norling, 2013). Its abundance often increases at a moderate disturbance level, at least in response to organic enrichment and physical disturbance (Borgersen et al., 2019). For instance, it has been recorded at very high densities at an old deposit site for mine tailings (Schaanning et al., 2019). At the same time, the species has been shown to be sensitive towards environmental pollutants like metals and oil-derived components (Bjørgesæter et al., 2008; Rygg, 1985a; Olsgard and Gray, 1995). Thus, the response may depend on the particular stressor involved. Here, the brittle star Amphiura filiformis and other echinoderms such as the large burrowing sea urchin *Bryssopsis lyrifera* mainly disappeared following thin-layer capping with powdered AC. Recovery of A. filiformis after disturbance has been shown to be a slow process. It takes about five years for the population to grow to maturity (Muus, 1981), and the process is largely dependent on the survival of new settling larvae (Sköld et al., 1994; Sköld et al., 2001). Here the population had not recovered yet, nine years after capping with AC.

The depleted benthic fauna may also impair the benthos functioning in the food web. Brittle stars including *Amphiura filiformis* are an important food source for several demersal fish and crustaceans, which feed on brittle stars arms protruding from the sediment (Duineveld and Van Noort, 1986; Sköld and Rosenberg, 1996). Considering the large density of adult animals in the population (>30 per 0.1 m²) and the regeneration capabilities of the species, it cannot be excluded that an elimination of the *Amphiura filiformis* population in AC treated areas could impact demersal fish and lead to trophic cascades if capping is performed over large areas.

In the deeper Eidangerfjord, the annelid *Spiophanes kroyeri* was the most dominating species at both sites, but at the AC site the abundance was less than half of the reference site (Table 2). This species is in general quite tolerant towards high loads of organic matter, but is sensitive towards metal pollution (Rygg, 1985b; Trannum et al., 2004). Most of the other dominating species also had lower abundance at the AC site than at the reference site. In contrast, the annelid *Chaetozone setosa* had highest abundance at the AC-site. This is in general a tolerant species, which often occurs under various forms of disturbances (Borja et al., 2000).

While free-living annelids had the same abundance at the AC and reference sites in Eidangerfjord, there were fewer tube-building annelids at the AC site (Fig. 2). In general, tube-building annelids have been assumed to be more sensitive towards disturbances than free-living annelids (Oug et al., 2012; Pearson and Rosenberg, 1978). For instance, during deposition of particles like drill cuttings and mine tailings, tube-building annelids have been observed to be particularly affected (Trannum et al., 2011, 2019, 2020). Such species may exhibit more sediment-specific preferences during settlement (Pinedo et al., 2000; Duchêne, 2010), which may explain their lower abundance at the AC site.

4.3. Index performance

As sediment-living organisms are mostly sessile, they integrate long-term effects of environmental change, and are therefore commonly used in benthic monitoring. Within EU's Water Framework Directive (WFD), benthic indices form the foundation for the ecological status classification (Council directive, 2000). Some indices are based on species diversity measures, while others also take into account the sensitivity of the organisms to disturbances (e.g. sensitivity to hypoxia). A common feature of these indices, however, is that they mainly respond to disturbance caused by organic enrichment, rather than caused by high concentrations of contaminants. This can be a challenge when assessing the environmental status of contaminated water bodies, and in the worst case give a misleading quality classification.

A notable finding in our study is that the benthic indices currently used in Norway for assessing the ecological status of benthic

communities did not well reflect the depleted benthic community that followed the AC capping (Figs. 3, 6). The Shannon-Wiener index (H') was identical at the AC and reference sites in the Ormerfjord in 2018, although the number of species was only half at the AC site. In the Eidangerfjord, H' was even slightly higher at the AC-treated site at this time, even though the number of species was significantly lower also here. In both fjords, the abundance and number of species was reduced at the AC-treated sites, though it was not detected by the diversity indices in our study. Many biodiversity measures, including H', are strongly influenced by species evenness, and when stress increases evenness due to an overall reduction in abundance, it can override the effect of species loss (Cao and Hawkins, 2005).

Some benthic indices are based on the species' degree of sensitivity or tolerance (AMBI, NSI, ISI₂₀₁₂) or a combination of species sensitivity and species diversity (NQI1, BQI). These indices were better in discriminating between the treatment and reference sites, but were still not highly sensitive to the disturbance of AC capping. These indices usually perform well in detecting faunal response to eutrophication and increased abundances of opportunistic species (Borja et al., 2011; Borja et al., 2015; Culhane et al., 2019), but appear to be less sensitive when the abundances in general are declining.

According to the Norwegian classification system, all locations obtained "good" condition (status class II) nine years after capping. However, the benthic fauna at the AC site in Ormerfjord was highly depleted, consisting of only 11 taxa and 27 individuals (grab average). In this case, the benthic indices seem to give a misleading assessment of the benthic communities and classify the water body with too high ecological status. It is therefore important to notice the limitations of these indices for an effective management of the marine environment. Our results show that these indices may be too conservative and understate disturbances to the benthic community and that they may need to be revised for assessing the impact of other stressors than eutrophication.

