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Large-scale eelgrass transplantation: a measure for carbon and nutrient sequestration in estuaries

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ABSTRACT: The accelerated global losses of seagrass meadows makes restoration increasingly important. This restoration study was conducted in a shallow Danish estuary and describes one of the rare examples of successful large-scale eelgrass *Zostera marina* restoration outside North America. A simplified 3-step site selection approach was successfully applied to locate an optimal site for large-scale transplantation. It consisted of (1) qualitative assessments of vegetation using aerial photos, (2) inspection of potential sites with assessments of stressor presence and potential growth conditions and (3) transplantation tests for a final assessment of site suitability and methodology. The large-scale transplantation was initiated at the test site with the highest shoot production. After transplantation, shoot densities developed rapidly, achieving a 70-fold increase in density after about 2 yr. A rapid edge expansion (0.32 m yr^{-1}) of the transplanted area was detected using drone-based monitoring. Both the final shoot density and edge expansion were comparable to those of natural eelgrass patches in the estuary. Eelgrass-transplanted areas accumulated more fine sediment particles and organic C, N and P than adjacent unvegetated sediment. Burial of organic C, N and P in eelgrass-transplanted sediments was 33 ± 7.5, 6.6 ± 0.9 and 3.0 ± 0.5 g m^{-2} yr^{-1}, respectively (mean ± SE). In addition, inorganic C and N were assimilated by eelgrass transplants at rates of 290 ± 22 and 12 ± 1.0 g m^{-2} yr^{-1}, respectively. The results highlight that important ecosystem services are already restored 2 yr after successful eelgrass restoration.

KEY WORDS: Seagrass · *Zostera marina* · Restoration · Carbon burial · Nitrogen burial · Drone

1. INTRODUCTION

Seagrasses provide key ecological functions in coastal areas. They are very productive and have high uptake and sequestration of carbon (C) and nutrients into slowly degradable seagrass tissue (Risgaard-Petersen et al. 1998, Flindt et al. 1999, Romero et al. 2006, Aoki et al. 2020). Eelgrass ecosystems therefore reduce the nutrient turnover compared with systems dominated by opportunistic and perennial macroalgae (Flindt et al. 1999, Banta et al. 2004). The leaf canopy dampens wave action, and in conjunction with continuously branching rhizospheres, seagrasses stabilize the sediment and limit sediment mobility (Fonseca & Fisher 1986, Fonseca et al. 2007, Potouroglou et al. 2017). High production of seagrass material with slow degradability, combined with increased sedimentation and burial of organic material in seagrass meadows, enhances their ability to accumulate and sequester C (Mcleod et al. 2011, Greiner et al. 2013). Furthermore, the mosaic of structures, shelters and surface areas in seagrass meadows serves as nursery ground and refuge for fish and invertebrates (Boström & Bonsdorff 1997, Bowden et al. 2001, Duffy 2006, Pihl et al. 2006).

Unfortunately, seagrasses and their associated ecosystem functions are lost globally at accelerated rates (Waycott et al. 2009, Short et al. 2011). Eelgrass *Zostera marina*, the dominant seagrass in Danish estuaries and coastal waters, has suffered major de-

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clines of 80–90% since 1900 (Boström et al. 2014). Eutrophication is a main driver for the loss of seagrasses, and hence nutrient reductions are crucial and necessary to support natural recovery of seagrasses (Nielsen et al. 2002). Unfortunately, natural recolonization of seagrasses is often lacking even when nutrient loadings are reduced (Leschen et al. 2010, Valdemarsen et al. 2010, 2011, Carstensen et al. 2013). Despite a few examples where nutrient reductions have promoted recolonization of eelgrass (Riemann et al. 2016), the general trend is that recolonization of eelgrass in Danish waters is absent (Hansen & Høgslund 2021).

A variety of eelgrass restoration projects have been performed in northern temperate areas to compensate for the accelerated loss. Restoration is performed either by broadcasting harvested seeds (Harwell & Orth 1999, Marion & Orth 2010, Orth et al. 2012, Infantes et al. 2016) or by transplanting shoots (Davis & Short 1997, Orth et al. 1999, Calumpong & Fonseca 2001), and both approaches require careful planning. Seed-based restoration requires development of efficient methods for harvest, maturation, storage and potential purification of seeds. The critical phase linked to seed broadcast methods is to combat stressors. Low hydrodynamic stress and low activity of reworking benthic fauna are essential for seeds to stay at their designated location (Valdemarsen et al. 2011, Flindt et al. 2016, Kuusemäe et al. 2018). The obvious benefit of a seed-based approach is the potential for large-scale and low-cost eelgrass restoration (Orth et al. 2012). However, tests in Danish estuaries using restoration based on seeds revealed that these approaches are disqualified due to a 99.9% loss of seeds after broadcast. Focus in these estuaries has therefore shifted to transplantation-based restoration of eelgrass (T. Lange et al. unpubl.).

Seagrass restoration efforts have shown varying degrees of success (van Katwijk et al. 2016), and restoration trials are highly dependent on 3 factors: site selection, scale of the restoration and method of transplanting shoot units (Fonseca 2011, Cunha et al. 2012, Park et al. 2013, van Katwijk et al. 2016). Careful selection of restoration sites by avoiding suboptimal environmental conditions is necessary to exclude stress factors and unfavourable growth conditions (Short et al. 2002). An alternative, but less resource-efficient, approach is to use protective measures to reduce harmful effects of specific stress factors (van Katwijk & Hermus 2000, Sousa et al. 2017, Wendländer et al. 2019). The use of conceptual suitability models combined with field screenings and establishment of small-scale transplantation is a proven approach for site selection (Short et al. 2002, Leschen et al. 2010, Valle et al. 2011, 2015). Aerial photos and qualitative field assessments of critical parameters (depth, exposure level and eutrophication, presence of stressors such as turbidity, bioturbation, drifting macroalgae and epiphytes) are valuable tools in the field screening phase. This is followed by test transplantations for a final empirical evaluation of the best-suited sites. This approach has been successfully implemented in various temperate and subtropical estuaries (Park et al. 2013, Wendländer et al. 2019), despite a variety of stress and growth-limiting factors in these systems (e.g. eutrophication, intense grazing and bioturbation). A sufficiently high number of test sites increases the chance of locating suitable growth conditions with low environmental stress (Short et al. 2002, Leschen et al. 2010, Wendländer et al. 2019).

