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Improved benthic fauna community parameters after large-scale eelgrass (*Zostera marina*) restoration in Horsens Fjord, Denmark

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ABSTRACT: Eutrophication is a key driver in the loss of marine ecosystems, and seagrass meadows are among the many ecosystems which have declined globally during the last decades. Seagrass restoration is being used worldwide in coastal areas to counteract the decline in areal extent and to promote biodiversity. This study assesses the spatial and temporal changes in benthic fauna composition after a successful large-scale eelgrass (*Zostera marina*) transplantation in Horsens Fjord, Denmark. Transplantation was done by anchoring individual shoots in the sediment. Subsequently, benthic fauna was compared among bare bottom (BB), transplanted eelgrass (TE) and a natural eelgrass (NE) meadow in Horsens Fjord. Species richness (S), abundance (N), Shannon-Wiener index (H'), Pielou's evenness (J') and biomass (B) of benthic fauna were significantly higher at TE and NE than at BB. S, H' and J' were not different between TE and NE, but N and B were. Furthermore, S, N and B showed significant year-to-year variation, with the highest values occurring the same year as peak eelgrass biomass at both TE and NE, and S, N and H' correlated positively with dry eelgrass biomass. Increases in community parameters were achieved at TE at least 1 yr 2 mo after transplantation, and a higher diversity of feeding groups was found. However, the ecological status of fauna at TE was in a transition state towards that at NE, according to the Water Framework Directive. The fast succession of benthic fauna proved that successful large-scale transplantation of eelgrass can restore fauna communities very quickly.

KEY WORDS: Macrofauna succession · Seagrass transplantation · Benthic invertebrates · Ecosystem restoration

1. INTRODUCTION

We are in the middle of a global biodiversity crisis which is mainly driven by loss of natural habitats, climate change and increased exploitation of natural resources by the growing human population (Pimm et al. 2014). Current extinction rates are estimated to be 1000 times higher than background rates (Pimm et al. 2014), and terrestrial, freshwater and marine ecosystems have deteriorated at alarming rates since the 1970s (Butchart et al. 2010). Among others, seagrass meadows, saltmarshes, mangrove forests, mussel reefs, kelp forests and coral reefs have all declined globally during the last century (Saunders et al. 2020), and the United Nations calls for ecosystem restoration to be the primary strategy to counteract this decline.

Interactions among habitat-forming structures and organisms are key drivers for the functioning of coastal ecosystems, and habitats with these structures or ecosystem engineers are home to diverse assemblages of flora and fauna ranging from micro-

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algae to vertebrates (Boström & Bonsdorff 1997, Sfriso & Facca 2011, Støttrup et al. 2014). Soft-sediment coastal habitats are typically dominated by seagrass meadows that form highly productive and diverse ecosystems (Duarte & Chiscano 1999). Seagrasses are ecosystem engineers and are recognized as a valuable coastal habitat with multiple ecosystem services (Orth et al. 2020). The 3-dimensional physical structure of seagrass meadows increases the surface area available for settling of sedentary organisms and provides shelter for a wide variety of invertebrates and fish (Boström et al. 2006). Furthermore, seagrass vegetation supports food webs through the net deposition of organic matter by dampening current speeds and preventing resuspension (Orth 1977, Fonseca & Fischer 1986). However, seagrasses have declined worldwide during the last century (Waycott et al. 2009). Although there has been a reversal in the decline for some seagrass species in Europe (e.g. *Zostera noltei*), other species such as *Z. marina* and *Cymodocea nodosa* continue to decline (de los Santos et al. 2019). Accordingly, ecosystem functions related to fauna that live in seagrass disappear when the ecosystem is lost (Hughes et al. 2002, Fredriksen et al. 2010).

Eelgrass *Z. marina* is the most common seagrass species in Scandinavian coastal waters, but the population has declined considerably in the last 90 yr (Boström et al. 2014). The decline was initially caused by the wasting disease of the 1930s (Cotton 1933, Tutin 1938) and later by human impacts, such as dredging, trawling, construction of harbors and, most importantly, eutrophication (Short & Wyllie-Echeverria 1996, Flindt et al. 1999). This has resulted in massive loss of eelgrass along the coasts of Finland, Estonia, Sweden, Norway and Denmark (Boström et al. 2014, de los Santos et al. 2019). The loss of eelgrass, as a key ecosystem engineer, is critical and has led to a functional collapse of many coastal ecosystems due to the loss of important ecosystem services. A major consequence is reduced biodiversity in coastal ecosystems (Boström & Bonsdorff 1997), because many soft-bottom coastal areas today remain unvegetated (Valdemarsen et al. 2010) and dominated by a few opportunistic species (Blomqvist & Bonsdorff 1986). Eutrophication combined with naturally occurring stressors prevents eelgrass from natural recolonization in Denmark (Valdemarsen et al. 2010), and the loss of eelgrass has led to a regime shift with a strong positive feedback mechanism, which further destabilizes the ecosystem in 2 ways: (1) The loss of a nutrient sink, with slowly degradable organic matter, destabilizes the system because a significant nutrient buffer is lost (Flindt et al. 1999). (2) Trophic cascades lead to the dominance of opportunistic species, e.g. the lugworm *Arenicola marina* or the European green crab *Carcinus maenas*, preventing natural recolonization of eelgrass by seed dispersal (Davis et al. 1998, Valdemarsen et al. 2011). To revert this regime shift to previous conditions, active ecosystem restoration approaches must be implemented in national water management plans.

Restoration of seagrasses, either by transplantation or by seeding, is occurring all around the world (Bastyan & Cambridge 2008, Lange 2020, Orth et al. 2020). One of the world's most successful seagrass restoration efforts, in the inshore lagoons of Virginia coastal bays, USA, has documented the return of several ecosystem services, e.g. reduction of water turbidity, increase in fish stocks, increase in invertebrate biomass and burial of carbon (C) and nitrogen (N) stocks (Orth et al. 2020). However, natural recolonization and transplantation of eelgrass remain difficult in Danish waterbodies that are heavily affected by high nutrient loading (Flindt et al. 2016, Lange 2020); eutrophication is only one of many stressors that complicate eelgrass transplantation in Denmark (Valdemarsen et al. 2010, 2011), and several eelgrass restoration campaigns have failed until recent years. The marine ecology group at the University of Southern Denmark (SDU) has developed a 3-step site-selection procedure which supports successful large-scale eelgrass transplantations in Danish waters and has established the first successful large-scale eelgrass transplantation in Horsens Fjord, Denmark (Lange 2020).

Large-scale eelgrass transplantation can potentially restore lost eelgrass areas and increase local fauna diversity, which improves the ecological indicators eelgrass depth limit and infauna diversity that are used in the European Union's Water Framework Directive (WFD). Increases in suspension-feeding fauna, following eelgrass restoration, can enhance water clarity and improve the ecological indicator chl *a*. Only 2 of 119 Danish waterbodies are currently in good or high ecological condition, mainly due to restricted eelgrass depth limit and low infauna diversity (MiljoeGIS, https://miljoegis.mim.dk/cbkort?profile=vandrammedirektiv2-2016; accessed 24 April 2021). If large-scale eelgrass restoration is successfully implemented as a management action in Danish waterbodies, it can potentially be a tool to improve these ecological indicators and achieve good ecological condition and fulfill the WFD.

