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Macrofaunal colonization after large-scale sand-cap and *Zostera marina* restoration of organic enriched sediments in a Danish fjord

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ABSTRACT: Decades of eutrophication in Danish estuaries have changed the dominant primary producers from eelgrass (*Zostera marina*) meadows to phytoplankton and ephemeral macroalgae. This has resulted in enrichment of sediments with organic matter, and frequent bottom-water hypoxia that negatively affects the macrofauna community. In this study, we propose that restoration by sand-capping is a solution to improve sediment conditions, which may facilitate eelgrass restoration and accelerate recovery of macrofauna communities. Sand-capping was carried out by adding a layer of approximately 10 cm of sand on top of 1.4 ha of organic-rich sediment. One year later, we transplanted 6000 eelgrass shoots onto the sand-cap; however, they did not survive more than a few months due to blooms of filamentous algae that smothered the eelgrass. The sand-cap remained intact and the colonization of macrofauna was investigated by analysis of species richness (*S*), abundance (*N*) and feeding guilds (FGs) in the sand-capped area and at uncapped sediment. *S*, *N* and diversity of FGs were significantly higher for infauna in the sand-capped area compared to uncapped sediment after 1 yr. Conversely, *S* of epifauna was highest on uncapped sediment and *N* of epifauna varied seasonally rather than being affected by the sediment conditions.

KEY WORDS: Marine infauna · Marine epifauna · Eelgrass restoration · Sand-capping · *Zostera marina*

1. INTRODUCTION

Danish estuaries and coastal waters have been heavily impacted by nutrient enrichment from agriculture and untreated wastewater in the past century, and discharges were unregulated until 1987, when Denmark received its first water action plan (Carstensen et al. 2006). The first water action plan targeted nutrient reduction from wastewater, while the following 2 plans included strategies to reduce diffuse nitrogen input (e.g. fertilizer reduction, buffer strips and wetland restoration) (Carstensen et al. 2006). The coastal eutrophication issues led to a switch in marine vegetation from eelgrass *Zostera marina* to phytoplankton and ephemeral macroalgae, followed by frequent bottom-water hypoxia (Carstensen et al. 2006). Bottom-water hypoxia occurred due to a decoupling between oxygen production (pelagic) and consumption (benthic) (Flindt et al. 1999) and was amplified by the decomposition of large quantities of ephemeral macroalgae that formed dense drifting mats along the seafloor (Flindt et al. 1999). These algal mats can smother eelgrass meadows and seedlings, so that natural recovery is inhibited (Rasmussen et al. 2012, Canal-Vergés et al. 2014, Moksnes et al. 2018).

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Ephemeral algal blooms can usually be constrained by high abundance of grazing fauna (Neckles et al. 1993). However, trophic cascades, linked to overfishing in Scandinavia, have altered food-web structure and reduced mesograzers to functional extinction, stimulating growth of emphemeral algae even more without the grazing control provided by fauna. These trophic cascades have severe negative consequences for eelgrass recovery (Moksnes et al. 2008, Baden et al. 2010, 2012). In addition, the benthic fauna community was negatively affected by bottom-water hypoxia. This was evident from a reduction in species richness, abundance and biomass in areas affected by short-term hypoxia, while areas that were exposed to long-term hypoxia (i.e. 4 to 6 wk) had a complete collapse of the benthic community (Conley et al. 2007). Furthermore, eutrophication has resulted in an alteration of ecosystem functions related to nutrient cycling, because of the change in dominant primary producers. When nutrients are no longer bound in slowly degradable *Z. marina*, they become available for other primary producers (i.e. phytoplankton and ephemeral macroalgae), with a much higher turnover rate, meaning that nutrients can be cycled multiple times during the growth season (Flindt et al. 1999, Kemp et al. 2005). The increased primary production results in increased deposition of fine-grained labile organic particles that enrich the sediment in estuaries. The labile fraction of the organic-rich sediment is quickly degraded, while the refractory portion accumulates over time and may currently constitute up to 95% of total organic carbon in Danish eutrophic estuaries (Valdemarsen et al. 2014). Natural recovery of these sediments would take between 23 and 50 yr if all nutrient discharge sources were removed (Valdemarsen et al. 2014). The low stability of the impacted sediments causes frequent resuspension events even at low current speeds (0.2 m s^{-1}) that reduces benthic light intensity and prevents *Z. marina* meadows from recovering naturally (Moksnes et al. 2018, Flindt et al. 2022). Today, after 36 yr of nutrient-reduction measures, it is evident that a state change from eelgrass dominance to bare sediment has resulted in a regime shift that is difficult to reverse only by reducing nutrient discharge (Carstensen et al. 2013, Riemann et al. 2016, Moksnes et al. 2018). We may need to resort to habitat restoration to recover important coastal habitats, and in light of the United Nations Decade on Ecosystem Restoration and the European Union's recent adoption of a Nature Restoration Law, we are likely to see a growing need for effective restoration methods that can be adopted by managers and stakeholders (Duarte et al. 2020, Orth et al. 2020, Saunders et al. 2020, Lange et al. 2022, Gagnon et al. 2023, Steinfurth 2023).

We propose sand-capping as a solution to restore degraded sediment condition in estuaries. It was tested for the first time on estuarine sediments heavily enriched with organic matter in the Danish estuary Odense Fjord (Oncken et al. 2022). Sand-capping was done by adding a layer of approximately 10 cm of sand on top of 1.4 ha of organic-rich sediment (Oncken et al. 2022). This method has previously been used to cap heavily polluted and toxic sediment in industrial harbors (Murakami et al. 2006, Katakura & Murakami 2015, Vidgren et al. 2015) or in eutrophic lakes to reduce phosphorus release (Kim et al. 2007, Kim & Jung 2010). Sand-capping is likely the best solution when sediment volumes are too large for dredging or natural recovery has very long prospects (Förstner & Apitz 2007, Flindt et al. 2022). This is the case in Odense Fjord, Denmark, where several square kilometers of seabed are enriched with slowly degradable organic matter (Valdemarsen et al. 2014).

Sand-capping provides several ecosystem functions such as decreased resuspension, which lowers turbidity and improves benthic light conditions (Flindt et al. 2022), as well as increased biodiversity and abundance of benthic invertebrates (Oncken et al. 2022). Furthermore, laboratory experiments have verified that sand-capping increases the anchoring capacity of eelgrass roots, making the plants more resilient against wave- and current-driven up-rooting (Flindt et al. 2022). However, sand-capping has not yet been tested in combination with eelgrass restoration *in situ*, and little knowledge exists on macrofauna colonization after sand-capping.

In this study, we aimed at restoring sediment conditions by sand-capping, recovering eelgrass meadows by transplanting eelgrass shoots and documenting changes in the benthic fauna communities after restoration activities (Fig. 1A). We investigated if the historical impoverishment of the infauna community structure (Oncken et al. 2022) can be reverted by restoring the sediment by sand-capping. Likewise, we intended to document changes in the epifauna community after both sand-capping and eelgrass transplantation. Unfortunately, the eelgrass transplants did not survive for more than a few months, so it was not possible to follow epifauna colonization of the eelgrass transplantation (Fig. 1B). Thereafter, the focus of this study was directed at macrofaunal colonization after sand-capping instead. The colonization of benthic macrofauna was monitored to assess if sand-capping had any positive effects on species diversity, animal abundance and functional diversity. Our

hypotheses were that (1) species richness, animal abundance and functional diversity (measured as diversity of feeding guilds in the community) of benthic macrofauna would increase in the sand-capped area to significantly higher levels than at an adjacent muddy site; and (2) species richness, abundance of infauna and functional diversity would be reduced on a gradient from the center of the sand-cap towards the edge, as the community composition closer to the edge would be affected by the mud habitat that surrounded the sand-cap.

2. MATERIALS AND METHODS

2.1. Study site

Odense Fjord is a shallow estuary on the island of Fyn, Denmark. It covers 62 km^2, with a 16 km^2 inner basin near Odense city and a 46 km^2 outer basin that connects to the Kattegat via a narrow opening called Gabet (Fig. 2A). The inner and outer basins have an average water depth of 0.8 and 2.7 m, respectively (Petersen et al. 2009). It is a microtidal system, with an average tidal amplitude of 0.25 m and an average water residence time of 1 mo (Petersen et al. 2009). The inner basin receives freshwater input from the river Odense Å, resulting in a salinity ranging from 5 to 18, while salinity in the outer fjord ranges from 18 to 25. The catchment area is 1095 km^2 and is dominated by runoff from agriculture (68% land use) and cities (16% land use) (Petersen et al. 2009). The nutrient discharge from land has historically been high, with the highest levels (3000 t N yr^{-1} and 300 t P yr^{-1}) in the 1980s, providing area specific N- and P-loadings of 48 g N m^{-2} yr^{-1} and 4.8 g P m^{-2} yr^{-1}, resulting in eutrophication with dominance of ephemeral macroalgae, phytoplankton and frequent bottom-water hypoxia (Petersen et al. 2009). Nitrogen and phosphorus discharges were reduced by approximately 30 and 80%, respectively, from 1990 to 2004 (Petersen et al. 2009), but no further reduction has been evident in the last 20 yr (Hansen & Høgslund 2021). Coastal habitats in Odense Fjord have deteriorated due to eutrophication, resulting in altered sediment conditions (Valdemarsen et al. 2014), reduction in areal extent of eelgrass meadows (from 44 to 1.5% of the total area of the fjord) (Petersen et al. 2009, Steinfurth 2023) and decreased infauna species richness and abundance from 1978 until today (Oncken et al. 2022). Eelgrass is not recovering despite reduced nutrient loading, and *Fucus vesiculosus* has increased in areal extent in the outer estuary instead (Petersen et al. 2009).