The transplantation process is relatively straightforward, where shoots are harvested from donor meadows and transplanted at a restoration site. However, there are several challenges in designing optimal methods for handling shoots to assure restoration success. Transplanted shoots are subject to multiple stressors hindering their establishment and development into new eelgrass meadows. Shoots can easily be dislodged by water currents, wave action and drifting macroalgae if they are transplanted without anchoring support (Valdemarsen et al. 2010, van Katwijk et al. 2016). Eelgrass transplants can also be protected by physical shielding from hydrodynamic exposure (van Katwijk & Hermus 2000) and bioturbating infauna (Sousa et al. 2017, Wendländer et al. 2019). Nets or cages can be used to reduce destructive effects from mobile epifauna and grazing fish (Davis & Short 1997, Davis et al. 1998, Wendländer et al. 2019). A key approach to overcome stress factors is by increasing the transplantation area, because this will (1) reduce the risks of failure and (2) provide self-sustaining feedback mechanisms. These benefits first arise at a scale of 1000–10 000 transplants, but become truly self-facilitative only above 10 000 transplants (van Katwijk et al. 2016), underlining the fact that size matters in seagrass restoration projects.

The present study describes a new large-scale eelgrass transplantation method for Danish estuaries. The main aim is to demonstrate a marine instrument that can support Danish national plans by facilitating nutrient retention in the estuaries through large-scale eelgrass transplantation. Rates of nutrient retention and burial were assessed together with other important ecosystem services, such as C burial and sediment particle retention, which were compared with unvegetated sediments and natural eelgrass meadows.

2. MATERIALS AND METHODS

2.1. Horsens Fjord

Horsens Fjord is a small (79 km^2), microtidal (±0.3 m) and shallow estuary in Denmark with an average depth of 3.0 m at mean sea level (MSL) (Fig. 1), and a yearly temperature and salinity range of 0–22°C and 14–27, respectively (national NOVANA database, Stn 94330002; https://odaforalle.au.dk/login.aspx). Average nutrient loadings were 893 and 35.3 t yr^{-1} for nitrogen (N) and phosphorus (P), respectively, in the period 2010–2014 (Windolf et al. 2013, SVANA 2016). The estuary receives freshwater inputs predominantly in the western inner part and has a clear estuarine gradient from the inner to the outer part where water exits the estuary into the Kattegat. Water residence time in the estuary varies from 5 to 10 d (Stedmon et al. 2006).

2.2. Site selection for large-scale transplantation

A 3-step site selection strategy was implemented, combined with testing of the appropriate protection method for adapting the eelgrass transplantation approach to the local conditions:

(1) Qualitative assessments were performed using aerial photos with focus on temporal coverage of eelgrass populations during the last 2 decades. This was based on national ortho-photos over the period 1999–2017 in 2 yr intervals. Areas with repeated loss and recolonization of eelgrass and with frequent macroalgal accumulations were avoided. Large bare areas with remnants of eelgrass and consistent vegetative expansion in radial growth patterns were targeted.

(2) Field inspections of the best-suited sites were conducted to qualitatively assess the environmental conditions for eelgrass transplantation. These included observations of presence and intensity of growth-limiting factors (epiphytes, opportunistic macroalgae, drifting perennial algae, bioturbation and shore crab abundance) and presence of seedlings or recently emerged eelgrass patches. In addition to determining fetch lengths, the degree of potential wave exposure was assessed based on visible sediment characteristics such as sediment coarseness. The sites should have a sufficiently large area (>1 ha) within a water depth range of 1.0–1.5 m MSL.

(3) Small-scale transplantation trials were applied as a final empirical assessment of the selected sites combined with tests of different protective approaches for transplantation (explained in Section 2.3). The final criterion of the site selection phase was positive developments of shoot density in transplantation trials.

2.3. Small-scale transplantation trials for site and method selection

Small-scale trials were conducted in April 2017 at 3 locations in Horsens Fjord (Fig. 1). Each trial consisted of 3 replicates according to the following treatments:

(1) Control plots (1 × 1 m) with 24 eelgrass transplants.

(2) Plots (1 × 1 m) with 24 eelgrass transplants surrounded by 2 cm mesh plastic net enclosures (3 × 3 m, 0.5 m high) with baited (0.5 l of crushed mussels) crab traps. A trap was placed both in- and outside of the net enclosure.

(3) Transplanting 24 eelgrass shoots within polypropylene cylinders (1 m diameter, 0.2 m high and 0.2 cm thick) secured halfway into the sediments with metal poles (1.1 m).

These 3 treatments were established in a randomized order with 2 m distance between replicates. The cylinder enclosures were not applied at Site A (Fig. 1) due to risk of high algal growth on the polypropylene structures which could negatively impact the eelgrass transplants at this nutrient-rich site. Water depths ranged from 1.0 to 1.4 m MSL at the trial sites. In each plot, eel-

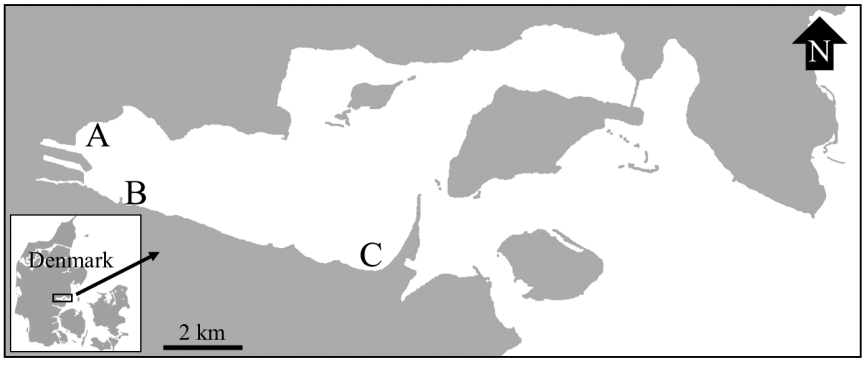

Fig. 1. Horsens Fjord, Denmark, showing locations (Sites A, B and C) of the 3 transplantation tests for identifying a suitable large-scale transplantation site. The large-scale eelgrass transplantation was established at Site C. The inset shows the location of Horsens Fjord within Denmark

grass shoots were transplanted with 3 rows of 8 shoots with 0.14 m between shoots and 0.5 m between rows. The transplants were anchored with pre-soaked (2 d) bamboo barbecue skewers (25 × 0.3 cm), bent into a V-shaped stake (hereafter V-stakes) that pushed the rhizome 2–5 cm into the sediment (Fig. 2A).