The aim of this study is to investigate the potential of eelgrass transplantation as an instrument to restore benthic fauna diversity. The succession of benthic fauna communities was monitored in a trans-

planted eelgrass (TE) area, a natural eelgrass (NE) meadow and adjacent bare bottom (BB). Our study placed special emphasis on species richness, abundance, diversity indices and biomass as well as feeding groups and ecological groups (EGs) according to disturbance. Our hypotheses were as follows: (1) The fauna succession in restored eelgrass meadows is rapid and proportional to eelgrass biomass. (2) Eelgrass transplantation restores benthic fauna diversity, abundance and biomass to levels comparable with natural meadows.

2. MATERIALS AND METHODS

2.1. Location

Horsens Fjord is a shallow estuary on the east coast of Jylland, Denmark (Fig. 1A). The fjord covers an area of 79 km^2 and opens into Kattegat and the strait of Lillebælt. The average depth of Horsens Fjord is 2.9 m (Miljoestyrelsen 2011), and the water residence time is approximately 18 d, with an average tidal amplitude of 0.3 m (Miljoestyrelsen 2011). The salinity of the estuary is affected both by seawater from Kattegat and by freshwater from approximately 18 streams and creeks as well as point source discharge from the city of Horsens. This results in a salinity gradient, from 14 in the inner part to 33 at the mouth of the fjord (Miljoestyrelsen 2011). The fjord has a catchment area of 791 km^2, and agriculture constitutes 75% of the land use (Miljoestyrelsen 2011). The average annual discharge of N and phosphorus (P) is 983 t N yr^{-1} and 21 t P yr^{-1}, respectively (Miljoestyrelsen 2011). Several decades of high nutrient discharge has led to a profound eutrophication history and a severe decline of eelgrass (*Zostera marina*) coverage (Miljoestyrelsen 2011), and today the fjord is in bad ecological condition according to the WFD (MiljoeGIS, 24-04-2021).

Large-scale transplantation of eelgrass was carried out in July 2017 at the study site Bisholt (Fig. 1A). Bisholt is located in the outer part of Horsens Fjord, close to the southern coastline, with salinity ranging from 20 to 26. The site has an average water depth ranging from 1.2 to 1.6 m and is located 450 m west of a large natural eelgrass meadow and 300 m off the coast (Fig. 1B). An area of 51 × 78 m was transplanted in a chessboard pattern with alternating vegetated and unvegetated squares of 3 × 3 m, in which transplantation was done on 4 m^2 (2 × 2 m), leaving the adjacent 5 m^2 bare for optimal space for natural vegetative recolonization. Transplantation was carried out by anchoring individual shoots, with either iron nails or bamboo skewers bent into a V-shape to lock the rhizomes in the sediment. No difference in development of shoot density was found between these 2 anchoring methods (Lange 2020). Eelgrass shoots were harvested locally from the natural eelgrass meadow east of the transplantation site (Fig. 1B). Harvest was done with garden rakes that withdrew small sections of shoots and rhizomes without removing sediment. For a more thorough description of the transplantation setup, consult Lange (2020).

2.2. Benthic fauna and eelgrass biomass sampling

Sampling of benthic fauna was carried out at Bisholt on 4 occasions: September 2018, November 2018, May 2019 and August 2019. Four random repli-

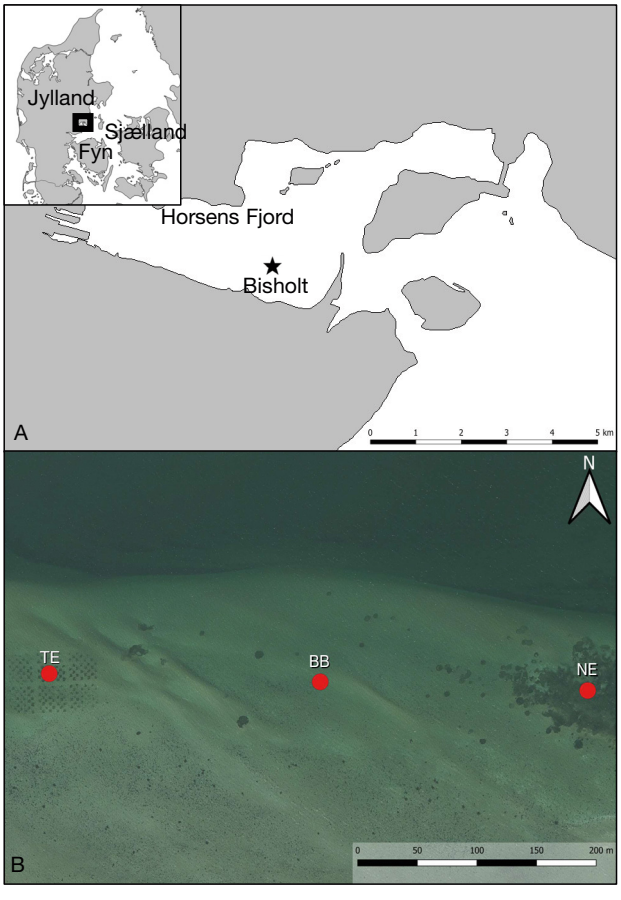

Fig. 1. (A) Horsens Fjord, Denmark, showing location of test site Bisholt (55.829447° N, 9.9924° E). (B) Bisholt test site with bare bottom (BB) 225 m away from both eelgrass habitats (55.829440° N, 9.9959° E). Transplanted eelgrass (TE) in a chessboard pattern (55.829447° N, 9.9924° E). Natural eelgrass (NE) meadow and donor meadow located 450 m east of the eelgrass transplantation site (55.829447° N, 10.000024° E) (Kortforsyningen.dk in QGIS 3.20.3)