2.2. Sand-cap construction

Sand-capping was performed in July 2018 at Egensedybet (55.512° N, 10.495° E) in the western part of the outer basin of Odense Fjord (Fig. 2A). About 1.4 ha (100 × 140 m) (Fig. 2B) of muddy sediment with organic content of 3–5% was sand-capped in an area with water depths ranging from 2.0 to 2.8 m (Oncken et al. 2022). Sand-capping was carried out by using a floating, moveable and anchorable platform with an excavator that deployed the sand with its shovel (Oncken et al. 2022). The sand was azoic and from a nearby geological sand formation (a terrestrial gravel pit), ensuring that no fauna was transferred to the location during construction. The median grain size of the sand was 0.340 mm, with organic content <1% and a silt and clay content (<0.063 mm) of 1.4% (Oncken et al. 2022). The sand covered the mud with an 8.8 ± 1.6 cm sand layer without any gaps and limited mixing at the sand/mud interface (Oncken et al. 2022).

2.3. Sediment sampling and analysis

Sediment cores were taken annually in September 2018, 2019 and 2020 with acrylic cylinders (α = 5 cm), along a transect with 4 sampling stations from the center of the sand-cap to the edge: 0 (center), 30, 40 and 50 m (edge) and supplemented with control samples on adjacent muddy sediment about 100 m away from the center of the sand-cap (100 m mud) (Fig. 2B). The sediment cores were brought to the laboratory and sectioned into 1 cm slices to a depth of at least 13 cm. The sediment slices were dried in an oven at 105°C for 24 h, and total organic matter was determined based on the loss on ignition (LOI) by combusting 2–3 g of dry sediment at 520°C for 6 h in preweighed and pre-burned crucibles. Sediment was sampled to verify that the sand-cap stayed intact during eelgrass restoration and the fauna sampling campaign. A single replicate at each station was deemed sufficient to verify this stability, because it was taken as a supplement to a larger sediment survey (n = 105) that was conducted simultaneously in a 20 × 20 m grid across the whole sand-capped area (Oncken et al. 2022).

2.4. Eelgrass transplantation

After confirming that the sand-cap stayed intact for at least 1 yr, eelgrass transplantation was carried out

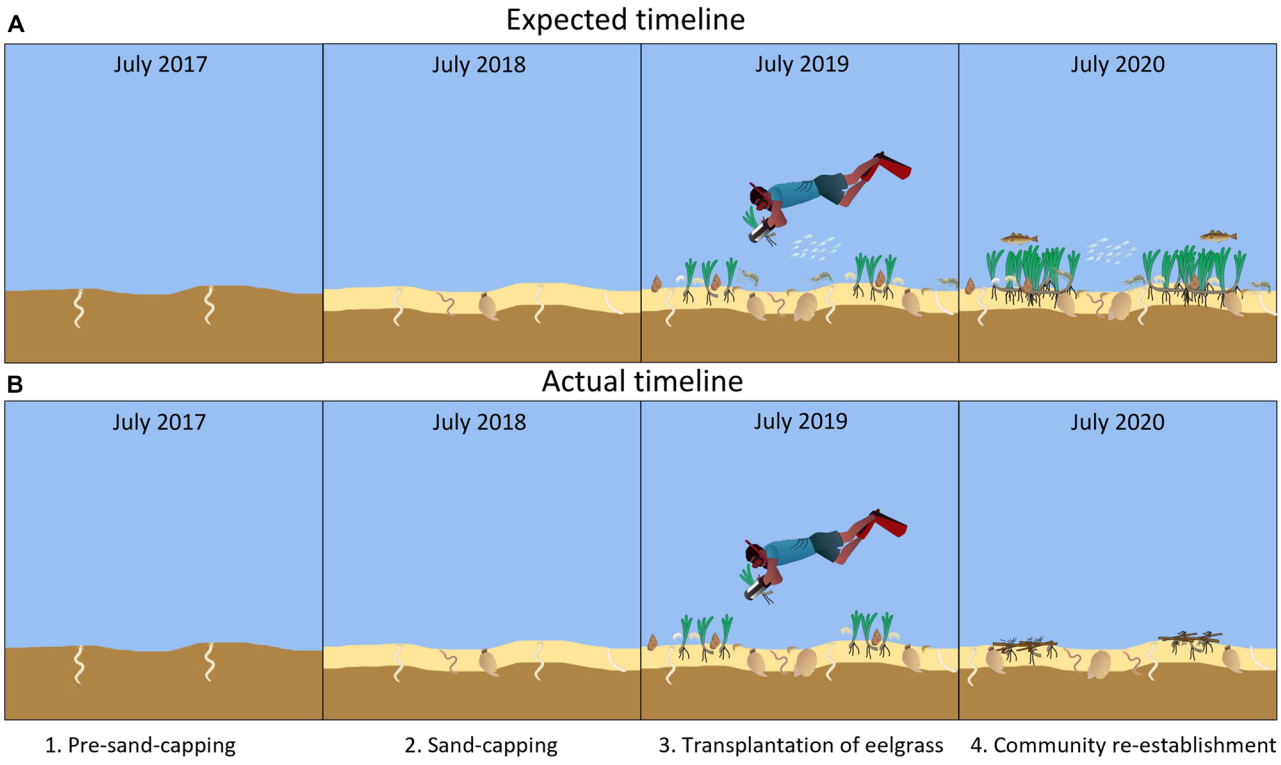

Fig. 1. Restoration activity in Odense Fjord, Denmark, with an (A) expected and (B) actual timeline of both habitat restoration and colonization of fauna (vector symbols: courtesy of the Integration and Application Network, University of Maryland, https://ian.umces.edu/media-library/)

in July 2019 in the shallowest part (2.0–2.2 m) of the sand-capped area (Fig. 2B). The shoots were harvested from the closest natural eelgrass meadow, approximately 1350 m northwest of the sand-capped area. Shoots were transplanted using weighted shoots, where the eelgrass rhizome (5–10 cm) was attached to an iron nail (8 × 0.3 cm, 5 g) with iron wire (10 cm long, 0.5 mm thick) according to Lange et al. (2022). This method has proven to work in Horsens Fjord and Vejle Fjord in Denmark, but at shallower depths (1–2 m) (Steinfurth 2023), and a review by van Katwijk et al. (2016) has shown that weighted shoots have the overall highest probability of resulting in successful restoration. A total of 60 transplantation plots were made (plot size: circle with $\alpha = 2$ m), with 100 planting units homogeneously transplanted in each by free divers (Fig. 2B). The plots were interspaced in an 8 × 8 m grid from center to center in each circular plot. This resulted in a total of 6000 planting units transplanted in an area that was 40 × 72 m (2880 m^2) (Fig. 2B).

Eelgrass was monitored by counting shoot densities (shoots m^{-2}) and assessing coverage (%) of epiphytes (e.g. *Ectocarpus siliculosus* and *Pylaiella littoralis*) and drifting macroalgae (e.g. *F. vesiculosus*, *Ulva lactuca* and *Chaetomorpha linum*). This was done approximately once every 2 wk from July 2019 to the end of October 2019 in a transect from the northernmost corner across the transplant area towards the southernmost corner (Fig. 2B). In total, 8 plots were monitored by placing a circular metal frame ($\alpha = 0.55$ m) centrally in the plot and counting all shoots inside and visually assessing epiphyte and macroalgae coverage. Monitoring was discontinued during winter, and no shoots could be found when we returned in spring 2020. Furthermore, a Garmin VIRB action camera was set up to take a picture of one of the transplant plots every 24 h for the duration of monitoring.