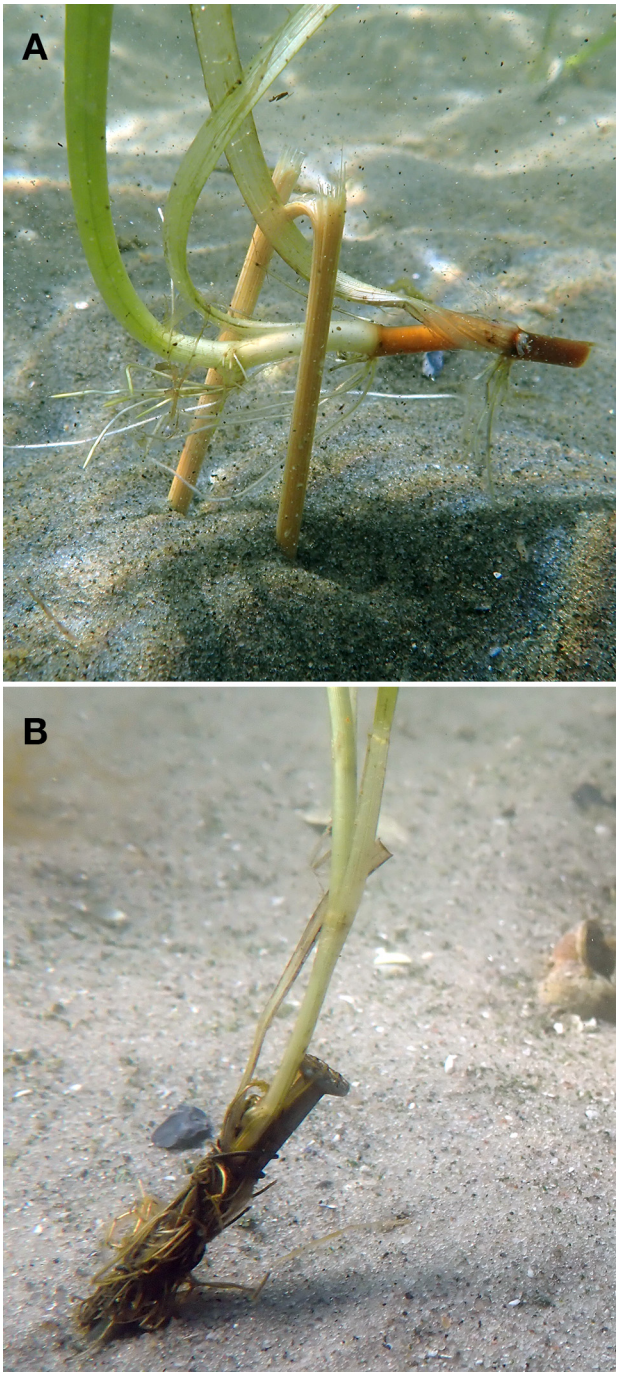

Fig. 2. Two applied methods for anchoring transplanted eelgrass shoots, shown before insertion into the sediment: (A) bamboo V-shaped stakes and (B) weighted shoots

2.4. Harvest of donor eelgrass for transplantation

The eelgrass donor meadow was located 450 m east of the large-scale transplantation area (WGS84: 55.829, 10.000). Shoots were harvested with garden rakes that recovered small sections of eelgrass mats (~0.3 × 0.3 m) containing both shoots and rhizomes but excluded sediment. This method is efficient and prevents large gaps in the donor meadows since no sediment is removed. Eelgrass shoot density in the donor meadow ranged from 800 to 1800 shoots m^{-2}, and during harvest, high-density inner parts of the meadow were targeted to avoid fragmentation, and the harvest ensured that <10 m^2 of eelgrass meadow was harvested for the large-scale transplantation. Eelgrass shoots to be anchored by V-stakes in the trials were transported directly to the transplantation area since no shoot preparation was needed. The eelgrass shoots harvested for large-scale transplantation were transported in large nets to shallow waters near the transplantation site where they were stored until further handling.

2.5. Large-scale transplantation

During site selection trials, a suitable site for large-scale transplantation was identified and the large-scale transplantation was established in the middle of July 2017. The large-scale transplantation area covered 51 × 78 m about 300 m from the coast at a water depth range of 1.2–1.4 m MSL. Within this area, 192 plots (2 × 2 m) were transplanted in an expanded checkerboard pattern with bare stretches of 4 m between the plots (Fig. 3). Two methods of transplant anchoring (V-stakes and weighted shoots, Fig. 2) were each randomly assigned into 3 subfields (24 × 24 m) containing 32 transplanted plots (2 × 2 m) (Fig. 3). There was 3 m spacing between the subfields. The 2 × 2 m plots consisted of 5 rows of 15 transplanted shoots with 0.14 m between shoots and 0.5 m between rows (Orth et al. 1999). The initial transplanted shoot density within each plot was 14 shoots m^{-2}, and the total number was 14 400 transplants with 17 300 shoots when multiple lateral shoots are included.

The 2 anchoring methods were (1) bent bamboo skewers as described in Section 2.3, and (2) weighted shoots where the eelgrass rhizomes (5–10 cm) were attached to nails (8 × 0.3 cm) with iron wire (10 cm long, 0.5 mm thick) (Fig. 2B). Uncoated iron materials were used, as they are eventually lost to corrosion. Small-scale tests indicated equal growth of eelgrass