cates of benthic fauna were taken around a GPS coordinate (radius <10 m) on each occasion at the following 3 types of habitats: BB, TE and the adjacent NE meadow located 450 m east of the transplantation site (Fig. 1B). The BB habitat was located between TE and NE, with 225 m distance to each of these eelgrass habitats (Fig. 1B). It was assessed that 225 m was sufficient distance for a control station to be unaffected by eelgrass, since benthic fauna is usually significantly different on bare bottom compared to eelgrass habitat, even if samples are taken as little as 2 m away from an eelgrass meadow edge (Surugiu et al. 2021). Sampling at TE was always carried out inside a vegetated square, and subsequent sampling was done in a new square for each replicate. Sampling at NE was done approximately 30 m inside the meadow. Fauna sampling was done with a handheld cylindrical aluminum corer (177 cm^2) to a depth of 30 cm, and all samples were sieved through a 1 mm mesh onsite. Fast-moving mobile fauna, e.g. crabs, shrimp and small fish, were excluded, because they could not be sampled quantitatively with the steel corer, while slow-moving epifauna, mostly gastropods and some amphipods, were included. Individuals smaller than 1 mm retained by the sieve were excluded as well. The combination of infauna, slow-moving epifauna and sessile fauna will be referred to as benthic fauna in this study. The retained material was stored in plastic containers, preserved in 4% buffered formaldehyde and brought to the laboratory for further handling. Eelgrass biomass was removed from the sample after no more than 72 h and washed clean of fauna and debris. Afterwards, dry weight (DW) was measured separately for leaves and rhizomes after drying in an oven at 105°C for 24 h. All benthic fauna samples were sorted, and recovered animals were identified to species level and feeding type when possible. Feeding types included deposit feeders, suspension feeders, omnivores, grazers and predators. Species exhibiting 2 feeding behaviors were categorized by the most frequent type according to literature, and species with 3 or more feeding behaviors were categorized as omnivores. All individuals of each species from every replicate were dried in an oven at 105°C for 24 h and thereafter combusted at 520°C for 6 h to determine ash-free DW. Invertebrates sampled in this study were not subject to any legislation which requires permission from the Danish Animal Experiments Inspectorate. However, all animals were handled with ethical responsibility to minimize suffering and euthanized using 4% buffered formaldehyde. No endangered or protected species were taken for this study.

2.3. Sediment analysis

Sediment cores for analysis of median grain size, silt–clay content and loss on ignition (LOI) as well as total C, N and P content were taken in August 2019 with acrylic cylinders (5 cm diameter). Three replicate cores from BB, TE and NE were brought to the laboratory and sliced in depth intervals of 0–2, 2–5, 5–10 and 10–15 cm. A sediment subsample was kept wet and used to determine grain size distribution (<2 mm) and silt–clay using a Malvern Master Sizer 3000. The rest of the sediment slices were dried in an oven at 105°C for 24 h. A subsample of ~25 mg dry sediment from each depth interval was packed in tin capsules and analyzed for total C and N content using a Thermo Flash 2000 elemental analyzer (Thermo Scientific). LOI was measured by combustion of 2 to 3 g dry sediment at 520°C for 6 h in preweighed and pre-burned crucibles. Total sediment P content in each depth interval was measured by boiling the ash at 120°C in 1 M HCl for 1 h. The extract was analyzed for phosphate by colorimetric analysis according to the molybdate blue method with ascorbic acid (Koroleff 1983).

2.4. Statistics and data processing

Five benthic community metrics were evaluated for each sampling time and station: species richness (S), numerical abundance (N), Shannon-Weiner index (H'), Pielou's evenness index (J') and biomass (B). The term species richness was used in 2 different ways: (1) total species richness found at each sampling time and station across all 4 replicates, and (2) sample-specific number of species found in each replicate. This latter approach was done to ensure that the number of species in each replicate could be related to the specific eelgrass biomass in each sample.

One-way ANOVA followed by Tukey's multiple comparison post hoc test was used to test for differences in sediment median grain size, silt–clay content, LOI and total C, N and P content in the sediment. One-way repeated measures ANOVA was used to identify differences in eelgrass biomass development between the 2 habitats TE and NE over time. Two-way ANOVA was used to test for temporal and between-habitat differences in eelgrass biomass, with habitat and sampling date as the 2 factors; this was followed by a pairwise Tukey's post hoc test for the factor sampling date. Two-way ANOVA followed by a pairwise Tukey's post hoc test was likewise conducted to identify temporal and spatial differences

among habitats for sample-specific number of species. Shapiro-Wilk's test and Levene's *F*-test indicated normal distribution and homogeneous variance in the respective datasets. *N* and *B* were not normally distributed even after transformation, and non-parametric statistics were used instead. Non-metric multidimensional scaling was used to produce 2 ordination plots based on Bray-Curtis similarity matrices for square root-transformed *N* and fourth root-transformed *B*. Non-parametric 2-way crossed ANOSIM was used to test for temporal and between-habitat differences for both *N* and *B*. This was followed by a SIMPER analysis to examine dissimilarities in community structures among habitats and sampling dates.

Benthic community structure was analyzed using the software AMBI (AZTI marine biotic index), which calculates biological indicators for the quality of soft-bottom fauna by grouping species into EGs from I to V according to tolerance against disturbance (Borja et al. 2000). The EGs correspond to the ecological quality status (EQS) provided by the WFD in the following way. Sensitive species are classified as EG I and are indicators of high EQS. Indifferent species are classified as EG II, which indicates good EQS. Tolerant species are classified as EG III and are indicators of moderate EQS. Opportunistic species and pollution-indicating species are classified as EGs IV and V, which correspond to poor and bad EQS, respectively (Borja et al. 2004). Differences in *N* in each of the 5 EGs across the 3 habitats were analyzed using a 2-way ANOVA with EG and habitat as the 2 factors, followed by a Tukey post hoc test. Shapiro-Wilk's test and Levene's *F*-test indicated normal distribution and homogeneous variance in the dataset.

Regressions were made to examine possible relationships between dry eelgrass biomass, *S*, *N*, *H'*, *J'* and *B*. The best fit was in all cases a linear relationship, and correlation analysis was carried out by performing Pearson's correlations tests. All tests were done in SigmaPlot 12.0 and Primer 6 with a significance level of $\alpha = 0.05$.

3. RESULTS

3.1. Sediment composition and eelgrass biomass

There was no significant variation in sediment composition among the 3 habitats, and all had sandy sediment with low silt and clay content and low organic content. Median grain size ranged from 289 to 384 µm (Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m687p065_supp.pdf), silt and clay content ranged from 0.6 to 5.7% (Fig. S2) and sediment organic content ranged from 0.4 to 1.1% (Fig. S3).

Sediment elemental composition (C, N and P) was not significantly different among habitats. Total C ranged from 74.5 ± 3.6 to 124.6 ± 2.3 g m^{-2} (mean ± SE) (Fig. S4), and total N ranged from 8.7 ± 2.2 to 16.7 ± 1.8 g m^{-2} (Fig. S5). Total P in the sediment ranged from 4.2 ± 0.09 to 12.3 ± 1.6 g m^{-2} (Fig. S6).

No significant differences between eelgrass biomass in the 2 habitats TE and NE were apparent at any sampling time ($p = 0.35$), but the biomass in both habitats increased significantly from September 2018 to August 2019 ($p < 0.001$). Eelgrass biomass at TE increased from 356 ± 86 to 872 ± 53 g DW m^{-2}, and eelgrass biomass at NE increased from 462 ± 61 to 732 ± 27 g DW m^{-2} (Fig. 2). The corners of the vegetated squares at TE had merged, and the unvegetated squares were partly covered by vegetative growth of eelgrass in August 2019 (Fig. 3). The transplanted area was approaching the appearance of a natural eelgrass meadow.