Previous eelgrass transplantations at this site, before sand-capping was initiated, have shown that the plants do not survive very well in the muddy substrate (R. C. Steinfurth et al. unpubl. data). However, a control station was needed at the muddy site to test whether eelgrass shoots survived better in sand-capped sediment compared to mud. To avoid spending excessive resources on transplanting another 6000 shoots that would likely die in the muddy sediment, 2 small-scale transplantations were made instead: one on the sand-

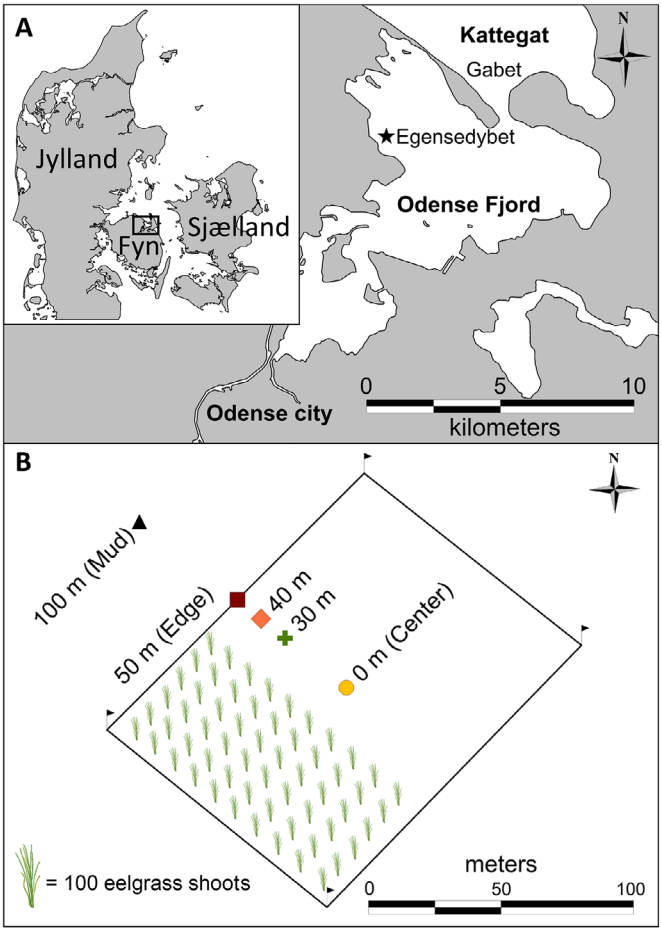

Fig. 2. (A) Odense Fjord in Denmark and the sand-capped location Egensedybet (55.512° N, 10.495° E); land in grey, sea in white. (B) Sand-capped area at Egensedybet with corner buoys (North: 55.513° N, 10.495° E; South: 55.511° N, 10.495° E; East: 55.512° N, 10.496° E; West: 55.512° N, 10.494° E), sampling locations: 0 m (Center), 30 m, 40 m, 50 m (Edge) and 100 m (Mud) and the eelgrass transplantation site (eelgrass vector symbol: courtesy of the Integration and Application Network, University of Maryland, https://ian.umces.edu/media-library/)

cap and one control station in the muddy sediment. This was done in order to have 2 equal-sized restoration plots that could be directly compared, without the bias of better eelgrass survival due to a larger restoration scale on the sand-cap, as described by van Katwijk et al. (2016). The 2 small-scale transplantations were placed approximately 20 m south-east from the 0 m (center) and 100 m mud station, respectively (Fig. 2B). This was assessed as sufficient distance to not interfere with fauna sampling. Each small-scale transplantation consisted of 3 plots (plot size: circle with $\alpha = 2$ m) with 100 shoots homogeneously planted in each. These were monitored by placing a circular metal frame ($\alpha = 0.55$ m) centrally in the plot and counting all shoots inside.

2.5. Fauna sampling

Infauna was sampled annually in September 2018, 2019 and 2020, along the same transect of 4 sampling stations where sediment samples were taken and supplemented with control samples on adjacent muddy sediment about 100 m away from the center of the sand-cap (100 m mud) (Fig. 2B). All samples were taken at similar depth (2.3 m) and hydrodynamic conditions. Four replicates were taken at each station every year. The samples were taken in a transect perpendicular to the transect from the center of the sand-cap to the control station on mud. Each replicate was taken approximately 5 m apart. Samples were taken with a hand-held cylindrical steel corer ($\alpha = 15$ cm and area = 177 cm^2) to a depth of 30 cm, and all samples were sieved through a 1 mm mesh on site. Individuals smaller than 1 mm retained by the sieve were excluded. The retained material was stored in plastic containers, preserved in 4% buffered formaldehyde and brought to the laboratory for further analysis. All infauna samples were sorted in the laboratory, and recovered animals were identified to species level and feeding guild when possible. Feeding guilds included deposit feeders, suspension feeders, deposit/suspension feeders, grazers and omnivores. The feeding guild deposit/suspension feeders was included in this study because several polychaete and bivalve species are known to switch between deposit feeding and suspension feeding. Species with 3 or more feeding strategies were categorized as omnivores.

Benthic epifauna was sampled seasonally in September 2018 (late summer), November 2018 (fall) and March 2019 (spring). Epifauna was not sampled along a transect, because the mobile nature of these organisms leads to a natural homogeneous distribution across the sand-capped area. Samples were only taken at the center of the sand-cap (0 m) and on the adjacent muddy sediment 100 m away from the center (Fig. 2B). In total, 5 replicates were taken by a diver with a rectangular hand-pushed shrimp net (50 × 20 cm) with stretched mesh size 4.5 mm. Each replicate consisted of a 5 m long push that trawled the seabed in the upper first centimeter of the sediment. This technique quantitatively captured individuals from 4.5 mm up to approximately 10–15 cm. The recovered samples were placed on ice in zip-lock bags on site and brought to the laboratory for further analysis. All epifauna samples were sorted in the laboratory, and

recovered animals were identified to species level and feeding guild when possible. Feeding guilds included grazers, omnivores and predators. Species that both scavenge and feed on detritus were categorized as omnivores.

All animals were handled with ethical responsibility to minimize suffering and were euthanized using 4% buffered formaldehyde or freezing. No endangered or protected species were taken for this study.

2.6. Statistics and data processing

Differences in infauna species richness (S_i) and epifauna species richness (S_e) were tested for by using univariate statistics. A 2-way ANOVA was used with years and stations as factors for S_i, with levels 2018, 2019 and 2020, and 0, 30, 40, 50 and 100 m, respectively. Seasons and stations were used as factors for S_e, with levels summer 2018, fall 2018, spring 2019 and 0 m, 100 m, respectively. A Tukey post hoc test was run on all significant test results from the 2-way ANOVA tests. A Shapiro-Wilk normality test and Levene *F*-test indicated a normal distribution and homogeneous variance (S_i: p = 0.502 and p = 0.891, S_e: p = 0.322 and p = 0.072). All tests were run in SigmaPlot version 12.0, with significance level $\alpha = 0.05$.

Infauna abundance (N_i) and epifauna abundance (N_e) were analyzed using multivariate statistics. N_i and N_e were not normally distributed, and permutational multivariate analysis of variance (PERMANOVA) non-parametric statistics were used to analyze these data instead. First, Bray-Curtis similarity matrices were constructed based on square root transformed data of N_i and N_e. Non-metric multidimensional scaling (nMDS) ordination plots were then produced based on the resemblance matrixes (Clarke & Warwick 2001). A 2-way crossed PERMANOVA design was used to test for differences in abundance, using years and stations as factors for infauna, with levels 2018, 2019 and 2020, and 0, 30, 40, 50 and 100 m, respectively, while seasons and stations were used as factors for epifauna with levels summer 2018, fall 2018 and spring 2019, and 0 m and 100 m, respectively. A pairwise comparison was carried out when main tests revealed significant differences or interactions between factors. The number of permutations was 9999 and permutation method was a permutation of residuals under a reduced model, while sum of squares was calculated using a type III partial method (Anderson et al. 2008). SIMPER analysis was run afterwards on square root transformed data of N_i and N_e, and all taxa contributing to dissimilarity were grouped by feeding guild to compare dissimilarities among feeding guilds between stations. Dissimilarities between seasons were also tested by SIMPER analysis for epifauna. These tests were run in Primer 6 with the PERMANOVA+ add on (Anderson et al. 2008).

3. RESULTS

3.1. Sediment composition

Sediment total organic matter (OM) at the sand-cap stations was initially <2% to a depth of 8–10 cm, which fits well with the 8.8 ± 1.6 cm sand-cap thickness measured by Oncken et al. (2022). This increased to 2–7% OM in the sand/mud transition zone (10–14 cm depth). The deepest sections of sediment (14–20 cm depth) below the sand-cap had steadily increasing amounts of OM with increasing depth, and it was up to 12.8% in some of the deepest sections (Fig. 3). A surface layer, which was richer in OM than the sand-cap, accumulated over time, and OM had increased to 1.8–3.5% in the upper 2 cm of the sand-capped sediment in 2020. This was especially pronounced at the edge of the sand-cap (50 m station) (Fig. 3C). However, the sand-cap below remained undisturbed, and no vertical mixing with the mud below was detected.

OM at the mud station was higher than at the sand-cap stations and ranged from 3.3 to 8.6%, from 3.4 to 10.5% and from 3.0 to 7.8% in 2018, 2019 and 2020, respectively (Fig. 3). Total OM at the mud station was between 3 and 7% to a depth of 9 cm, which was similar to the sand/mud transition zone at the sand-cap stations. Sediment sections from 9–13 cm at the mud station had OM up to 10.5%, which was similar to the deepest sediment below the sand-cap (Fig. 3).