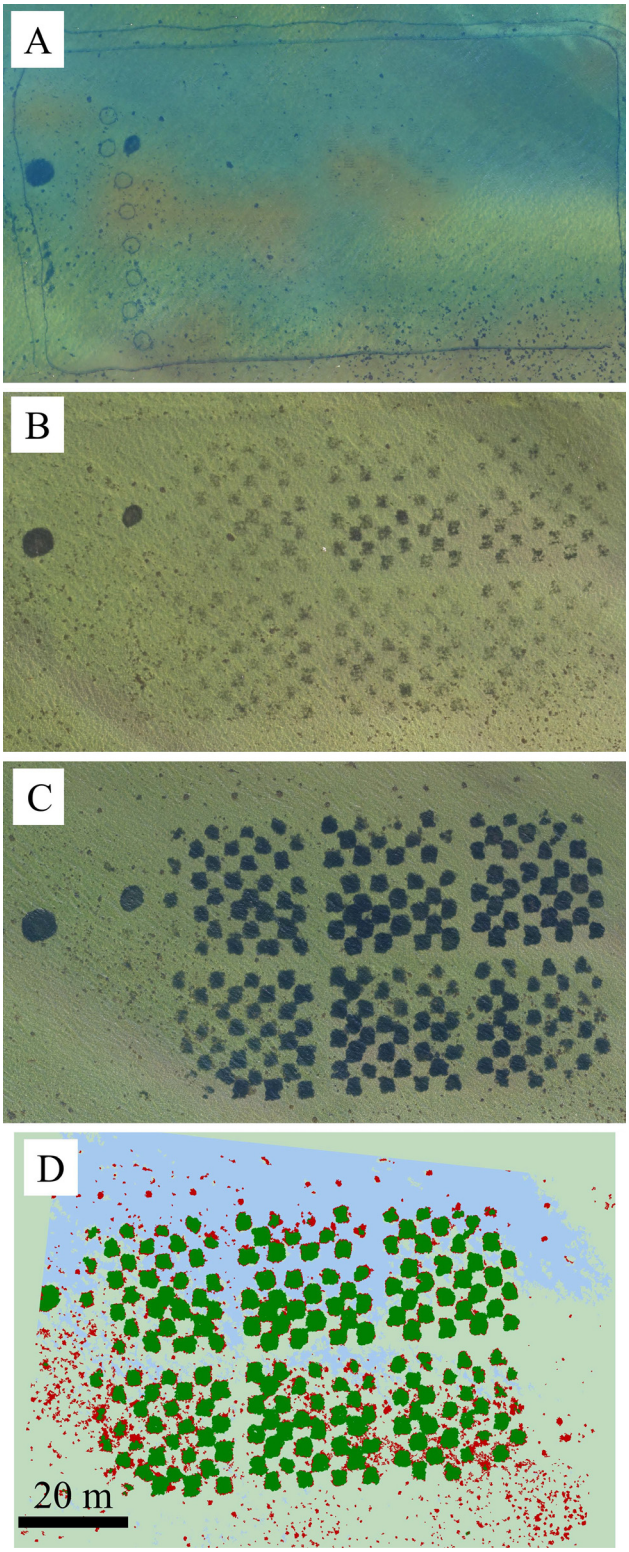

Fig. 3. Drone mapping of the large-scale transplantation in the years (A) 2017, (B) 2018 and (C) 2019, and (D) the output from the image analysis of vegetation types in 2019 where eelgrass is shown in green and macroalgae in red. All maps are at the same approximate scale

transplants anchored with V-stakes and weighted shoots (T. Lange unpubl.).

Two of the same protective approaches as used for the trials, i.e. net enclosures and mussel-baited crab traps, were used along the perimeter of the large-scale transplantation. There was a buffer of ~15 m between the transplants and the net (Fig. 3A). Crab traps were placed along the outside of the net every ~15 m to reduce destructive effects from shore crabs (Davis et al. 1998). Crab traps were emptied weekly during the first 2 mo after transplantation and then entirely removed. The nets were destroyed by storms in early winter and retrieved.

The large-scale transplantation lasted 5 working days with 10 people involved continuously. The time spent on harvest, shoot preparation, road transport of the crew, establishment of planting guides and the actual transplantation was ~2.4 min per shoot. This excludes site selection, project planning, placement of net enclosure, establishment of crab traps and monitoring.

2.6. Monitoring of shoot density

Shoot density was monitored in both the transplanted and natural eelgrass patches (reference patches). Monitoring of the transplanted eelgrass was done by random selection of 4 plots (2 × 2 m) in each 24 × 24 m subfield for both anchoring methods (V-stake and weighted shoots). Two circular counting frames (internal diameter of 40 cm) were thrown haphazardly within each 2 × 2 m plot. For each of the 6 subfields (24 × 24 m), shoot densities were quantified from a total of 8 counting frames. Three neighbouring natural eelgrass patches (3–15 m patch diameter) were monitored with lower temporal resolution using 2 counting frames in each patch.

2.7. Drone-based monitoring and image analysis

Coverage of the transplanted area and surrounding natural patches was quantified using images obtained with a DJI Phantom 4 Pro multi-rotor drone (www.dji.com). The images were collected at 100 m altitude with >70% overlap and sidelap, and stitched using the software Agisoft Metashape (www.agisoft.com). The resulting orthomosaic was subsequently analysed in the software eCognition Developer v. 9.3 (Trimble Geospatial, www.ecognition.com), by a multiresolution segmentation (scale 40, colour weight 0.8) and classification by *K* near-

est-neighbour machine learning algorithm (2 classes and 30 training samples per class).

2.8. Biomass sampling

Sampling of transplanted eelgrass biomass was done in October 2019 using a cylindrical (internal diameter of 15 cm and height of 35 cm) steel sediment sampler that uses vacuum to allow the extraction of sediment cores without losing sample material. Eelgrass recovered in each sample was split into (1) live above-, (2) dead above- and (3) below-ground biomass. In addition, all nodes on the rhizomes were counted, and 5 leaf lengths were measured for biomass estimates (described below). Furthermore, samples of sloughed leaves were collected in the transplanted area, of which 40 leaf sections were cut (10 cm long) to achieve a weight–length ratio of sloughed leaves. All leaves and roots were carefully cleaned of epiphytes and fine particles before they were dried (60°C until constant weight), weighed and stored for later C and N content analysis using an elemental analyser (Thermo Analytical, Flash EA 2000 Series).

The biomass of sloughed leaves after transplantation was estimated by multiplying the area-specific count of rhizome nodes where leaves had been attached by the mean leaf length and the weight–length ratio of sloughed eelgrass leaves. This estimate is possible because the number of produced leaves equals the number of rhizome nodes. The total produced biomass of the transplanted eelgrass was estimated as the sum of the live aboveground, live belowground and sloughed leaf biomasses.

2.9. Sediment sampling and analysis

Sediment cores for C, N and P pools were taken with acrylic core-liners (internal diameter of 5 cm) about 2 yr (October 2019) after transplantation. The sediment was kept cold (5°C) and sectioned into depth intervals (0–2, 2–5, 5–10, 10–15 cm) within 3 d after sampling. Sediment cores were taken in pairs with close proximity in the transplanted and unvegetated sediments. This means that for each sample taken in a transplanted patch, a sample was taken in the adjacent unvegetated sediment ~2 m away from the transplanted patch. Visible fauna, live plant material, large shells and stones were removed before further sediment analysis. Sediment grain size distributions were determined with a Malvern Master Sizer 3000. C and N content was analysed on dried samples (60°C until constant weight) from sectioned sediment cores in an elemental analyser (Thermo Analytical, Flash EA 2000 Series). Sediment organic matter (loss on ignition) was analysed by combusting (520°C for 6 h) 2–3 g of dry sediment. Total P in each sediment interval was determined spectrophotometrically after extraction in boiling acid (1 M HCl) for 1 h at 120°C.