3.2. Species richness

A total of 36 species of benthic fauna were identified across all 3 habitats at the Bisholt test site in Horsens Fjord during all seasons. These included 13 polychaetes, 1 oligochaete, 7 gastropods, 1 Polypla-

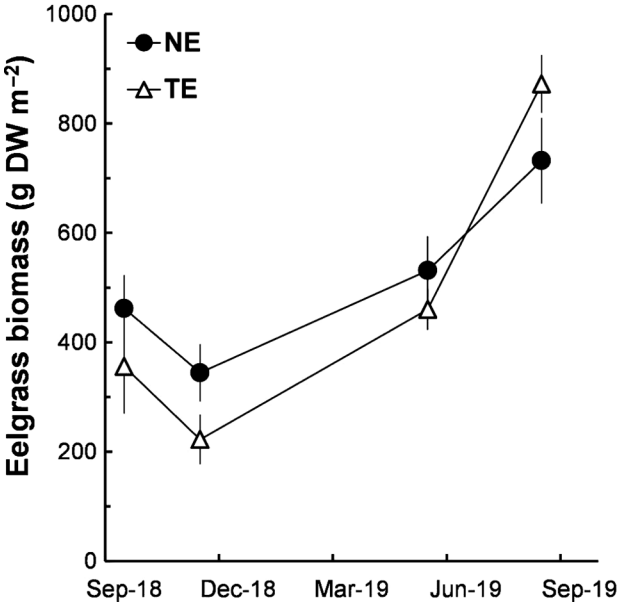

Fig. 2. Temporal development of natural eelgrass (NE) and transplanted eelgrass (TE) biomass from September 2018 to August 2019 (±SE, n = 4). DW: dry weight

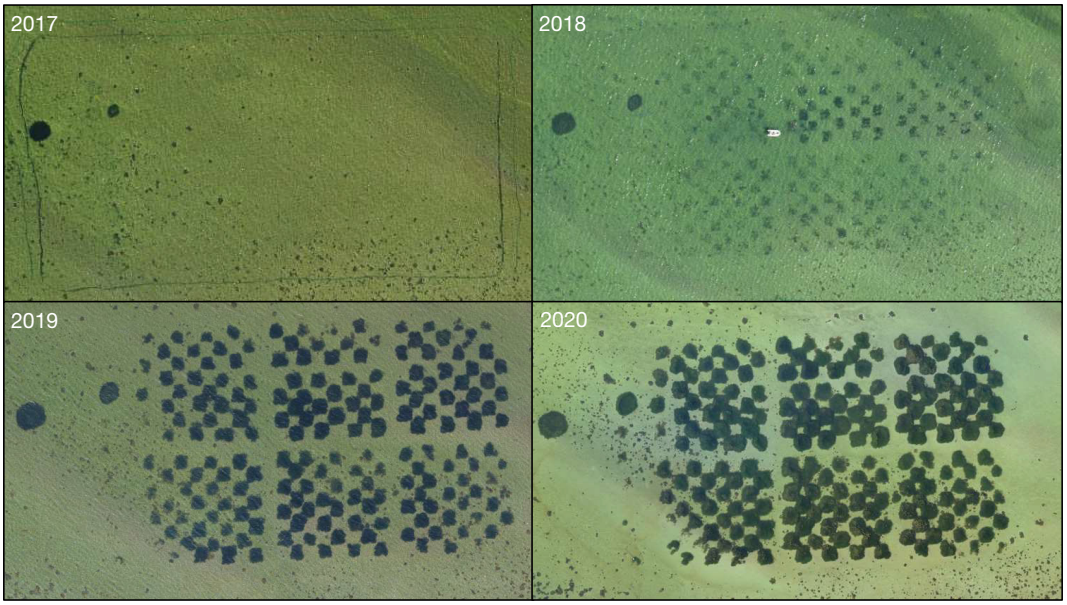

Fig. 3. Annual development of eelgrass transplantation at Bisholt test site, from 2017 to 2020 (drone images by Niels Svane, Department of Biology, University of Southern Denmark)

cophora, 10 bivalves, 3 crustaceans and 1 Asteroidea (Fig. 4A). The 36 species covered 5 feeding types: deposit feeders, suspension feeders, omnivores, grazers and predators (Table S1). Species richness was always higher in vegetated habitats compared to bare bottom and ranged from 6 to 14 at BB, 12 to 24 at TE and 13 to 23 at NE (Fig. 4A).

Average number of species per sample remained low at BB during the entire sampling period, ranging from 3.5 to 7.0 (±0.3–0.7 SE) with no significant change over time ($p > 0.05$). Species per sample was always significantly higher at TE and NE compared to BB ($p < 0.05$), except for November 2018, but never significantly different from each other. Species per sample showed significant year-to-year variation in both eelgrass habitats and was 106 and 66% higher in August 2019 compared to September 2018 at TE and NE, respectively (Fig. 4B). While BB was dominated by deposit-feeding polychaetes, the benthic fauna at TE and NE was more diverse and evenly distributed

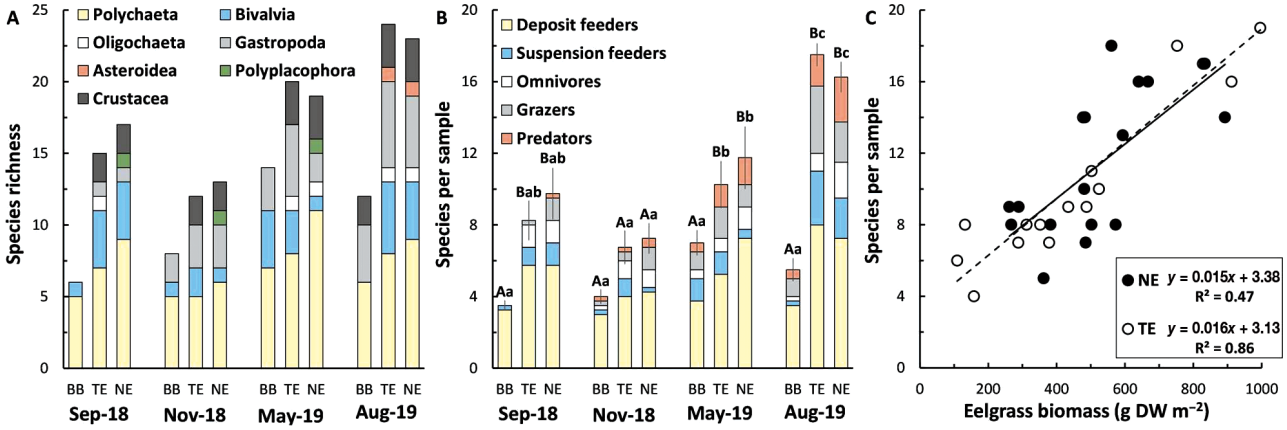

Fig. 4. (A) Species richness, with colors of bars indicating grouping by taxonomy (Polychaeta, Bivalvia, Oligochaeta, Gastropoda, Asteroidea , Polyplacophora and Crustacea), and (B) average number of species per sample (±SE, n = 4), with colors of bars indicating grouping by feeding strategy (deposit feeders, suspension feeders, omnivores, grazers and predators), on bare bottom (BB), transplanted eelgrass (TE) and natural eelgrass (NE) during each sampling period. Capital letters denote significant difference between habitats and lowercase letters between sampling dates. (C) Correlations between dry eelgrass biomass and number of species per sample for NE and TE, including linear regression lines for NE (solid black) and TE (dashed black). DW: dry weight; AFDW: ash-free DW

among the 5 feeding groups, with a high frequency of suspension-feeding bivalves, grazing gastropods, omnivorous polychaetes and predators (Fig. 4B). Species per sample showed a significant linear relationship with dry eelgrass biomass in both habitats (TE: r^2 = 0.86, p = 2.8 × 10^{-7}; NE: r^2 = 0.47, p = 0.003) (Fig. 4C).