3.2. Eelgrass transplantation

Eelgrass shoot densities in the transplantation area declined from the initial transplantation in July 2019 to the end of October 2019. Eelgrass shoots died at a faster rate in muddy substrate compared to the sand-cap and at a faster rate in the small-scale transplantations compared to the large-scale ones (Fig. 4A). The large-scale sand-cap transplantation initially had 52 shoots m^{-2}, which declined to 24 shoots m^{-2} in October. The 2 small-scale transplantations had initial shoot densities of 45 shoots m^{-2} (sand-cap) and 51 shoots m^{-2} (mud). The small difference in initial

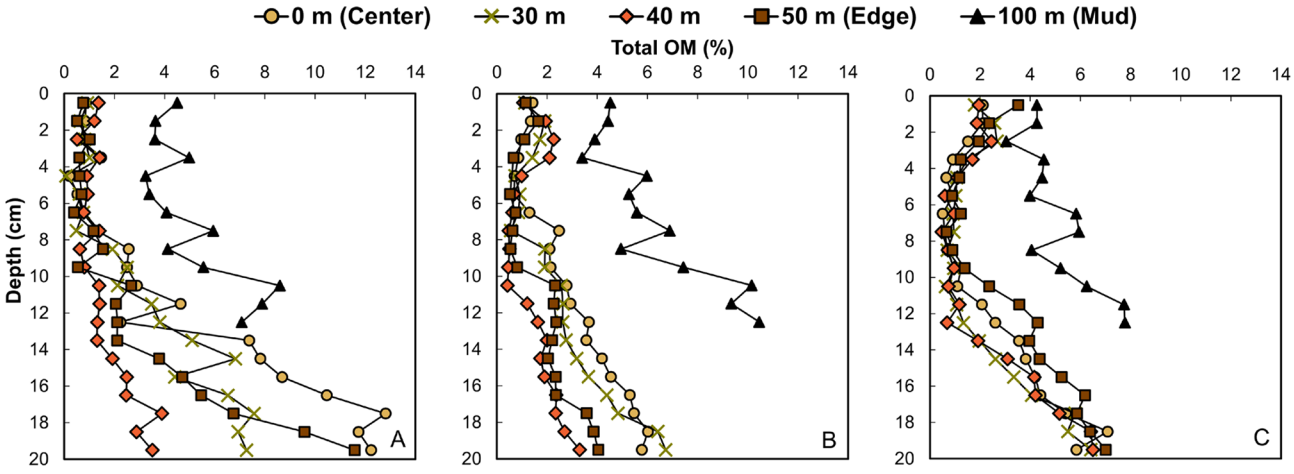

Fig. 3. Depth profiles of organic matter (OM) in the sediment from stations on a transect from the center of the sand-cap (0 m) to adjacent uncapped mud (100 m), in the years (A) 2018, (B) 2019 and (C) 2020

density was due to side shoots on some planting units. By late October, the shoot densities declined to 14 and 1 shoot m^{-2} for sand-capped and muddy substrate, respectively (Fig. 4A). Monitoring was discontinued during winter, and no shoots could be found when we returned in spring 2020. Epiphyte coverage on the eelgrass leaves increased from 5% in July to 45% in August and 25–35% in September, before it

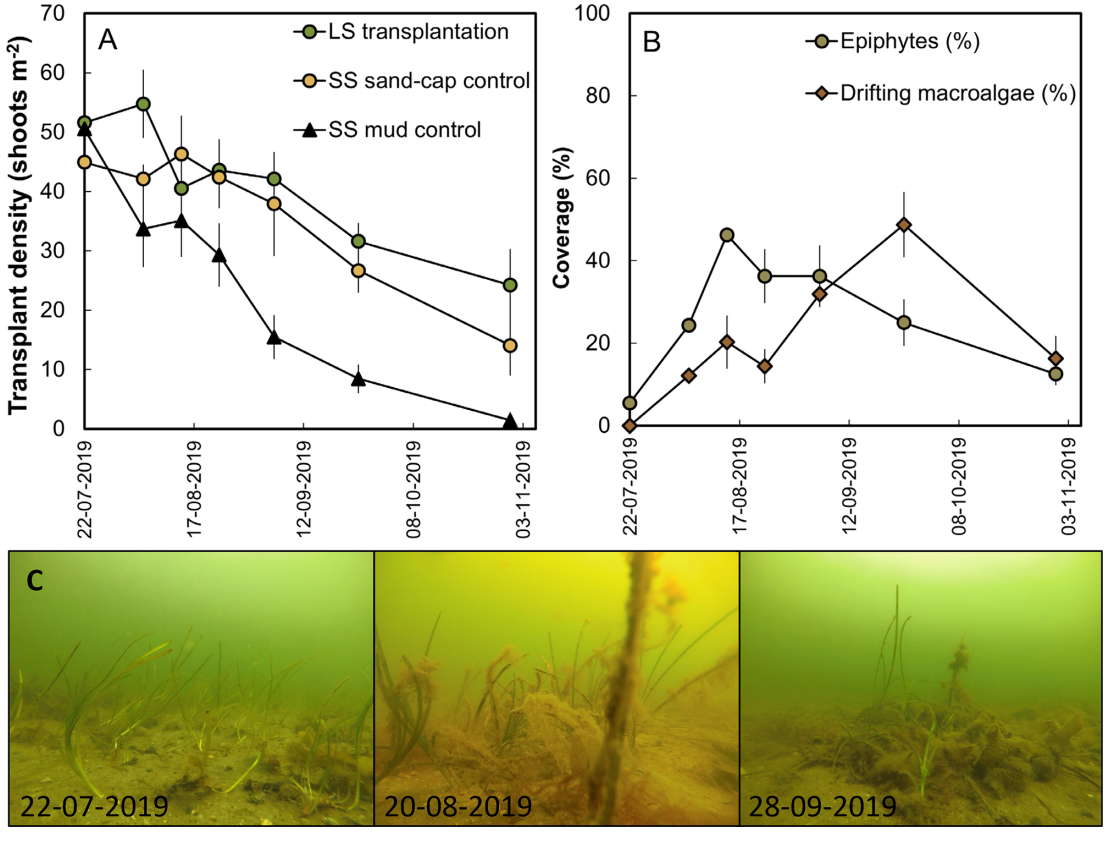

Fig. 4. (A) Temporal development of eelgrass transplant densities planted in large-scale (LS) and small-scale (SS) plots on the sand-cap and an adjacent small-scale (SS) mud control in 2019. (B) Temporal development in coverage of epiphytes and drifting macroalgae in the transplanted eelgrass. (C) Pictures from the action camera time series, documenting epiphytes and macroalgae smothering the eelgrass transplants. Dates given as d-mo-yr

steadily declined to 15% in October. Simultaneously, there was an increase in drifting macroalgae from 0% in July to 20% in August and 50% in September, which declined to 20% in October (Fig. 4B). The presence of these stressors coincided with decreasing eelgrass shoot densities. The time series of pictures taken in one of the eelgrass plots revealed that eelgrass shoots were initially smothered by epiphytes and later by a combination of epiphytes and drifting mats of macroalgae (Fig. 4C).

3.3. Infauna colonization

A total of 25 species of infauna were found in this study. They included 9 polychaetes, 1 oligochaete, 2 gastropods, 11 bivalves and 2 crustaceans (Table S1 in the Supplement at www.int-res.com/articles/suppl/m729p063_supp.pdf). Species richness at the sand-cap stations ranged from 8 to 15 in 2018, from 13 to 21 in 2019 and from 10 to 13 in 2020, while lower values of 3 in 2018, 10 in 2019 and 9 in 2020 were recorded at the 100 m mud station (Table S1).

