



Where does the salt marsh start? Field-based evidence for the lack of a transitional area between a gradually sloping intertidal flat and salt marsh

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ABSTRACT

Salt marshes are vegetated ecosystems between land and sea, hosting unique plant and animal communities, contributing to crucial habitats for birds and providing numerous other ecosystem services. They form a sustainable nature-based coastal protection, and its protective value increases with the width of the system. Salt marshes and their adjoining tidal flats are often studied separately. At present, there is a lack of studies that describe the features of these two systems in unison and in relation to each other. This study descriptively assesses an array of abiotic and biotic variables that potentially affect successful vegetation establishment. Unfavourable soil conditions may limit establishment as well as bioturbation of infauna. This is related to the marsh expansion potential and could aid in salt marsh restoration and marsh growth stimulation projects. In a naturally developing salt marsh, we sampled the elevation gradient from the tidal flat to the low marsh for benthic species composition, plant seed availability and abiotic variables. The abundance of benthos was highest landwards of the transition zone, in the pioneer zone. Distinct meiobenthic groups occurred in the different zones along the tidal flat to low marsh gradient, but macrobenthos was largely absent from the muddy soil. In the sparsely vegetated transition zone, the abundance of salt-marsh seeds was low, similar to the tidal flat. It suggests that, even though a seed source was in proximity, seed availability in spring was insufficient to achieve lateral marsh expansion. Clustering and nMDS analyses showed that an identifiable transition zone was lacking. The transition zone resembled the bare tidal flat in terms of its abiotic and biotic conditions. This was mainly driven by significant changes in soil oxygenation and seed availability.

1. Introduction

The risks posed by current and future sea-level rise calls for improved coastal protection (Bouma et al., 2014; Möller, 2006). Nature-based coastal defence, such as dunes and salt marshes, provide a sustainable and cost-effective extension to current practices (Temmerman et al., 2013). To keep pace with enhanced sea level rise, salt marshes depend on sufficient sediment accretion rates. Most studies estimating long-term survival of salt marshes have focused on vertical marsh accretion (Kirwan and Guntenspergen, 2010; Marani et al., 2013; Mariotti and Fagherazzi, 2013), however lateral (i.e. seaward) expansion is just as crucial for the preservation of salt marshes (Balke et al., 2016; Bouma et al., 2014, 2016). Nowadays, many marshes are constricted inland by hard structures and lateral expansion can solely occur on the seaward side of marshes.

The salt marsh to the tidal flat interface is generally divided into multiple zones based on plant species and vegetation cover (Petersen et al., 2014). The high marsh is characterized by one or a few late-successional plant species with high cover and density. At the low marsh, a diverse variety of plant species occur with varying degrees of cover. Then follows the pioneer zone, which is dominated by pioneer species with cover >5%. The boundary between the pioneer zone and the tidal flat can be either sharp if a marsh cliff is present (van der Wal et al., 2008b) or gradual on a gently sloping marsh. Just beyond the pioneer zone lies the transition zone on the tidal flat, with only a few pioneer plants per m² present, i.e. a cover <5%. This zone is also referred to as the pre-pioneer zone (Baptist et al., 2016; Marion et al., 2009). Pioneer vegetation establishment on the bare intertidal flat preceding the marsh is essential for salt marsh development (van der Wal et al., 2008b). However, there is a lack of studies that describe the

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conditions and characteristics of the transition zone in unison with both the tidal flat and the salt marsh.

Scientific studies have identified multiple abiotic factors that affect salt marsh vegetation establishment. The harshest conditions for vegetation establishment occur on the intertidal flat, with soil anoxia and high soil salinities. These stressful conditions gradually decrease with increasing elevation towards the upper marsh, allowing the establishment of marsh plants typical for low and high marshes (Bertness and Hacker, 1994). To successfully establish, annual species depend on yearly seed recruitment but even perennial species benefit from establishment by seeds to colonize of new areas. For seedling establishment, a minimum bed level elevation is required, estimated at 0.5 m below mean high water level (Wang and Temmerman, 2013). The local elevation is closely linked to inundation characteristics which govern a disturbance-free window of opportunity necessary for seedling establishment (Balke et al., 2014; Wiehe, 1935). High soil salinity reduces seed germination and hinders the emergence of seedlings for marsh vegetation in general (Baldwin et al., 1996; Elsey-Quirk et al., 2009; Ungar, 1978). When soil salinity is reduced, for example through precipitation, conditions for seedling establishment improve. Tolerance to anoxic soil conditions differs for each marsh species, with pioneer species being more tolerant towards anoxia (Davy et al., 2011; Holmer et al., 2002). Furthermore, grain size can affect settlement and survival of pioneer marsh species through soil stability. Muddier soil is less prone to erosion than sandy soil and thus favourable for pioneer seedling establishment and survival (Houwing et al., 1999).