2.10. Statistical procedures

Normality and equal variances were assessed with Shapiro-Wilk's test and Levene's test, respectively, prior to 1- and 2-way ANOVAs with Tukey HSD post hoc comparisons. Temporal developments in shoot density (transplantation tests and in the large-scale transplantation) were analysed with Wilcoxon's pairwise repeated measures procedure with Bonferroni-corrected significance. Statistical analyses of sediment data were tested on sediment concentrations (e.g. µg N cm^{-3} sediment) since these are unaffected by thickness of the depth interval. Outliers (>1.5× the interquartile range) due to calcium carbonate shell fragments were excluded. Fixed effects 2-way ANOVAs were used to test for the differences in, for example, sediment C content from the 3 habitats. One-way ANOVAs were applied on pooled sediment data (1 pooled data value for each core, i.e. replicate) to test for differences between habitats irrespective of sediment depth. All statistical tests were performed with the software PAST (Hammer et al. 2001) at a 5% level of significance, and all data are presented as means ± SE.

3. RESULTS

The highest shoot production among test-transplantation sites was evident at Site C in the outer part of the estuary (Fig. 4). The protection methods for eelgrass transplants that included nets and baited crab traps had significantly higher shoot numbers than all other treatments ($p < 0.05$), except for the control treatment at Site C (Fig. 4). The highly positive shoot development in the net-protected treatment at Site C was the basis for choosing this site and protection method for the subsequent large-scale eelgrass transplantation which was established 3 mo later in July 2017.

Shoot densities in the large-scale transplantation increased from 14 shoots m^{-2} in July (Month 0) to

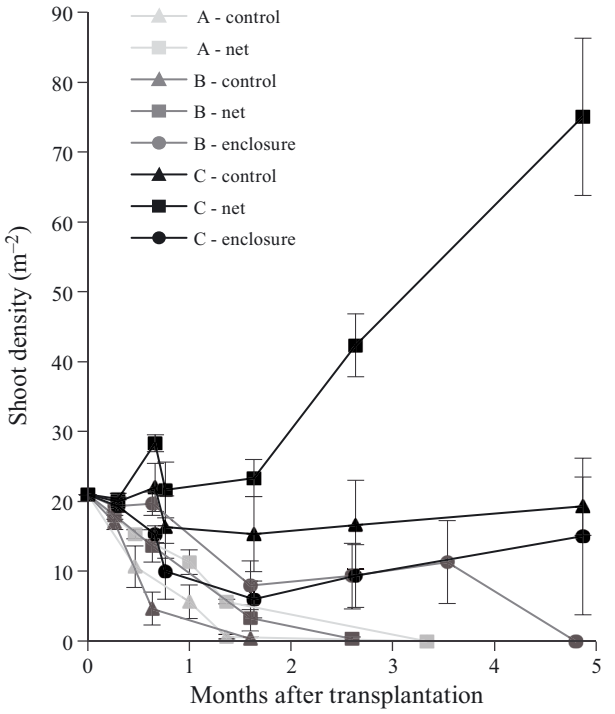

Fig. 4. Transplantation trials for site and method selection at 3 sites (A, B and C) with different protection methods for eelgrass transplantation in Horsens Fjord. The trials were initiated in April 2017 (mean ± SE, n = 3). In the net treatment, transplants were surrounded by mesh plastic net enclosures with baited crab traps placed both in- and outside of the net; in the enclosure treatment, shoots were transplanted within polypropylene cylinders. Site A was unsuited for enclosures (see Section 2)

28 shoots m^{-2} in December 2017 (Month 5) (Fig. 5A) and they survived a relatively severe storm (30–35 m s^{-1} wind gusts) during winter. The shoot densities increased the next spring and summer (Months 13–14) to above 500 shoots m^{-2}. After a slight decline during the second winter (Months 17–19), densities increased rapidly again and were similar to those of natural eelgrass patches after 24 mo ($p > 0.05$). The 2 anchoring methods provided similar results ($p > 0.05$). Transplants expanded horizontally at a rate of 0.32 m yr^{-1} (Fig. 5B) and formed joined rhizospheres within rows within the first 5 mo (Fig. 6A), and the rows started merging after 12 mo in most plots (Fig. 6B). This is equivalent to a typical vegetative expansion of 25 cm yr^{-1}. For comparison, drone mapping and subsequent image analysis of drone photos estimated the same vegetative expansion of 0.32 m yr^{-1} (Fig. 5B). The expansion rates in transplanted patches were similar to those of natural patches ($p > 0.05$). The total number of shoots in 2019 (after 24–28 mo) was estimated to be ~1 005 000 shoots from shoot density counts (Fig. 5A: 784 shoots m^{-2}) and drone mapping of the total area covered (Fig. 3: 1282 m^2). This is equivalent to a 70-fold increase in number of shoots since the transplantation in 2017. The transplants were established in expanded checkerboard patterns and initially covered 768 m^2 or 20% of the transplanted area, which expanded vegetatively to 1282 m^2, equivalent to 30% of the transplanted area by September 2019 (Fig. 3).

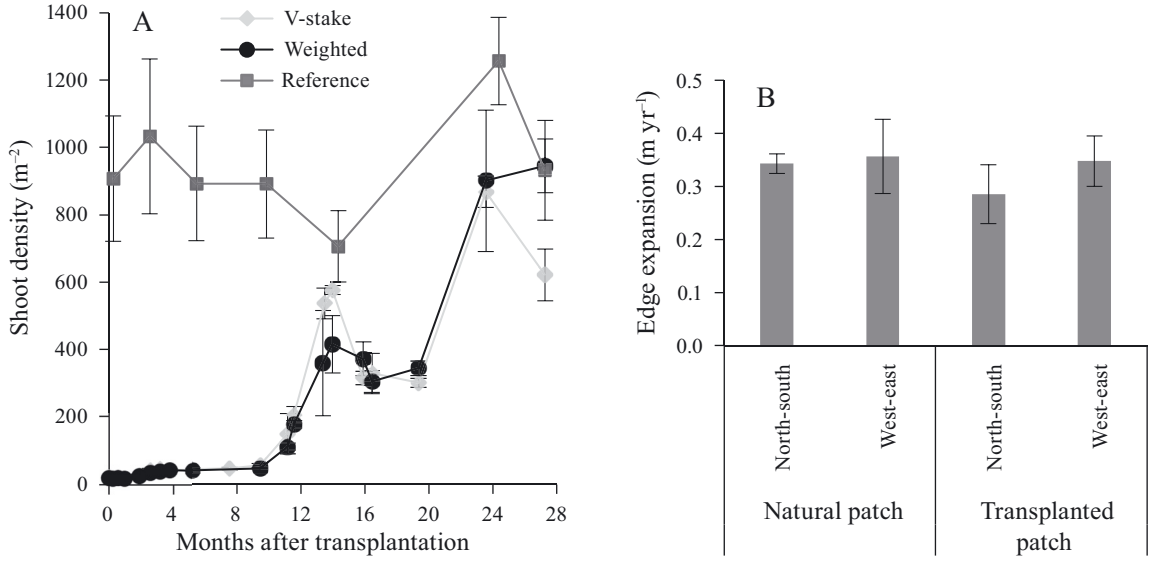

Fig. 5. (A) Shoot density development in the large-scale transplantation in Horsens Fjord for the 2 anchoring methods (V-stake anchors and weighted shoots) and in natural eelgrass patches (Reference). The transplantation was established in mid-July 2017 (mean ± SE, n = 3). (B) Vegetative edge expansion rates of natural eelgrass and transplanted patches in 2 ordinal directions. Horizontal edge expansion was measured using drone imagery analysis from 2018 and 2019 (mean ± SE, n = 5 and 12, respectively)