A significant interaction was found between the 2 factors year and station for S_i (ANOVA: $F_{8,45} = 4.178$, $p < 0.001$). A pairwise comparison of the interactive effects revealed no significant temporal change in S_i at the mud station, but a significant change in S_i at the sand-cap stations. S_i increased significantly from 2018 to 2019 at all sand-cap stations by 55–175% but decreased again from 2019 to 2020 to levels comparable to 2018 (Fig. 5A). Furthermore, S_i was significantly higher at the 0 and 30 m stations than at the 100 m station in both 2018 and 2020. S_i was significantly higher at all sand-cap stations than at the 100m (mud) station in 2019 (Fig. 5A). Polychaetes dominated the community at all stations, and *Alitta succinea*, *Hediste diversicolor* and *Heteromastus filiformis* were found across all stations and years. In contrast, *Scoloplos armiger*, several Spionidae (e.g. *Polydora cornuta*, *Pygospio elegans* and *Streblospio shrubsolii*) and *Tharyx robustus* were almost exclusively part of the sand-cap community (Table S1). Bivalves primarily colonized the sand-cap during 2019 and attained similar species richness as polychaetes during this year, but their richness and abundance dwindled in 2020. Juvenile *Mya arenaria* and *Parvicardium minimum* were some of the most abundant bivalves in 2019 (Table S1). The amphipod *Microdeutopus gryllotalpa* and the gastropod *Ecrobia ventrosa* also appeared in 2019 at the sand-cap station but disappeared again in 2020. The colonization of different species of mollusks was

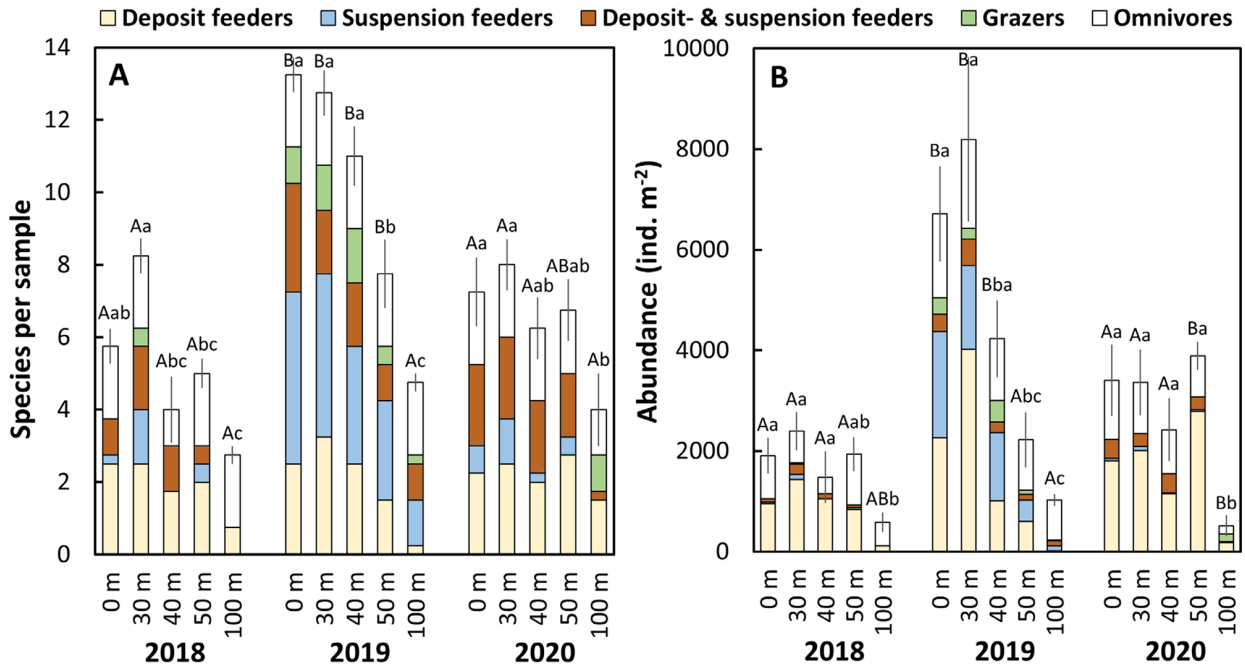

Fig. 5. (A) Number of infauna species per sample and (B) animal abundance on a gradient from the center of the sand-cap (0 m) to adjacent uncapped mud (100 m) in the years 2018, 2019 and 2020 (mean ± SE, n = 4), grouped according to feeding guilds: omnivores, grazers, deposit and suspension feeders, suspension feeders and deposit feeders. Capital and lowercase letters denote significance between years within stations and between stations within years, respectively. Same letters indicate no significant difference

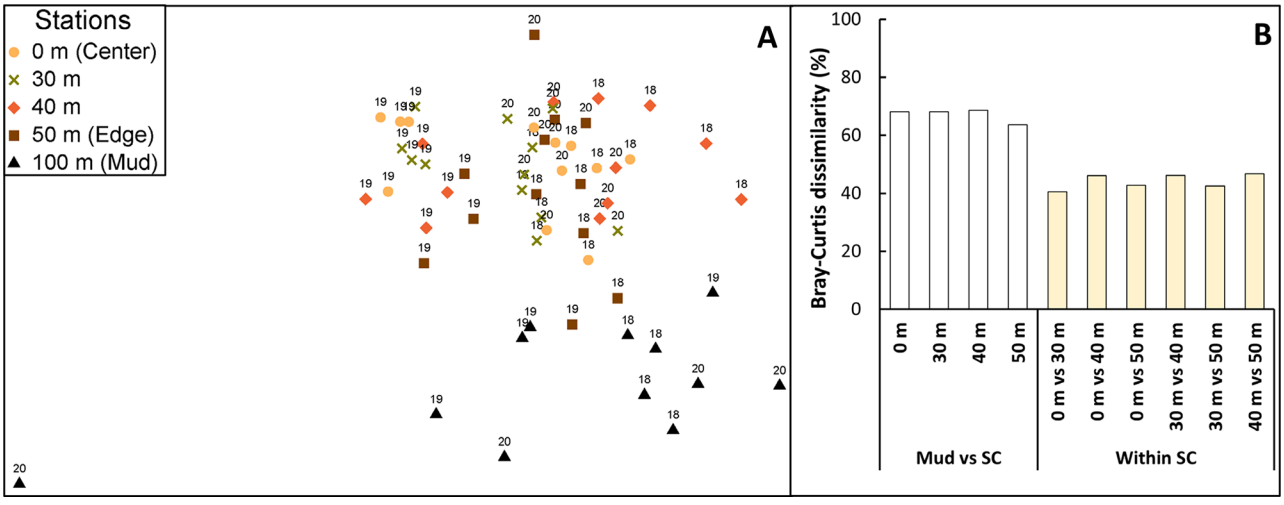

Fig. 6. (A) Non-metric multidimensional scaling plot (stress 0.14) of infauna abundance (N_i) at stations 0 m (center), 30 m, 40 m, 50 m (edge), 100 m (mud); labels indicate years from 2018 to 2020. (B) Bray-Curtis dissimilarities (%) of N_i. Dissimilarity between 100 m (mud) and sand-cap (SC) stations and within SC stations

primarily driving the increase in species richness at all sand-cap stations in 2019 (Fig. 5A).

A significant interaction between stations and years was found for N_i (pseudo-F = 2.387, p = 0.0001). Pairwise test of the interaction showed that the 0, 30 and 40 m stations all had significantly higher abundance than the 100 m station during all 3 years. N_i at the 50 m station was not different from N_i at the 100 m station in 2018 and 2019 but was significantly higher in 2020 (Fig. 5B). Furthermore, a significantly higher N_i was found at the 0, 30 and 40 m stations in 2019 compared to the 2 other years, which was driven by an increased abundance of deposit feeding polychaetes and suspension feeding bivalves (Fig. 5B). N_i at the 50 m station was significantly higher in 2020 than 2018 and 2019, because of an increase in the deposit feeding polychaete *T. robustus* (Fig. 5B).

N_i was relatively low in 2018 and ranged from 1482 to 2394 ind. m^{-2} at all sand-cap stations but was still higher than the 584 ind. m^{-2} at the 100 m station (Fig. 5B). N_i increased to 4232–8194 ind. m^{-2} at the 0, 30 and 40 m stations in 2019 (Fig. 5B). This was mainly driven by colonization of *M. arenaria*, *H. diversicolor* and *T. robustus* in high numbers (Table S1). The 50 and 100 m stations had significantly lower N_i that ranged from 1026 to 2223 ind. m^{-2} in this year (Fig. 5B), because of lower abundance of the 3 previously mentioned species. A decrease in N_i occurred in 2020, and N_i at all of the sand-cap stations ranged from 2423 to 3890 ind. m^{-2}, while N_i at the 100 m station was 513 ind. m^{-2} (Fig. 5B). *H. diversicolor* and *T. robustus* accounted for 69–84% of the abundance on the sand-cap in 2020.

The nMDS plot revealed that the 100 m (mud) community was dissimilar to the community at all sand-cap stations. Only a few replicates from the 50 m station overlapped with the 100 m station (Fig. 6A). Furthermore, the community in 2019 was dissimilar to the community structure in 2018 and 2020 on the sand-cap. The SIMPER analysis revealed that the infauna community had a different structure of feeding guilds at the 100 m station compared to the sand-cap stations. The 100 m station was always more than 64% dissimilar to the sand-cap stations, while dissimilarity between stations within the sand-cap ranged from 41 to 47% (Fig. 6B). Stations closest to the center of the sand-cap (0 and 30 m stations) had the most similar distribution of feeding guilds. Deposit feeding fauna accounted for more than 40% of the dissimilarity between the 100 m station and sand-cap stations (Table 1). This feeding guild was up to 23 times more abundant on the sand-cap compared with the 100 m station (Table 1). Deposit feeders, omnivores and suspension feeders explained 75–82% of the total dissimilarities between the sand-cap stations and the 100 m station. Omnivores were about twice as abundant, while suspension feeding fauna had up to 22 times higher abundance on the sand-cap compared with the 100 m station (Table 1).