Next to the abiotic factors, several biotic variables influence successful vegetation establishment on the bare tidal flat. Soil stability can be enhanced by microphytobenthos, which produce extracellular polymeric substances thereby increasing the cohesiveness of the surface sediment (Blanchard et al., 2000). Stable soils are favourable for establishment of marsh vegetation (Bouma et al., 2016). For seedling establishment the availability of seeds is a prerequisite, especially for annual plants (van Regteren et al., 2019). Mature vegetation provides a seed source, as most seeds disperse close to the parental environment (Chang et al., 2007; Rand, 2000; Wolters et al., 2008). Retention of seeds is highest in dense but flexible vegetation (Chang et al., 2008). Nonetheless, bare restoration sites show quick colonization by annuals such as *Salicornia* spp. from nearby marshes, while other marsh species appeared within a few years (Erfanzadeh et al., 2010a; Mossman et al., 2012a; Wolters et al., 2008). This indicates the transport of seeds between marshes as well (Huiskes et al., 1995). Seed availability and successful establishment of pioneers can be hindered by bioturbation of benthic infauna, such as amphipods, polychaetes and oligochaetes (Delefosse and Kristensen, 2012; Gerdol and Hughes, 1993; van Regteren et al., 2017). Burrowing benthos can dislodge rooting seedlings and even cause mutual exclusion of infauna and vegetation within a similar intertidal habitat (van Wesenbeeck et al., 2007). In contrast, bioturbation increases the soil oxygenation and other resource flows beneficial for seedling establishment (Reise, 2002; van Regteren et al., 2017). In the field, all these previously mentioned physical and biological environmental conditions are likely to change across the intertidal flat to salt marsh interface.

The aim of this study is to understand how environmental conditions, seed availability and the benthic community in the transition zone may influence salt marsh expansion potential. Therefore, abiotic and biotic characteristics were examined along a gradient from a bare tidal flat to vegetated salt marsh in a naturally developing salt marsh. The following hypotheses were tested: 1) abiotic characteristics will show gradients from the intertidal flat to the marsh, with the transition zone being intermediate; 2) the abundance of benthic fauna is highest in the transition zone, consisting of a mixture of salt marsh and tidal flat species; 3) seed abundance is highest in the low marsh and decreases towards the tidal flat and 4) a combination of ameliorated stressful conditions and proximity to a seed source allow pioneer seedlings to establish in the transition zone.

2. Methods

2.1. Study site

A naturally developing salt marsh in the Wadden Sea of approximately 30 ha was used as the field study site. It is located along the Dutch mainland at Westhoek, The Netherlands (53°16.31N, 5°33.14E). The landward boundary is formed by a dyke. Since its formation in 1996, the marsh has been expanding step-wise, with stable periods alternating with periods of expansion, both horizontal along the dyke and lateral onto the intertidal flats (Baptist et al., 2019b). The vertical accretion rate was $1.6 \pm 0.7 \text{ cm yr}^{-1}$ (mean \pm se) for the period 2015–2019 (Baptist et al., 2019b). The vegetation in the pioneer zone is dominated by pioneer species *Salicornia* spp. and *Spartina anglica*. The most frequently occurring plants on the low marsh are *Aster tripolium*, *Puccinellia maritima*, *Suaeda maritima*, *Atriplex prostrata* and *Halimione portulacoides* (Table 1). The high marsh is characterized by *Elymus athericus* and the more brackish *Phragmites australis*.

2.2. Field sampling

Sampling was conducted during spring in May 2016, the establishment period of marsh vegetation. In a gradient from tidal flat to salt marsh 14 transects encompassing 5 zones were sampled (Fig. 1). Four zones were defined by vegetation cover and species composition, following the classification by Petersen et al. (2014). These included: low marsh, pioneer zone, transition zone and tidal flat. The tidal flat was divided into waterlogged hollows and higher elevated hummocks based on visual observation of topographic heterogeneity (Fig. 1). In total, 67 plots were sampled, on the north-eastern and south-western extremities of the study site, the low marsh zone had not yet established (Fig. 1). In the next sections first the abiotic factors are described, followed by the biotic factors.

Elevation of each plot was measured in NAP (Dutch Ordnance Datum, approximately mean sea level) with a Real-Time-Kinematic-DGPS with a vertical accuracy of 0.016 m. Soil oxygenation was estimated visually discriminating between oxic brown sediment and anoxic black sediment (Rosenberg, 2001). Depth of the oxygenated layer was measured to the mm using a soil corer of 26 cm in length, replicated thrice per plot and averaged. If the oxygenation reached deeper than 26 cm, this value was still noted as the maximum. Sediment cores, 4.6 cm

Table 1

Mean cover (%) of salt-marsh plants (mean \pm sd) given for each zone.