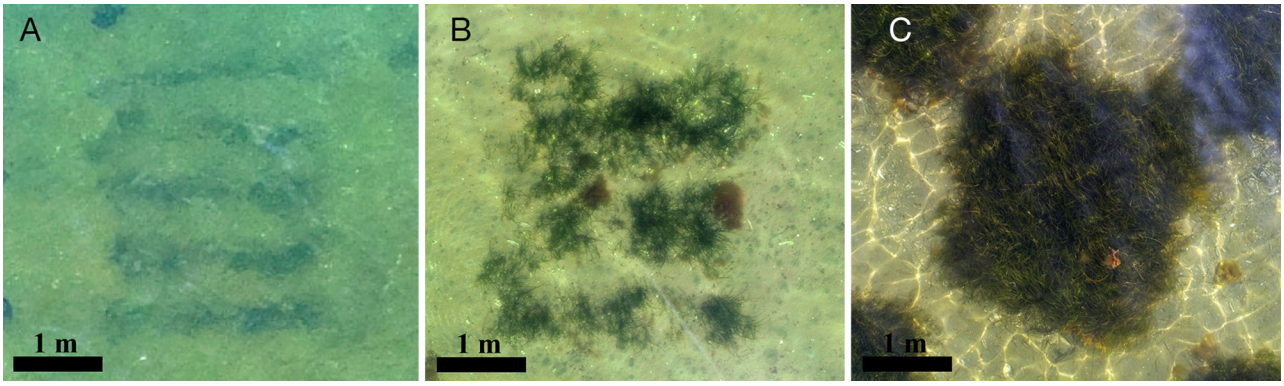

Fig. 6. Low-altitude (5 m) drone monitoring of the development in coverage and areal expansion of one specific 2 × 2 m transplanted plot in the years (A) 2017, (B) 2018 and (C) 2019

The biomass of transplants developed considerably from ~4 g dry weight (DW) m^{-2} initially to 721 ± 67 g DW m^{-2} after 2 yr. The C content in the above-ground, below-ground and sloughed leaf biomasses was 38.1 ± 0.12, 36.0 ± 0.6 and 36.7 ± 0.2%, respectively, while the N content was 2.4 ± 0.1, 0.9 ± 0.1 and 1.8 ± 0.0%, respectively. The biomass increase, over the monitoring period of 2.25 yr, is therefore equivalent to a yearly plant C and N assimilation rate of 290 ± 22 and 12 ± 1 g yr^{-1}, respectively (Fig. 7).

Remarkable differences were found in median grain size (Fig. 8A) and organic matter content (Fig. 8B) between eelgrass-transplanted and unvegetated sediments. Eelgrass-transplanted sediments had a 23% smaller median grain size, compared to unvegetated sediments in the upper 2 cm sediment interval, confirming a significant accumulation of fine particles in the transplanted plots (Fig. 8A). The significant differences in grain size between eelgrass-transplanted and unvegetated sediments continued down to 10 cm depth. The sediment with eelgrass transplants and the natural patches had similar ($p > 0.05$) grain size and organic matter in the upper 2 cm layer, but the difference diminished deeper down where finer particles were consistently higher in the natural meadow. Sediment organic content was consistently higher in the vegetated than the unvegetated areas, although only significant in the 2 middle sediment intervals (2–5 and 5–10 cm) for the transplanted area. Depth-integrated pools of organic matter were significantly higher ($p < 0.05$) in both natural eelgrass and transplanted sediments than unvegetated sediments, while the 2 vegetated areas showed no significant difference ($p > 0.05$). An increased accumulation of C, N and P was found in eelgrass-transplanted sediments after 2.25 yr (Fig. 9). However, the depth-integrated sediment pools of C in the 0–15 cm interval were not significantly different between eelgrass-transplanted and unvegetated sediment but were both significantly lower than natural eelgrass sediment. N pools were higher in eelgrass-transplanted than unvegetated sediment at all depth intervals but only significantly so in the upper layer. Depth-integrated N pools in unvegetated sediments were significantly lower than in both natural eelgrass and transplanted sediments, while no significant difference was evident between the latter two. Significantly higher P pools in eelgrass-transplanted sediment than unvegetated sediment were only evident in the deepest sediment interval (10–15 cm). The effect of eelgrass transplantation on C and nutrient sequestration was estimated from the excess increase in nutrient

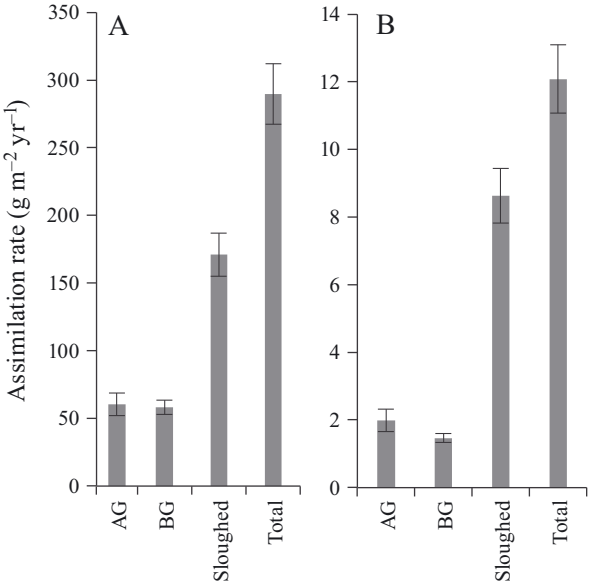

Fig. 7. Assimilation rates of (A) carbon and (B) nitrogen in above-ground (AG), below-ground (BG), sloughed leaf (formerly assimilated, but lost) and total biomass of eelgrass transplants during the first 2.25 yr after transplantation (mean ± SE, n = 5)