3.4. Epifauna colonization

Total epifauna species richness and abundances were remarkably low during the whole study. A total of 13 epifauna species were found, including 4 gastro-

Table 1. SIMPER analysis of difference in abundance (ind. m^{-2}) between the 100 m (mud) station and the sand-cap stations (0, 30, 40 and 50 m) for each feeding guild of infauna — deposit feeders (D), omnivores (O), suspension feeders (S), deposit and suspension feeders (D/S) and grazers (G). Average abundance (*N*, ind. m^{-2}) at each station, contribution to dissimilarity (Cont. diss.) and cumulative contribution to dissimilarity (Cum.) are given

Feeding guild	100 m	0 m			30 m			40 m			50 m		
	N	*N*	Cont. diss. (%)	Cum. (%)	*N*	Cont. diss. (%)	Cum. (%)	*N*	Cont. diss. (%)	Cum. (%)	*N*	Cont. diss. (%)	Cum. (%)
D	105	1677	41	41	2490	43	43	1073	40	40	1411	45	45
O	475	1230	19	60	1135	16	59	808	21	61	941	22	67
S	33	727	18	78	618	19	78	456	14	75	166	15	82
D/S	38	266	17	95	328	17	95	233	17	92	138	13	95
G	57	109	5	100	81	5	100	143	8	100	29	5	100

pods, 6 crustaceans, 1 Asteroidea and 2 species of small fish. Total species richness was 4–8 and 8–10 at the 0 m and 100 m station, respectively (Table S2). S_e did not change significantly between seasons at any of the 2 stations, but S_e was significantly higher at the 100 m station than at the 0 m station during all seasons (ANOVA: $F_{1,24} = 16.989$, $p < 0.001$; Tukey post hoc: $p < 0.001$) (Fig. 7A).

N_e was significantly different both between seasons and stations, with a significant interaction between the 2 factors (pseudo-$F = 4.604$, $p = 0.0001$). Pairwise testing revealed that N_e at the 0 m station decreased significantly from summer, through fall to spring (Fig. 7B). N_e did not change at the 100 m station from summer to fall but increased slightly in spring 2019 (Fig. 7B). Furthermore, N_e was significantly higher during summer and lower during spring on the sand-cap, while no difference in N_e between stations was detected in fall (Fig. 7B). There was a clear dominance of meso-predators, and the shrimp *Crangon crangon* and gobid *Pomatoschistus minutus* were the 2 most abundant species during summer 2018. *C. crangon* was still the most abundant species in fall 2018, while *P. minutus* decreased in abundance as did the shore crab *Carcinus maenas*, which disappeared completely at both stations. The gastropods *Littorina litto-*

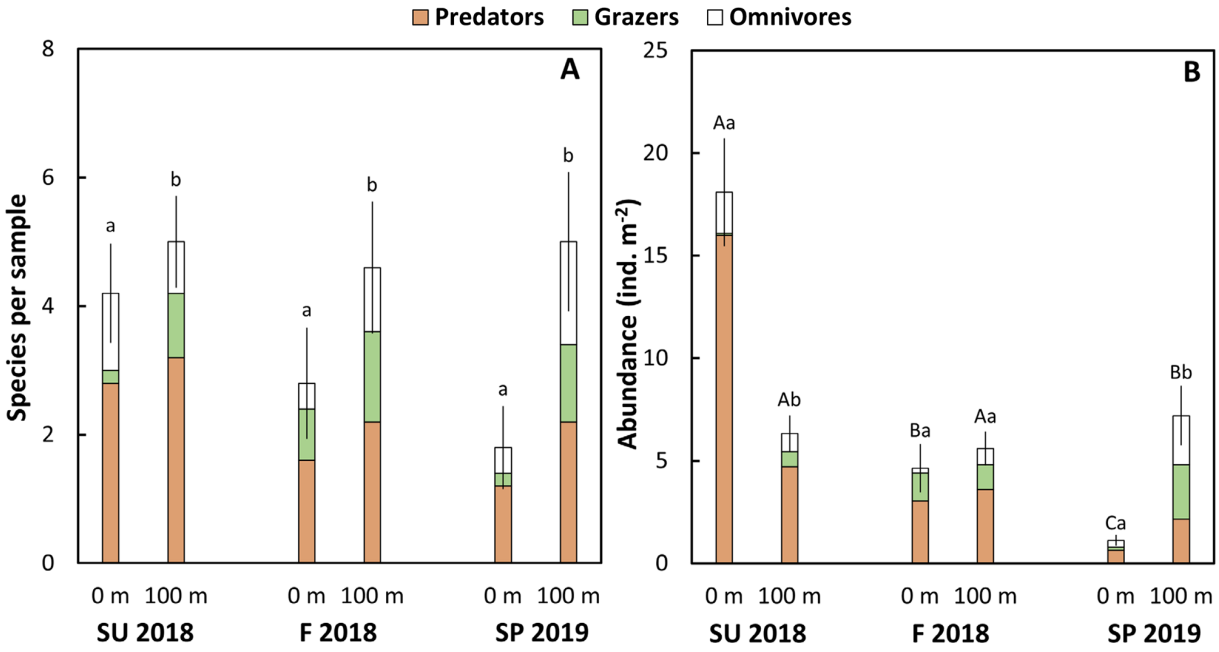

Fig. 7. (A) Number of epifauna species per sample and (B) fauna abundance on the sand-cap (0 m) and adjacent mud (100 m) (mean ± SE, n = 5) during 3 seasons: summer (SU 2018), fall (F 2018) and spring (SP 2019), grouped according to feeding guilds: predators, grazers and omnivores. Capital and lowercase letters denote significance between seasons within stations and between stations within seasons, respectively; same letters indicate no significance

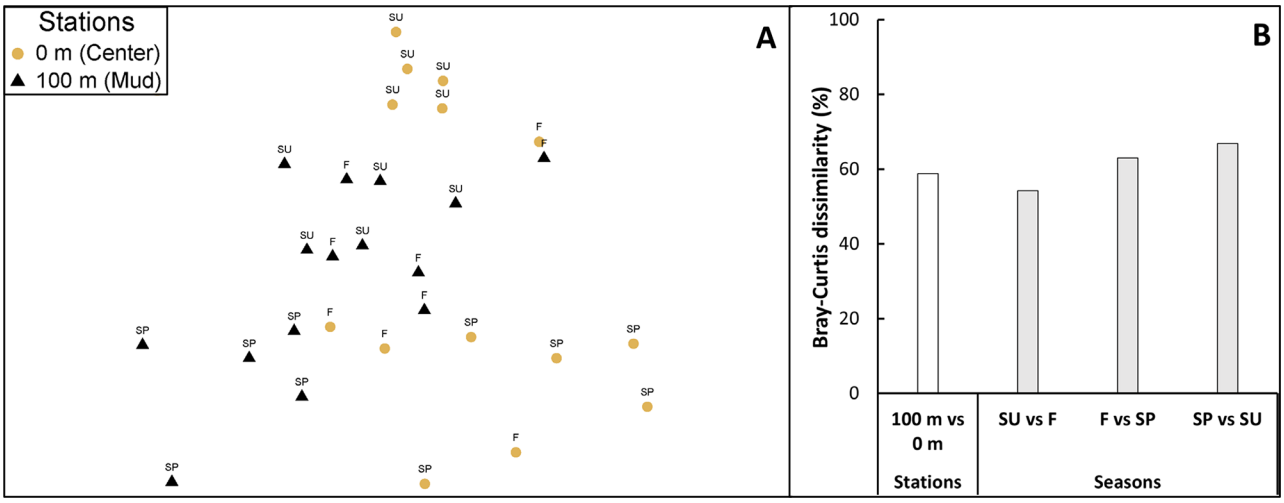

Fig. 8. (A) Non-metric multidimensional scaling plot (stress 0.17) of epifauna abundance (N_e) for the stations 0 m (center) and 100 m (mud). (B) Bray-Curtis dissimilarities of N_e. Dissimilarity between 100 m (mud) and 0 m (center of sand-cap station) and between seasons: summer (SU), fall (F) and spring (SP)

rea and *Tritia reticulata* had the highest abundance of all species during spring 2019 at the 100 m station (Table S2).