Plant species	Tidal flat Hollows	Tidal flat Hummocks	Transition zone	Pioneer zone	Low marsh
<i>P. maritima</i>	0	0	0	0.6 \pm 0.8	21.5 \pm 36.6
<i>A. tripolium</i>	0	0	0	1.5 \pm 1.8	45.4 \pm 24.3
<i>A. prostrata</i>	0	0	0	0.14 \pm 0.36	5.1 \pm 11.7
<i>S. anglica</i>	0	0	0	8.8 \pm 13.7	2.0 \pm 3.4
<i>Salicornia</i> spp.	0	0.07 \pm 0.27	0.9 \pm 0.36	26.2 \pm 23.5	11.6 \pm 13.8
<i>S. maritima</i>	0	0	0	0.4 \pm 0.5	0.8 \pm 0.8
<i>Spergularia</i> spp.	0	0	0	0.07 \pm 0.27	0.6 \pm 1.2
<i>E. athericus</i>	0	0	0	0	0.5 \pm 0.8
<i>T. maritima</i>	0	0	0	0	0.09 \pm 0.3
<i>A. stolonifera</i>	0	0	0	0	1.9 \pm 6.0
Total cover (%)	0	0.07 \pm 0.27	0.86 \pm 0.36	37.8 \pm 22.7	89.5 \pm 21.9
Sample size	14	14	14	14	11

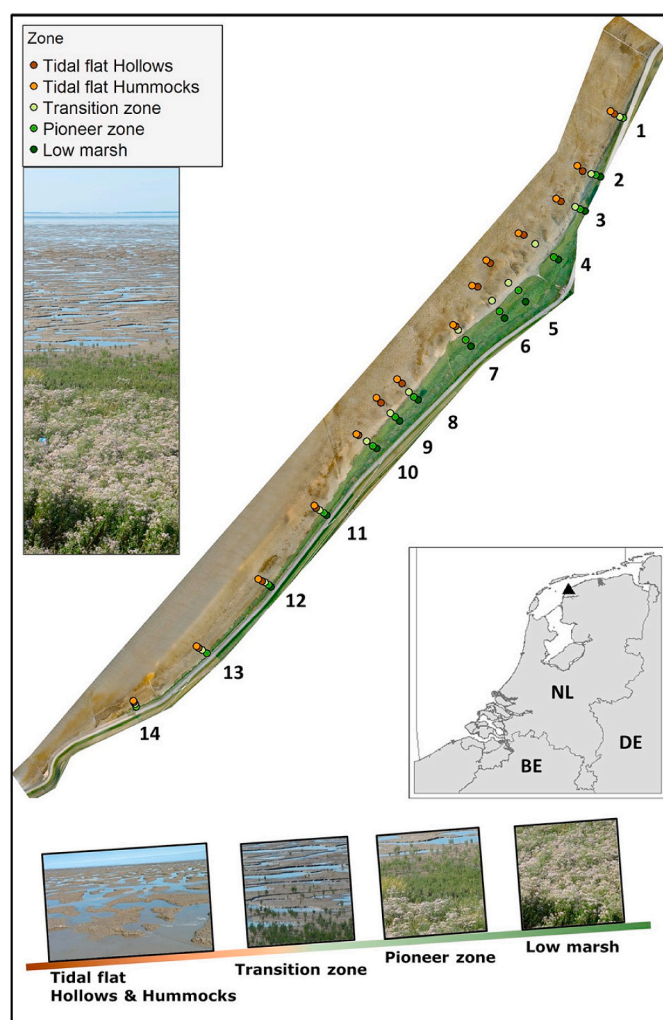


Fig. 1. Map of sampling stations, zonation indicated with coloured dots. Transects are numbered from north-east to south-west. The triangle indicates the location of the Westhoek salt marsh, The Netherlands. The lower excerpts exemplify the zonation.

wide and 5 cm deep (volume of 83 cm³), were taken to measure bulk density, water content and organic matter content. Sediment cores were frozen until further analyses in the laboratory. Additionally, three soil samples were taken to estimate surface soil salinity. One sediment sample of 40 mL was taken for grain size analysis. Median grain size (d50) and mud content (fraction smaller than 63 µm) were extracted from this analysis.