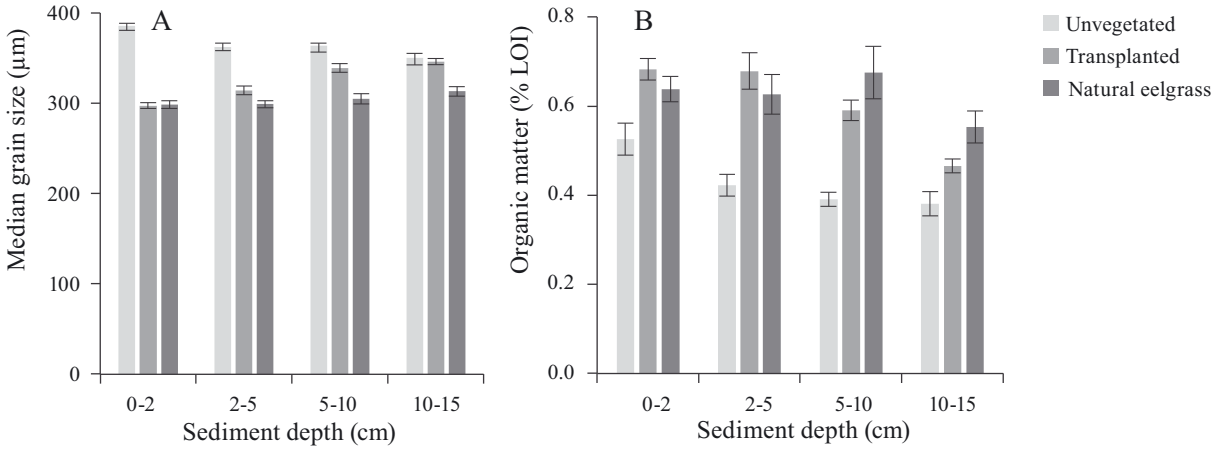

Fig. 8. (A) Sediment median grain size and (B) sediment organic matter (%LOI: percent loss on ignition) in unvegetated, eelgrass-transplanted and natural eelgrass-vegetated sediments 2.25 yr after transplantation (mean ± SE, n = 5–6)

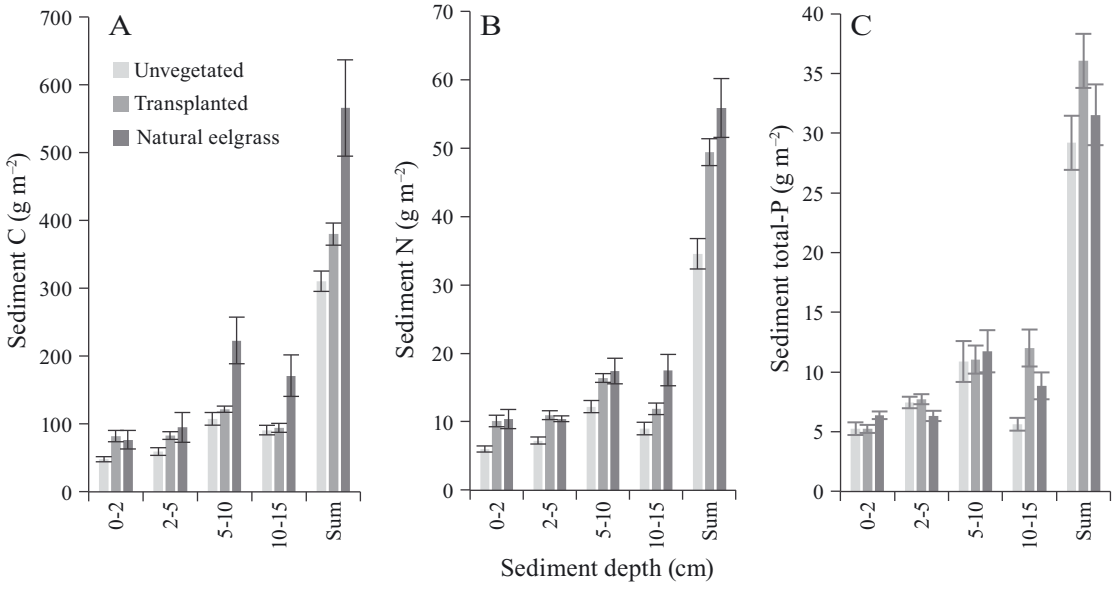

Fig. 9. Sediment stocks of (A) carbon, (B) nitrogen and (C) phosphorus in unvegetated, eelgrass-transplanted and natural eelgrass-vegetated sediment 2.25 yr after transplantation (mean ± SE, n = 5–6)

pools in eelgrass-transplanted sediment over time since transplantation. Burial rates in eelgrass-transplanted sediments were estimated to be 33 ± 7.5 , 6.6 ± 0.9 and 3.0 ± 0.5 g m^{-2} yr^{-1} for C, N and P, respectively.

4. DISCUSSION

4.1. Importance of site selection for large-scale transplantation success

Restoration ecologists widely agree on the importance of site selection in restoration projects. This study has outlined a simple approach that can give successful results. The same simple approach is currently used in 3 other large-scale transplantation projects in Denmark (6000–8500 shoots transplanted): one in Lunkebugten and 2 in Vejle Fjord (T. Lange et al. unpubl.). These have shown positive shoot developments during the first 6 mo, with a doubling in shoot density, which is similar to the early development in the successful Horsens Fjord transplantation (Fig. 5A). This result supports the view that the outlined site selection approach is generally applicable in Danish estuaries. The same site selection approach was also successfully used in subtropical Australian estuaries where suitable sites and methods were identified for transplantation of *Zostera muelleri* (Wendländer et al.

2019). All of these results should be viewed in the context that, to our knowledge, successful large-scale restoration attempts of eelgrass meadows are rare outside of North America (Cunha et al. 2012, Tan et al. 2020). Hence, these site selection approaches including method optimization represent important advances for future eelgrass restoration projects.

A high number of test sites are required for locating suitable sites, but the effort is valuable and provides a high chance of large-scale success. Other studies have also shown that a relatively high number of transplantation tests is necessary for identifying sites with adequate growth conditions (Short et al. 2002, Leschen et al. 2010, Wendländer et al. 2019). Ideally, test sites should be allowed to develop over a full year to cover all potential stress factors (e.g. algae growth, bioturbation, storm seasons, etc.) associated with the different seasons before deciding on a location for large-scale transplantation.