The nMDS plot revealed that the community at the 0 m station was not similar during different seasons, even though the samples from the fall were quite scattered in the ordination plot. Meanwhile, samples from the 100 m station were similar in summer and fall but dissimilar during spring (Fig. 8A). Differences between stations were likewise affirmed by dissimilarities between the 0 and 100 m stations during summer and spring, although they were similar in fall (Fig. 8A). The SIMPER analysis showed that the 2 stations were 59% dissimilar in terms of feeding guilds (Fig. 8B). Most (77%) of this difference was explained by a 2 times higher abundance of meso-predators at the 0 m station and a 3 times higher abundance of grazers at the 100 m station (Table 2). The seasonal dissimilarities were largest between spring and summer (67%) and lowest between summer and fall (54%) (Fig. 8B). Differences in meso-predator abundance explained 66 and 62% of these dissimilarities, respectively (Table 3), and meso-predator abundance was highest during summer. Conversely, meso-predator abundance was much lower during fall and spring, and differences in meso-predator abundance between fall and spring explained only 50% of the dis-

Table 2. SIMPER analysis of difference in abundance (ind. m^{-2}) between the 100 m (mud) station and the sand-cap station (0 m) for each feeding guild of epifauna — predators (P), grazers (G) and omnivores (O). Average abundance (*N*, ind. m^{-2}) at each station, contribution to dissimilarity (Cont. diss.) and cumulative contribution to dissimilarity (Cum.) are given

Feeding guild	100 m	0 m		
	N	*N*	Cont. diss. (%)	Cum. (%)
P	3.5	6.6	50	50
G	1.5	0.5	27	77
O	1.4	0.9	23	100

Table 3. SIMPER analysis of difference in abundance (ind. m^{-2}) between seasons — summer (SU), fall (F) and spring (SP) — for each feeding guild of epifauna — predators (P), grazers (G) and omnivores (O). Average abundance (*N*, ind. m^{-2}) at each station, contribution to dissimilarity (Cont. diss.) and cumulative contribution to dissimilarity (Cum.) are given. Note that omnivores and grazers are in opposite order for dissimilarity between spring and summer

Feeding guild	SU	F			F	SP			Feeding guild	SP	SU		
	N	*N*	Cont. diss. (%)	Cum. (%)	*N*	*N*	Cont. diss. (%)	Cum. (%)		*N*	*N*	Cont. diss. (%)	Cum. (%)
P	10.4	3.3	62	62	3.3	1.4	50	50	P	1.4	10.4	66	66
G	0.4	1.3	21	83	1.3	1.4	30	80	O	1.4	1.4	21	87
O	1.4	0.5	17	100	0.5	1.4	20	100	G	1.4	0.4	13	100

similarity. Grazer abundance increased during spring and differences in grazer abundance explained 30% of the dissimilarity between fall and spring (Table 3).

4. DISCUSSION

4.1. Eelgrass transplantation

The eelgrass transplantation experiment failed, despite using restoration methods that have proven to be successful in other systems (Fonseca et al. 1998, Orth et al. 2006, van Katwijk et al. 2016, Lange et al. 2022). The eelgrass was smothered by epiphytes and drifting ephemeral macroalgae mats, which indicated that eutrophication is still a mayor issue for eelgrass restoration in Odense Fjord. Simultaneously, drifting mats of *Fucus* sp. also acted as a stressor on the restored eelgrass. *Fucus* sp. is not an indicator for eutrophication and has increased in the outer part of Odense Fjord instead of *Zostera marina* after the reduction in nutrient loading (Petersen et al. 2009). However, the physical stress from drifting *Fucus* spp. has a major negative impact on both eelgrass seedlings and meadows because the rocks that the plants attach to cause ballistic impacts on eelgrass when they are transported all over the seabed by waves and currents (Valdemarsen et al. 2010, Canal-Vergés et al. 2014). Nevertheless, it was evident that sand-capping alters the physical properties of the sediment, and previous work has shown that the sand increases erosion thresholds, anchoring capacity of eelgrass roots and benthic light, through a reduction in sediment resuspension, which can facilitate eelgrass restoration (Flindt et al. 2022, Oncken et al. 2022). In this study it contributed to 29% slower decline of transplanted eelgrass shoots on the sand-cap compared with muddy sediments.

It is well described in the literature that eutrophication problems or other potential eelgrass stressors should be dealt with before attempting to restore eelgrass meadows (Fonseca et al. 1998, van Katwijk et al. 2016). Nutrient discharge was reduced in Odense Fjord, but it appears that the reduction in nitrogen and phosphorus discharges of approximately 30 and 80%, respectively (Petersen et al. 2009), is currently insufficient for eelgrass to recover or be restored even when sediment conditions are restored by sand-capping. This is because eelgrass recovery is not only impacted by eutrophication and impoverishes sediment conditions, but it is challenged by multiple stressors (Flindt et al. 2016). These include resuspension-driven reduction in benthic light (Canal-Vergés et al. 2010, 2014), seedling mortality due to physical impact from drifting macroalgae (Valdemarsen et al. 2010, Canal-Vergés et al. 2014) and burial of seeds and seedlings by the lugworm *Arenicola marina* (Valdemarsen et al. 2011), which complicates restoration because multiple issues need to be resolved simultaneously before restoration can work.

4.2. Successful infauna colonization

Infauna communities generally react to organic enrichment of the seabed with decreasing species richness, functional diversity and dominance of tolerant species (Pearson & Rosenberg 1978, Grall & Chauvaud 2002, Bolam et al. 2004), especially in areas where eutrophication leads to bottom water hypoxia (Conley et al. 2007, Bon et al. 2021). Our study confirms that restoration of the seabed with sand can revert such structural community changes, as illustrated by a swift colonization of the sand-capped area. This led to higher species richness (S_i), abundance (N_i) and higher functional diversity of infauna at the sand-capped site compared to adjacent organic-rich muddy sediment.

S_i in the muddy sediment of Odense Fjord was impoverished and comparable to fauna communities found in areas where oxygen deficiency is detected frequently (Karlson et al. 2002). Conversely, S_i in the sand-capped area was higher and comparable to that in other low-organic sandy sediments (Blomqvist & Bonsdorff 1986) and historical data from Odense Fjord before eutrophication (Oncken et al. 2022). The colonization of infauna after sand-cap restoration was rapid, as indicated by a community composition resembling natural estuarine sediments at least after 1 yr (Blomqvist & Bonsdorff 1986, Oncken et al. 2022). However, colonization may have occurred even quicker, but we cannot tell with the temporal resolution that samples were collected at in this study. Furthermore, future work should include a longer time series of sampling in the sand-capped area to support the fact that the restored habitat persists and keeps its ecological functions and high biodiversity. Longer time series would also contribute to a better understanding of year-to-year variability in the restored sediment and could reveal if the small reduction in species richness and abundance from 2019 to 2020 reflected a downward trend or simply year-to-year variation. The relatively short lifecycle of many of the invertebrates that inhabit the coastal zone contributes to substantial annual variation in community composition, especially in the early years after habitat resto-

ration (Valdemarsen et al. 2018, Steinfurth et al. 2022, Gagnon et al. 2023).

The suspension feeders that dominated the early colonization of infauna on the sand-cap can contribute to increased water clarity and are known to control water quality when they are abundant and are thus a vital feeding guild in eutrophic estuaries (Hily 1991, Petersen et al. 2008, Grizzle et al. 2018). Regaining this ecological function could potentially facilitate cleaner water and recolonization of eelgrass. However, suspension feeding bivalves decreased in numbers 2 yr after construction of the sand-cap, and a more stable community is needed to retain this ecological function. A similar 'boom and bust' colonization pattern of polychaetes and bivalves has been observed for invasive species (Delefosse et al. 2012) or colonizers of newly flooded coastal lagoons in Denmark (Valdemarsen et al. 2018). This pattern could indicate high initial recruitment, but also that juvenile survival may be low in the newly restored area. This recruitment mechanism assures that at least some individuals successfully survive the initial recolonization period (Pearson & Rosenberg 1978). However, it is difficult to assess these mechanisms with the current sampling design, as it lacks biomass and cohort measurements of the community. The abundance of deposit feeders (e.g. *Tharyx robustus*) and omnivores (e.g. *Hediste diversicolor*) remained high throughout the study. Their burrowing activity is expected to contribute substantially to improve the continued infauna colonization by increasing the redox potential in the sediment via bioturbation (Volkenborn et al. 2007, Hartmann et al. 2009, Valdemarsen et al. 2018). This study showed that colonization by infauna was most successful in the central part of the sand-cap. Conditions near the edge were likely affected by the adjacent mud; this was clear from the accumulation of a thin layer of organic-rich sediment on top of the sand-cap in 2020 at the 50 m station. This left a 10–20 m wide perimeter where species richness of infauna was in a transition state between the 2 habitats. It is therefore likely that sand-capping large areas (e.g. entire embayments) with a low perimeter to area ratio would reduce the transition zone and improve biodiversity on a seascape level. Capping at such a large scale calls for more cost-effective methods as described in Section 4.4.

4.3. Epifauna colonization

Colonization by epifauna was not detectable on the sand-cap, and species richness was always highest at the 100 m station. There was a strong decrease in meso-predator abundance during winter at both stations, in accordance with seasonal migration of several meso-predators (e.g. *Crangon crangon*, *Carcinus maenas* and *Pomatoschistus minutus*) (Hinz et al. 2004). Furthermore, there was a slight increase in gastropods *Littorina littorea* and opossum shrimp *Praunus flexuosus* abundance during spring, indicating that season rather than substrate was the structuring factor for the epifauna community.