3.3. Fauna abundance and biomass

Benthic fauna abundance was significantly different both between sites (p < 0.001, R = 0.77) and temporally (p < 0.001, R = 0.65) (Fig. 5A). Abundance at BB was always lower compared to TE and NE, but the latter 2 habitats also had significantly different abundance (Fig. 5A). Seasonal and annual variation in abundance was evident, and abundance was significantly higher in all habitats in 2019. Deposit-feeding polychaetes dominated the abundance at all 3 habitats, and suspension feeders, grazers and predators were most abundant in eelgrass habitats (Fig. 5A). Abundance of benthic fauna and dry eelgrass biomass followed significant positive linear correlations for both TE (r^2 = 0.71, p = 4.5 × 10^{-5}) and NE (r^2 = 0.39, p = 0.009) (Fig. 5B).

Benthic fauna biomass was significantly different both between sites (p < 0.001, R = 0.59) and temporally (p < 0.001, R = 0.49) (Fig. 5C). Despite high variability, fauna biomass was consistently higher at TE and NE compared to BB, except for May 2019, and biomass at NE was significantly higher than at TE, except for November 2019 (Fig. 5C). Large deposit-feeding polychaetes such as *Arenicola marina* contributed most to the biomass at BB during all seasons except for May 2019, where large individuals of the bivalve *Ensis* spp. dominated the biomass. High biomasses for suspension-feeding organisms were otherwise only found at TE and NE (Fig. 5C). TE and NE were dominated not only by *Ensis* spp. but also by *Cerastoderma glaucum*, *Mya arenaria* and *Mytilus edulis*. The largest invertebrate grazers were *Littorina littorea*, while *Tritia reticulata* had the highest biomass among predators. There were no significant correlations between benthic fauna biomass and eelgrass biomass (Fig. A1 in the Appendix).

There was a significant difference in the abundance of fauna assigned to AMBI EGs III and V among all habitats. BB had the highest abundance of fauna in group V and NE the lowest abundance. The opposite was true for EG III, where NE had the highest abundance and BB the lowest (Fig. 6).

According to the SIMPER analysis, the highest average dissimilarity of benthic fauna abundance was evident between BB and NE (70%), while TE was almost equally dissimilar to both BB (56%) and NE (53%) (Fig. 7). The SIMPER analysis also revealed annual variation in benthic fauna abundance, with lowest dissimilarities within years (2018: 46%; 2019: 50%) and highest between years (>57%) (Fig. 7).

SIMPER analysis for benthic fauna biomass revealed highest dissimilarity between BB and NE (73%) and lowest dissimilarity between TE and NE (58%). The dissimilarity between BB and TE was remarkably high (68%), which indicated that TE was more similar to NE than to BB (Fig. A2). The SIMPER analysis also revealed annual variation in benthic

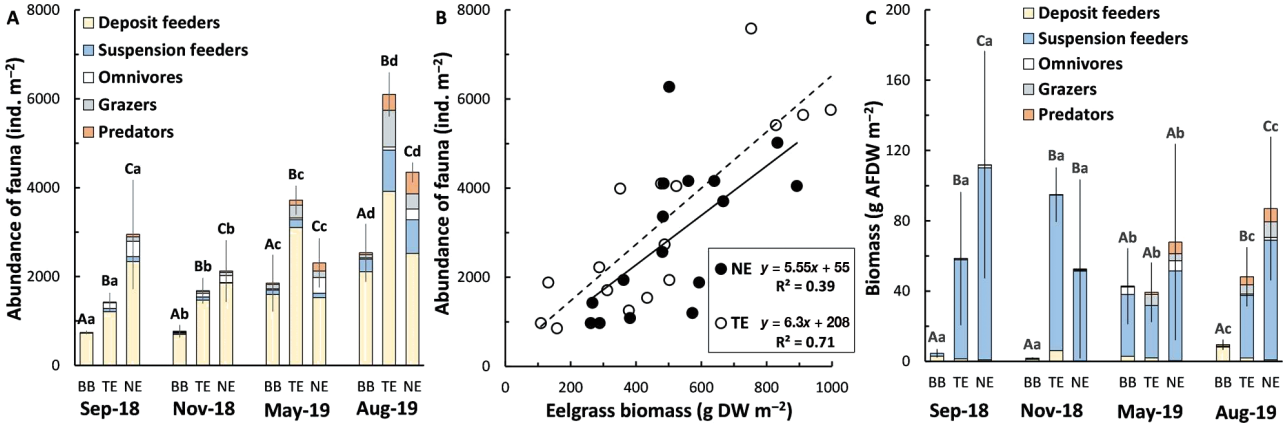

Fig. 5. (A) Average abundance of benthic fauna (±SE, n = 4) on bare bottom (BB), transplanted eelgrass (TE) and natural eelgrass (NE) during each sampling period. (B) Correlations between dry eelgrass biomass and abundance of benthic fauna for NE and TE, including linear regression lines for NE (solid black) and TE (dashed black). (C) Average biomass of benthic fauna (±SE, n = 4) on BB, TE and NE during each sampling period. Colors of bars indicate grouping by feeding strategy (deposit feeders, suspension feeders, omnivores, grazers and predators). Capital letters denote significant difference between habitats and lowercase letters between sampling dates. DW: dry weight; AFDW: ash-free DW

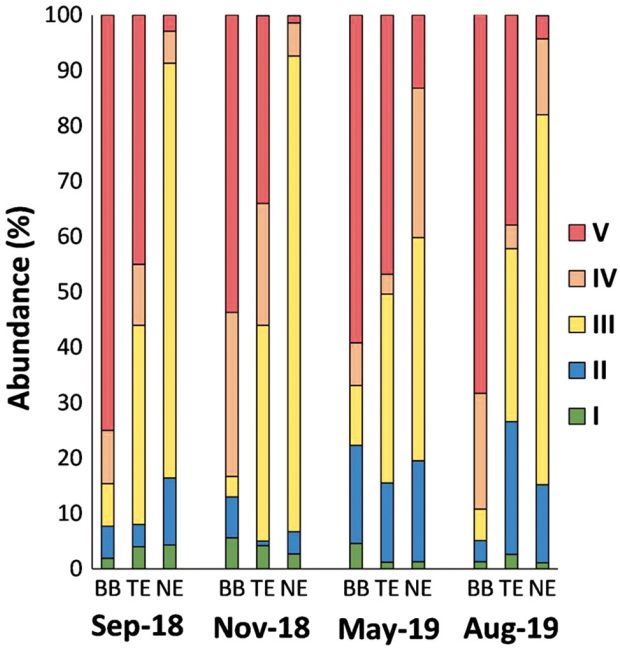

Fig. 6. Distribution of benthic fauna abundance into 5 ecological groups (I–V) by the software AMBI on bare bottom (BB), transplanted eelgrass (TE) and natural eelgrass (NE) during each sampling period

fauna biomass, with highest dissimilarity between years (>64 %) and lowest dissimilarities within years (2018 and 2019: 57 %) (Fig. A2).