The BQI-index used for Swedish status assessment was better suited at assessing the depleted benthic community at the AC sites. The AC site in the Ormerfjord obtained only "poor" condition in 2018, while the reference site was classified as "moderate". However, in the Eidangerfjord, both sites were assessed as "good" (class II) ecological status, which is in line with the more moderate disturbance in the Eidangerfjord and in accordance with the Norwegian benthic indices. As the Norwegian NOI1, BOI combines species tolerance values, abundance and diversity (Rosenberg et al., 2004), but differs from NQI1 in several ways. NQI1 uses the AMBI index as the sensitivity component, while BQI uses species tolerance values based on ES50_{0.05} (Leonardsson et al., 2009). Diversity is quantified by species richness in BQI, and by SN (a logarithmic function incorporating both species richness and abundance) in NQI1 (Josefson et al., 2009). Since the BQI emphasizes the number of species (S) more than NQI1, the BQI index value responds more strongly than NQI1 when species number decrease.

4.4. Recommendations for monitoring of a depleted benthic community

We recommend that the indices commonly used to assess the status of macrobenthic communities should be further evaluated in cases where the disturbance leads to a depleted fauna (i.e. to a lower species abundance and number of species). The indices usually perform well in detecting faunal responses to eutrophication, which typically increases abundances of opportunistic or tolerant species. They are less sensitive when the abundances and numbers of species decline. This may in the worst case give a misleading assessment of the benthic communities. An index designed to detect decreases in both abundance and numbers of species caused by other stressors than organic loading would benefit from a stronger emphasis on the number of species rather than species diversity or abundance.

One potential improvement could be to assess the functional responses in environmental monitoring, which is also recommended in other studies (Elliott and Quintino, 2007; Rand et al., 2018; Liu et al.,

2019; Trannum et al., 2019). Trait based ecotoxicology may offer a tool for predicting sensitivity of benthic invertebrates and the benthic community to sediment-associated contaminants (Baird et al., 2008; Archaimbault et al., 2010; van der Meer et al., 2017). Such approaches are also in line with several studies emphasizing the species' functional roles, rather than the structural diversity, in mediating productive and efficient ecosystems (Diaz et al., 2006; Karel et al., 2008; Oug et al., 2012; Fleddum et al., 2013; Gagic et al., 2015; Beauchard et al., 2017).

Other future improvements could be to include biomass in the assessments. In the present study, the biomass showed far larger difference between the treatments than the indices. It is not a tradition to measure biomass in Norwegian ecosystem monitoring, but it should be considered in accordance with recommendations by Nilsen (2007). Particularly in cases with a depleted fauna, the biomass will provide important information with regard to the benthic faunas' productivity and role in the foodweb. It could also be interesting to have a status-classification index that includes biomass. Recently, functional indices, i.e. indices that calculate potential bioturbation and bioirrigation, have been developed where biomass is included as a factor (Queirós et al., 2013; Renz et al., 2018; Wrede et al., 2018). It should therefore be possible to have a similar index for ecological status classification that could help us capture changes in the benthic community in a better way, especially when the disturbance is not coupled to eutrophication. Thus, a combination of the more traditional biodiversity indices with functional indices, where also biomass is incorporated, could be a way forward, and at the same time represent a link to the ecosystem services provided by the benthic community.

5. Conclusion

The benthic communities in both fjords were still negatively affected by thin-layer capping with a mixture of clay and powdered activated carbon (AC) almost a decade after capping. Capping with powdered AC led to a reduced species abundance, reduced number of species and a reduced biomass, as well as significant differences of the community composition compared to untreated reference sites. The negative effects of the AC capping were generally less pronounced in the deeper Eidangerfjord (80-95 m) compared to the more shallow Ormerfjord (30 m). Most likely, this was a result of higher species diversity and, accordingly, higher resilience of the benthic community in Eidangerfjord. In Ormerfjord, key species such as the brittle star *Amphiura filiformis* were still depleted nine years after capping with AC. The benthic indices currently used in Norway for assessing the ecological status did not well reflect the impaired benthic communities by AC capping. This highlights the need for better indices to assess ecological status of benthic communities with regard to other disturbances than eutrophication.

To our knowledge, this is the first study that shows that thin-layer capping with powdered AC causes a decrease in biodiversity of benthic communities almost a decade after treatment. The slow recovery and the long-lasting elimination of the key species *Amphiura filiformis* raise concern about long-term effects of powdered AC on benthic ecosystem functions such as regeneration of nutrients and food provision for demersal fish. Thus, despite its benefits for reducing contaminant release and bioavailability, capping with powdered AC should be carefully weighed against its detrimental long-term disturbance of the benthic community before decisions are made on remediation with activated carbon over large areas or entire water bodies with viable benthic communities. More research is needed to see if less harmful effects can be achieved if another type of less ingestable AC is used, i.e. of coarser grain size than the powdered AC used here, but at the same time be efficient in contaminant sequestration.

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Declaration of competing interest

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influenced the work reported in this paper.

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