Furthermore, drifting macrophyte mats (*Fucus* sp.) were observed on the muddy seabed, while the sand-cap remained bare (visible on ortho-photos; Fig. A1 in the Appendix). Such mats are known to support invertebrate epifauna at densities comparable to those found in seagrass beds (Norkko et al. 2000). This may explain the higher species richness at the mud station and the increased abundance of *L. littorea* and crustaceans (e.g. *P. flexuosus* and *Palaemon adspersus*) during spring. Furthermore, it shows how important vegetation is in shallow estuaries. Biodiversity of epifauna depends strongly on the persistent presence of seagrass or perennial macroalgae, because a majority of invertebrate epifauna and small fish are canopy dwellers (Fredriksen et al. 2005, Christie et al. 2009, Steinfurth et al. 2022). Odense Fjord has lost approximately 98% of the historical cover of *Zostera marina* due to eutrophication and associated siltation, which have reduced benthic light conditions (Petersen et al. 2009, Delefosse 2012, Steinfurth 2023). It is expected that such a decline in areal extent of suitable habitat affects the invertebrate epifauna community substantially.

4.4. Sand-capping perspectives

Sand-capping large areas would be very costly if it was performed with sand from gravel pits as in this study, but the cost would be reduced if non-contaminated sand from dredging local navigation channels is used, rather than sand from distant terrestrial or marine sources (Flindt et al. 2022). The frequent stormy winters in Denmark lead to substantial beach drift of sandy sediment along the northern coastline of Funen, which primarily ends up in the outer part of the navigation channel, and the harbor authorities in Odense Fjord report that they have to restore the navigation channel yearly by dredging about 90 000 m^3 yr^{-1} (Danish Environmental Protection Agency, https://edit.mst.dk/media/srsa5dgj/klaptilladelse-odense-havns-sejlrende.pdf; accessed 5 December 2023) of which about 50 000 m^3 are non-contami-

nated sediment from the outer part. The sand is dredged, loaded to barges and shipped to distant dumping sites. These activities are expensive in labor, shipping equipment and fuel, and the sand would be better utilized if it was used to consolidate muddy areas in the fjord by sand-capping. The annual amount of dredged material would be enough to create a 50 ha sand-capped area (10 cm thick) annually. This would constrain the cost of sand-capping to construction costs and make sand-capping much cheaper. Furthermore, beach nourishment alone accounts for 3 239 000 m^3 sand replenishment per year at the west coast of Jutland in Denmark (Danish Coastal Authorities, https://kyst.dk/klimatilpasning/sandfodring-paa-vestkysten; accessed 1 December 2023); if this sand material was used for sand-capping instead it would make it feasible to cap extensive areas. The approach has recently been tested in the coastal lagoon of Gyldensteen, a coastal realignment project in Northern Funen, where 2500 m^3 of drifting sand were taken from outside the lagoon and used for capping. The sand was tested to assure that the levels of heavy metals complied with national regulation standards, to avoid negative environmental impact during sand-cap construction. Furthermore, we assessed that the risk of spreading invasive species was low, as long as local dredged material is used. On the contrary, fauna colonization could potentially be accelerated if the dredged material already contains fauna that could contribute to community development.

Dredged material has already been utilized for terrestrial tasks, such as capping landfills, where it has proven to be a cost-effective method for land reclamation because the dredged material is readily available from routine dredging and provides a beneficial use for the dredged material instead of dumping it at distant marine sites (Mohan et al. 1997). Furthermore, dredged material is already being used for various products or tasks that are categorized as 'beneficial use', such as construction products, landfill, reconstruction of agricultural soil, beach nourishment and habitat building (i.e. creation of shoals, spits and bars, oyster reef restoration, bathymetric recontouring, restoration of intertidal marshes and mudflats, filling of bird and wildlife islands and remediation of upland habitats) (Yozzo et al. 2004, Solanki et al. 2023). However, up to 90% of dredged material is currently still either being dumped into the sea or used for land reclamation (Solanki et al. 2023), and it appears that the general perception is that dredge material is a waste product rather than a resource. This perception might need to change before the general public will accept that dredge material is dispersed on the sea floor in coastal waters and estuaries.

Capping with other materials, such as clay mixed with powdered activated carbon (to reduce release of contaminants), has been suggested previously, but it can have severe negative effects on benthic fauna and reduce species richness, abundance and biomass even further and delay natural recovery (Trannum et al. 2021). It has also been proposed that a lack of OM in capping materials limits natural recovery of benthic fauna and that organic enrichment is beneficial for faunal colonization (Kvassnes et al. 2009, Kvassnes & Iversen 2013). However, others have demonstrated that such organic enrichment of capping material has little effect on recolonization (Sweetman et al. 2020). Our results suggest that sandy material with low organic content (<1%) provides adequate basis for substantial improvement of infauna biodiversity and ecosystem functioning in Danish estuaries.

5. CONCLUSION

In this study, our aims were to restore sediment conditions by sand-capping, recover eelgrass meadows by transplanting eelgrass shoots and document changes in the benthic macrofauna communities after restoration activities. We succeeded at restoring sediment conditions for at least 3 growth seasons, but it was an insufficient solution to facilitate recovery of eelgrass due to the presence of eutrophication-related stressors, which killed the eelgrass transplants over the course of 3.5 mo. Nevertheless, the improved sediment conditions at the sand-cap facilitated the colonization of an infauna community that was more species rich, with higher animal abundance and a more diverse functionality (e.g. higher diversity of feeding guilds) compared to muddy sediment. It did not improve the epifauna community significantly. There were indications that the infauna community exhibited a colonization gradient from the center of the sand-cap towards the edge and that the community at the edge had lower species richness and animal abundance than in the center, but these parameters were still higher at the edge than at adjacent muddy sediment. We conclude that sand-capping can improve infauna communities, but that studies with longer time series are needed to assure that it has long-term prospects as a habitat restoration tool for managers and stakeholders.

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Appendix.

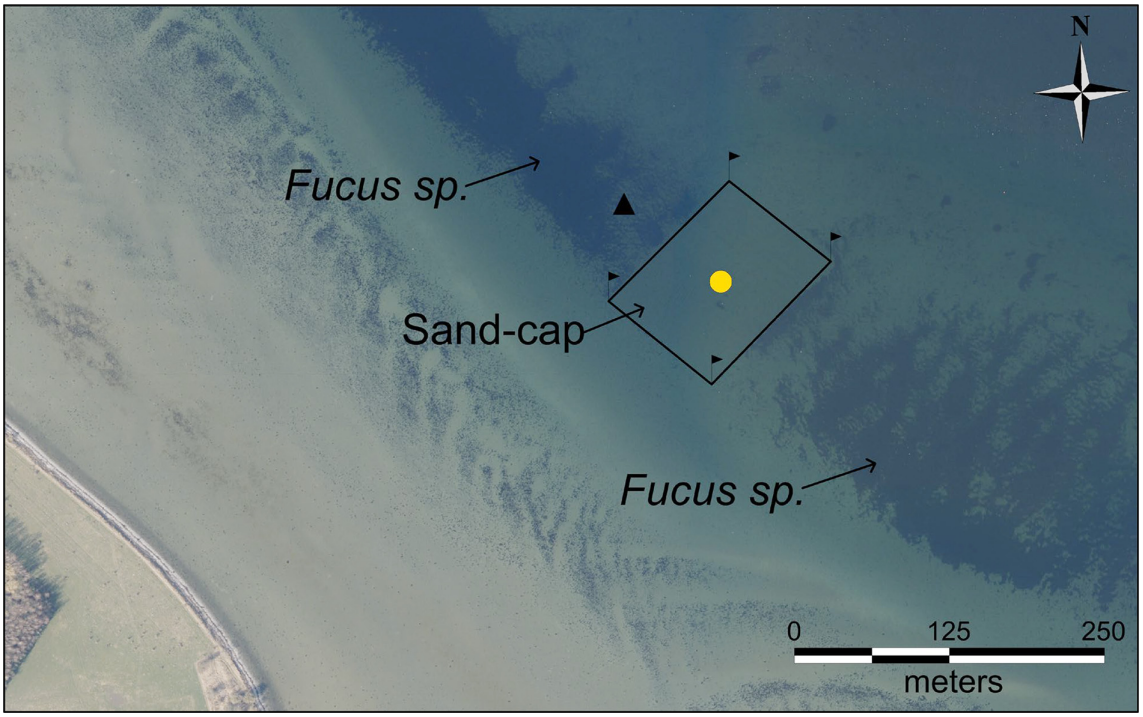

Fig. A1. Ortho-photo showing a sharp outline of the sand-cap, with drifting *Fucus* sp. mats north-west and south-east of the sand-cap. Epifauna sampling stations are shown as a yellow circle (0 m) and black triangle (100 m). Source: Dataforsyningen, https://dataforsyningen.dk/data/981; accessed 13 January 2023

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