H′ was always higher in vegetated habitats than in BB areas (Fig. 8A). A significant positive linear correlation with eelgrass biomass was found for *H′* at TE ($r^2 = 0.61$, $p = 0.0003$) but not at NE (Fig. 8B). Pielou's evenness followed a similar trend as *H′* and was also generally highest in vegetated habitats compared to BB, except for TE in May 2019 (Fig. A3). *J′* did not correlate significantly with eelgrass biomass, neither for TE nor for NE (Fig. A3).

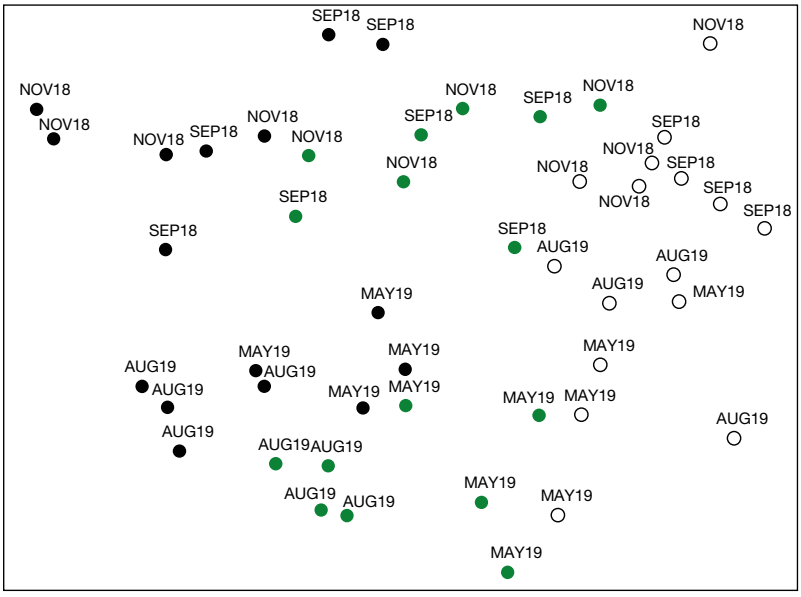

Fig. 7. Two-dimensional non-metric multidimensional scaling plot based on Bray-Curtis similarity matrix performed on benthic fauna abundance (stress 0.18), grouped into bare bottom (BB, white), transplanted eelgrass (TE, green) and natural eelgrass (NE, black) and sampling dates

4. DISCUSSION

4.1. Succession of benthic fauna after eelgrass transplantation

Benthic fauna showed remarkably increased diversity, abundance and biomass 1 yr 2 mo after the successful eelgrass transplantation in Horsens Fjord. Furthermore, the community structure of benthic fauna at TE was in a continuous transition state between that at BB and NE. It was undergoing succession from a community dominated by opportunistic species (AMBI EGs IV and V) to climax species (AMBI EGs I–III) (Borja et al. 2000). Species richness and diversity indices *H′* and *J′* at BB were comparable to previously reported values from bare bottom in the Baltic Sea, while these benthic fauna parameters at TE and NE were higher than in other Baltic eelgrass beds (Boström & Bonsdorff 1997), coincident with denser eelgrass stands in Horsens Fjord. Successful transplantations of *Zostera marina* and *Halodule wrightii* in North Carolina, USA, resulted in similar enhancement of species richness and abundance of benthic fauna within a year compared to bare bottom and unsuccessful transplants (Homziak et al. 1982). Our results confirm that succession of benthic fauna, after seagrass transplantation, occurs at similar speed in North American and Danish waters. These findings affirm that ecosystem restoration, such as transplantation of eelgrass, can be considered a successful instrument to regain biodiversity that was once lost and to improve ecological conditions according to the WFD.

4.2. Ecological implications

Positive ecological interactions and facilitation cascades are important and mostly unexplored in relation to sea-

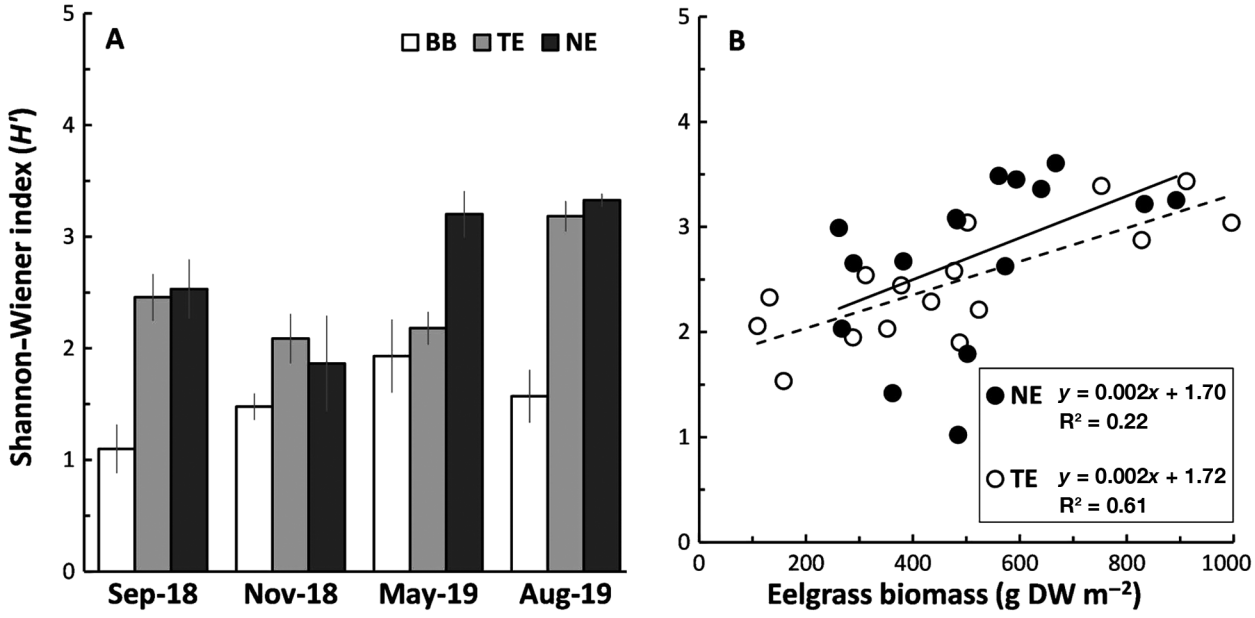

Fig. 8. (A) Shannon-Wiener index (*H'*) for each of the 3 habitats, bare bottom (BB), transplanted eelgrass (TE) and natural eelgrass (NE), during each sampling period (±SE, n = 4). (B) Correlations between dry eelgrass biomass and *H'* for both NE and TE, including linear regression lines for NE (solid black) and TE (dashed black). DW: dry weight