4.2. Transplantation method considerations

The design of large-scale transplantations is typically a trade-off between the wish for sufficiently high transplant density in the largest possible area and limited funds for conducting the labour-intensive work. Stressor resilience is gained by high transplant density, and the risk of losing the transplanted meadow to stochastic events is lower in large restoration areas (van Katwijk et al. 2016, Paulo et al. 2019). However, transplantations in spaced out checkerboard patterns will allow years of vegetative expansion (about 0.32 m yr^{-1}) of individual patches before gaps are closed between them. Resilience of transplantation plots is assured by spacing out 2 × 2 m patches with high transplant density. Paulo et al. (2019) transplanted seagrass with spacing between plots and found a transplant patch size of 11 m^2 to be the critical minimum size at a highly exposed transplantation site. The present study in the less physically exposed Horsens Fjord revealed success with 4 m^2 patches (plots) and lower transplanting densities than used by Paulo et al. (2019). At the current edge expansion rate, the patches in the present large-scale transplantation will be completely merged 6 yr after transplantation. Subsequently, only the outer perimeter of the new meadow will expand, leading to slower increases in associated ecosystem services, while giving increased resilience to stress. This means that compromises must be taken between transplant plot sizes, plot spacing, density and total size of the transplantation area. Each parameter has benefits and disadvantages partly dependent on the local environment in each transplantation project. High-density transplantation has high stress tolerance but high costs. Tight plot spacing will also have high stress tolerance but provide low areal gain in the first years and hence low return of area-dependent ecosystem services.

Transplantation tests in Horsens Fjord with net enclosures and baited crab traps had significantly higher shoot growth compared with controls. This approach was applied in the large-scale transplantation attempt and may have facilitated initial growth and expansion of transplanted eelgrass plots. However, due to lack of unprotected large-scale transplantations, no final conclusions can be made on potential positive effects of net enclosures and crab removal on the large-scale transplantation.

4.3. Drone-mapping of the large-scale transplantation area

Drone-mapping of the transplanted area in Horsens Fjord revealed an impact of physical stress on the outer boundaries of the large-scale transplantation area. Fragmentation due to physical stress was most pronounced at the corners of the area (Fig. 3C). The strong western wind fetch potential at the transplanted area in Horsens Fjord was clearly evident as more severe fragmentation and loss of plots along the exposed western boundary. Accordingly, eelgrass transplantations must be large enough to have an outer perimeter that can protect the central area, allowing transplants to thrive and develop. The drone imagery of large-scale transplantations is a valuable tool for surveying the fate of large-scale restorations (i.e. self-facilitation and spread of risks), which was also emphasized by van Katwijk et al. (2016).

4.4. Sediment stabilization and sequestering of C and nutrients after transplantation

Important ecosystem services were achieved 2 yr after the successful large-scale eelgrass transplantation. The new eelgrass meadow retained substantial amounts of C, N and P from the water column, leading to long-term sequestration of plant-bound C and N in the expanding plant biomass and permanent sequestering of particles deposited in the sediment. The latter compartment accounted for a large fraction of the total retention (5 and 33% for C and N, respectively) in the Horsens Fjord transplantation

and functions as a permanent sink for C (Mcleod et al. 2011, Greiner et al. 2013) and nutrients (Aoki et al. 2020). Nutrients retained in the standing (above- and below-ground biomass) eelgrass biomass comprise a transient and relatively constant pool once the eelgrass has reached maximum shoot density (similar to natural patches). The standing biomass varies with season in Denmark, with the highest level during summer and a 30% reduction during winter. Hence, the highest nutrient retention in the standing eelgrass biomass occurs during summer which typically coincides with the timing of macroalgal blooms. As these algae require a steady supply of nutrients, the uptake and storage of these nutrients into slowly degradable eelgrass tissue may dampen nutrient dynamics and hamper growth of opportunistic algae (Flindt et al. 1999, Banta et al. 2004). This means that nutrients stored in the standing eelgrass biomass and sloughed leaves are temporarily immobilized and will not lead to immediate eutrophication. A large fraction of the nutrients stored in sloughed leaves may be removed from the estuarine nutrient cycling by being either washed ashore as beach wrack or transported by currents out of the estuary (Flindt et al. 1997, 2004). These highly important functions of the eelgrass are regained by transplantations and can counteract eutrophication by making nutrients functionally unavailable for phytoplankton and opportunistic macroalgae. This suggests that eelgrass transplantations can promote a reversal in the primary producer community away from the dominance of phytoplankton and opportunistic macroalgae in otherwise eutrophic estuaries (Duarte 1995).

The retention of fine particles by the transplanted eelgrass canopy is the main driver behind burial of C, N and P in sediments. The observed lowered grain size in the eelgrass-transplanted sediments after 2 yr substantiates the canopy impact on hydrodynamics and sediment mobility. Consequently, migrating sand ripples and sediment resuspension induced by wave action are dampened within eelgrass-transplanted areas and instead deposition of fine particles is facilitated. In fact, the sediment surface within eelgrass meadows is often several cm higher than in neighbouring unvegetated areas. This is supported by the fact that median grain sizes of eelgrass-transplanted and unvegetated sediments in the present study first are similar at a sediment depth of 10–15 cm. In contrast, fine particles from surrounding unvegetated sediments are lost to wave action and water currents. The final result of the dampened hydrodynamics within eelgrass meadows is increased burial of organic C, N and P in the sediment.

When the C and nutrient stocks of the young transplantation in Horsens Fjord are compared with mature eelgrass meadows in Denmark, it is obvious that the burial has only reached the initial phase in the transplanted area. The estimated C burial rates in the Horsens Fjord transplanted area are similar to those from other studies on restored seagrasses (Greiner et al. 2013, Marbà et al. 2015) although relatively low compared to global averages for seagrass meadows (Mcleod et al. 2011). A nationwide study in Denmark revealed that sediment C, N and P stocks in mature eelgrass meadows are 1013 ± 116, 109 ± 12 and 17 ± 1 g m^{-2}, respectively (Kindeberg et al. 2018). This is several times higher, particularly for C and N, than observed here in the 2 yr old transplanted area. Based on the high burial potential in mature eelgrass meadows (Kindeberg et al. 2018), continuous nutrient accumulation in the eelgrass-transplanted sediments is expected for the following years.

Despite the general lack of large-scale eelgrass restoration success outside of North America, our study has shown that large-scale transplantation of eelgrass certainly is feasible in a Danish estuary. However, the long-term transplantation success is highly dependent on appropriate site selection, shoot planting and protective approaches. Furthermore, large-scale transplantations have the potential to initiate a return of eelgrass ecosystem services arising with increased transplant biomass such as sequestration of C and nutrients. Eelgrass transplantation is a promising nature-based management solution that can support national plans for nutrient reduction and climate change mitigation using the abilities of eelgrass meadows to store and retain C and nutrients.

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