grass restoration (Valdez et al. 2020). However, their impact on biodiversity is measurable, and secondary foundation species enhance the overall diversity of ecosystems (Thomsen et al. 2018). This was evident in Horsens Fjord, where increases in suspension-feeding fauna and invertebrate grazers occurred after transplantation. Suspension-feeding fauna are known to control water quality in coastal areas if biomass and abundance are sufficiently high (>2000 ind. m^{-2}) (Alpine & Cloern 1992). The 2 vegetated locations in Horsens Fjord had ~1000 suspension-feeding ind. m^{-2}, and the biomass was 480 and 918 times higher at TE and NE, respectively, than at BB. This was probably caused by the more favorable living conditions created by eelgrass as an ecosystem engineer (Orth 1977, Fonseca & Fischer 1986, Orth et al. 2020). The diverse assembly of suspension feeders found in the eelgrass beds provides a filtration capacity proportional to their biomass (Riisgård 2001), leading to higher water clearance within eelgrass beds compared to the adjacent bare bottom. Accordingly, eelgrass restoration provides an essential ecosystem service on water transparency, apart from passive particle retention by the canopy and C and nutrient storage in plant biomass (Flindt et al. 1999, Greiner et al. 2013). Enhanced benthic light conditions are a prerequisite for the natural expansion of eelgrass to deeper waters (Benson et al. 2013), and the positive interactions between eelgrass transplants and suspension feeders promote such positive feedbacks in eutrophic coastal areas (Maxwell et al. 2017), leading to improved ecological conditions according to the WFD. However, excessive eutrophication may exceed the filtration capacity of suspension feeders and lead to alternate deteriorating states with dominance of opportunistic macroalgae and epiphytes (Valiela et al. 1997, Flindt et al. 1999). The presence of invertebrate grazers is therefore important for buffering against such alternative states, because they can mitigate the shading caused by epiphytes and increase eelgrass photosynthesis (Neckles et al. 1993, Benson et al. 2013). The abundance of grazers required to control epiphytes is reported to be in the range of 900 to 11 400 ind. m^{-2} (Howard & Short 1986, Neckles et al. 1993). The abundance of invertebrate grazers in the present Horsens Fjord study was in the lower range (TE: 827 ind. m^{-2}; NE: 342 ind. m^{-2}) of these values 2 yr after transplantation, probably because mobile invertebrate grazers were not monitored quantitatively. Although suspension feeders and invertebrate grazers can mitigate the effects of eutrophication to some extent, it is obvious that further expansion of eelgrass area and depth distribution can only be assured if nutrient discharge from land is reduced considerably (Krause-Jensen et al. 2008), so that alternate deteriorated states are avoided.

Transplantation of eelgrass in Horsens Fjord restored benthic fauna communities and provided up to 53 times higher benthic fauna biomass than adjacent bare bottom areas. This increase in benthic

fauna ensures better food supply than that in unvegetated areas, for commercially important fish species like Atlantic cod *Gadus morhua*, European plaice *Pleuronectes platessa* and European flounder *Platichthys flesus* (Pihl & Wennhage 2002, Pihl et al. 2006). Eelgrass meadows also act as juvenile nursing areas for many fish species (Gotceitas et al. 1997, Manderson et al. 2000, Bertelli & Unsworth 2014), and juvenile Atlantic cod feed on some of the prey species that were abundant at TE and NE in Horsens Fjord, e.g. the polychaetes *Hediste diversicolor* and *Alitta* spp. and the amphipod *Corophium* spp. (Pihl 1982). These species also act as an important food source for plaice and flounder, together with bivalves like *Mya arenaria*, *Mytilus edulis* and *Cerastoderma* spp. (Devlas 1979, Pihl 1982, Evans 1983), which were primarily found in vegetated habitats in Horsens Fjord. It is therefore expected that the restoration of eelgrass by transplantation is beneficial for the productivity of local fish stocks, because local habitat availability and food supply is enhanced. This was evident in Virginia coastal bays, USA, where fish biomass became several thousand times higher after restoration of seagrass (Orth et al. 2020).

4.3. Eelgrass biomass as a structuring factor for benthic communities

Benthic fauna community parameters *S*, *N* and *H′* followed seasonal fluctuations in eelgrass biomass closely and provided significant linear correlations. This suggests that increased availability of space by the habitat-forming eelgrass is directly proportional to diversity and abundance of fauna. A study in North Carolina found the same proportionality for infauna at low eelgrass biomasses, but the relationship turned asymptotic at higher biomasses (Homziak et al. 1982). Epifauna also follow an asymptotic relationship with transplanted *H. wrightii* and *Syringodium filiforme* shoot densities (Fonseca et al. 1996). This suggests that benthic fauna in Horsens Fjord will most likely be restricted by space limitation at higher eelgrass biomasses than those measured in the present study. These relationships indicate that eelgrass as an ecosystem engineer is a structuring factor for benthic fauna communities in coastal soft-bottom areas, because eelgrass meadows provide shelter for a wide range of fauna (Boström et al. 2006), increase food availability, reduce resuspension (Orth 1977, Fonseca & Fischer 1986) and provide the structural complexity needed for ecosystem niches to develop (Heck & Wetstone 1977). Sediment characteristics are also known to influence assemblages of benthic fauna communities (Bowden et al. 2001), but this effect was not evident in Horsens Fjord, because of similarity in sediment characteristics at the 3 locations (Figs. S1–S6).

The relationships between eelgrass biomass and community parameters (*S*, *N* and *H′*) were more distinct for TE than for NE. This suggests that community parameters at NE do not depend closely on site-specific eelgrass biomass but rather on size and form of the meadow from a landscape perspective. Even though an eelgrass transplant experiment in the lower Chesapeake Bay, USA, found no effect of either patch size or perimeter:area ratio on species richness and abundance of epifauna (Lefcheck et al. 2016), contradicting that patch size and transplantation pattern should affect community parameters in transplanted eelgrass. Even so, community parameters (*S*, *N* and *J′*) increased with time after transplantation (Lefcheck et al. 2016), similar to Horsens Fjord (*S*, *N* and *B*), indicating that time after transplantation may be more important than patch size and transplantation pattern for the development of community structure in transplanted eelgrass, until the transplantation becomes similar to a natural eelgrass meadow.

The ecosystem services provided by eelgrass as an ecosystem engineer are very well documented in natural eelgrass meadows, but the present study clearly shows that these effects develop rapidly after ecosystem restoration by eelgrass transplantation, even in fragmented transplantation patterns such as TE in Horsens Fjord. Increases in the areal gain of eelgrass by seed dispersal or vegetative growth, in the years following restoration, further increase ecosystem services (Lange 2020, Orth et al. 2020). However, the rate at which the extra ecosystem services are gained will diminish over time in proportion to the pace by which the meadow expands.

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Appendix. Additional data

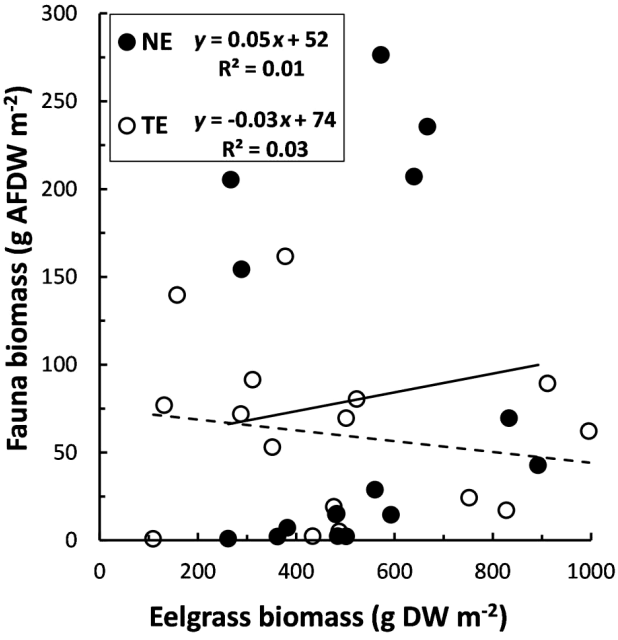

Fig. A1. Correlations between dry eelgrass biomass and fauna biomass for both natural eelgrass (NE) and transplanted eelgrass (TE), including linear regression lines for NE (solid black) and TE (dashed black). DW: dry weight; AFDW: ash-free DW

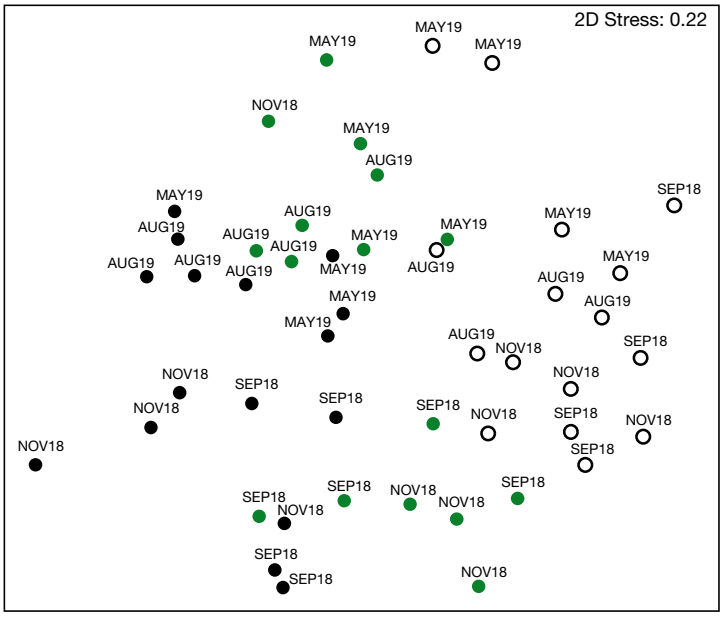

Fig. A2. Two-dimensional non-metric multidimensional scaling plot based on Bray-Curtis similarity matrix performed on benthic fauna biomass (stress 0.22), grouped into bare bottom (BB, white), transplanted eelgrass (TE, green) and natural eelgrass (NE, black) and sampling dates

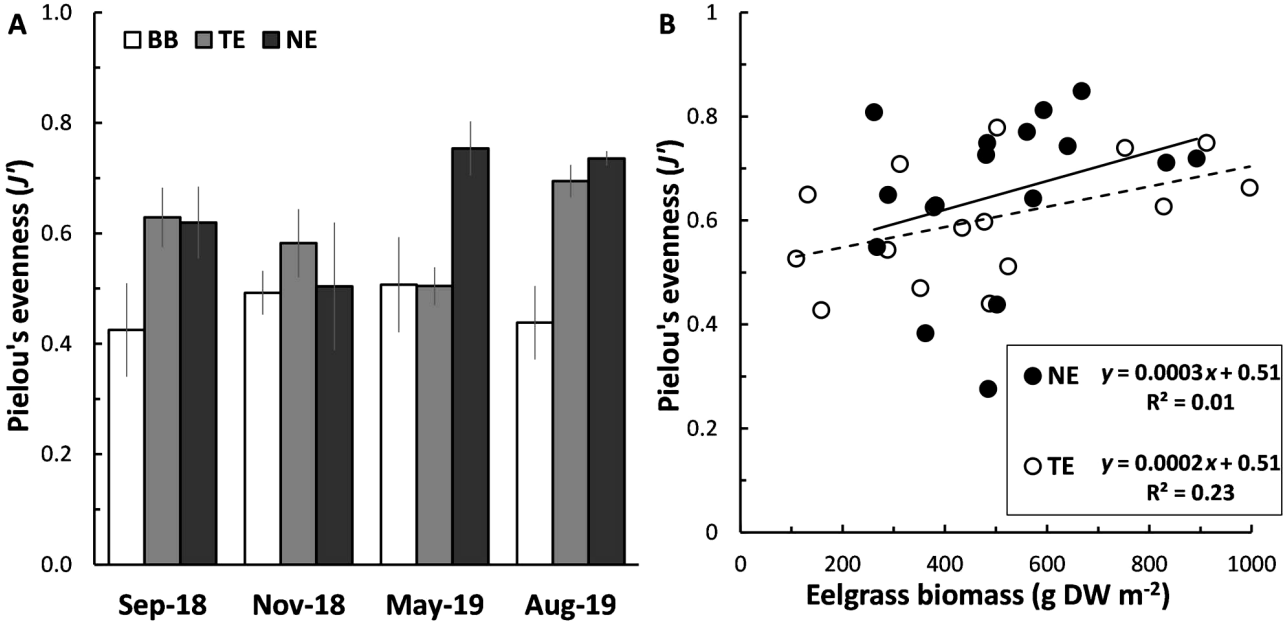

Fig. A3. (A) Pielou's evenness (*J′*) for each of the 3 habitats, bare bottom (BB), transplanted eelgrass (TE) and natural eelgrass (NE), during each sampling period (±SE, n = 4). (B) Correlations between dry eelgrass biomass and *J′* for both NE and TE, including linear regression lines for NE (solid black) and TE (dashed black). DW: dry weight

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