Vegetation was scored in 2 m by 2 m plots using the Londo decimal scale for permanent quadrats (Londo, 1976). This method first scores the presence of species, then the cover of each species by visual inspection and is widely used (Kiehl et al., 1997; Nolte et al., 2019). Soil cores, 5.6 cm in diameter and 10 cm deep (volume of 246 cm³), were taken to analyse both the benthos community and seed abundance. They were preserved in a 6% formaldehyde solution with rose Bengal to stain the live animal tissue. Microphytobenthos (µg cm⁻²), consisting of diatoms and cyanobacteria, was measured on the soil surface with a BentoTorch fluorometer (bbe Moldaenke GmbH), replicated thrice per plot and averaged.

2.3. Laboratory analyses

The sediment cores were weighed to obtain wet weight (WW), dried for 24 h at 105 °C and weighed again to obtain dry weight (DW)

(Lappalainen and Kangas, 1975). WW and DW were used to calculate mass water content (%). Subsequently, they were incinerated for 8 h at 560 °C to obtain the ash-free dry weight (AFDW) (Lappalainen and Kangas, 1975). To estimate organic and carbonate content of the sediment, loss on ignition (LOI, % of AFDW) was measured (Heiri et al., 2001). Dry bulk density (g cm⁻³) was calculated following Flemming and Delafontaine (2000). Soil surface salinity was analysed by putting 15 g of soil into 60 mL of demi-water. This mixture was shaken vigorously and left overnight for the sediment to settle. Once settled, salinity was measured with a Hach HQ40D multi-meter using the Practical Salinity Scale (‰). The average of the three samples was taken as the value for soil salinity. Grain size distribution was analysed by the Netherlands Institute for Sea Research (NIOZ) with a Coulter Beckman LS 13 320. Median grain size (d50) and mud content (fraction < 63 µm) were extracted from the grain size distribution data.

The benthos/seed cores were sieved over a 250 µm sieve, transferred into a transparent tray and placed on a light table. All organisms and seeds were collected and counted. Benthic species were determined up to order level and stored in 70% ethanol. Nematodes and foraminifera were excluded because the sieve was too coarse to retain them quantitatively. Seeds were identified up to species level when possible, otherwise categorised as unidentified.

2.4. Statistical analyses

The protocol designed by Zuur et al. (2010) was used for data exploration and validating the assumptions. Analyses of variance (ANOVA) were used to test whether zones differed from each other based on abiotic and biotic variables, the abundances of benthic groups and seed availability. Zone was used as a predictor variable. When the ANOVA yielded significant results, post hoc Tukey HSD tests were used to explore which zones differed from each other. These were used to test the first three hypotheses.

The clustering and ordination analyses were used to assess hypothesis 4. Cluster analyses were used to validate the zonation defined by plant species, plant cover and topographic heterogeneity. A selection of non-collinear environmental variables was made based on variance inflation factors (Table 2). The following environmental variables were used: elevation, dry bulk density, soil salinity, mud content, loss on ignition and microphytobenthos. Soil oxygenation was excluded due to collinearity with elevation, similar to mass water content collinear with dry bulk density and d50 collinear with mud content (Supplementary Fig. 1). Clustering on environmental variables and benthos community was performed using Ward's hierarchical clustering on an Euclidean distance matrix. Environmental data were thus standardised and scaled (Clarke and Warwick, 2001). Benthos community data were also analysed using non-metric multidimensional scaling (nMDS) based on Bray-Curtis similarity (Bray and Curtis, 1957). The performance of the nMDS was assessed using a Shepard-Diagram.

The relationship between the benthic community structure and abiotic environmental variables was examined using the BioEnv procedure (Clarke and Ainsworth, 1993) to define which combination of

Table 2

Variables used in cluster and nMDS analyses and significance in BioEnv analysis. Stars (*) indicate variables not included in the analyses due to collinearity.

Environmental variable	Used in cluster and nMDS	Significant in BioEnv
Elevation (m NAP)	Yes	Yes
Soil oxygenation depth (mm)	* collinear elevation	NA
Soil salinity (‰)	Yes	No
Dry bulk density (g cm ⁻³)	Yes	Yes
Mass water content (%)	collinear dry BD	NA
Mud content (% < 63 µm)	Yes	No
d50 (µm)	* collinear mud content	NA
Loss on ignition (%)	Yes	Yes
Microphytobenthos (µg cm ⁻²)	Yes	No

Table 3

Mean number of seeds sampled in the top 10 cm soil layer, given per 246 cm³ for each zone and species (mean \pm sd).

Plant species	Tidal flat Hollows	Tidal flat Hummocks	Transition zone	Pioneer zone	Low marsh
<i>P. maritima</i>	0	0	0	0	6.0 \pm 19.9
<i>A. tripolium</i>	0.07 \pm 0.27	0.21 \pm 0.58	0.07 \pm 0.27	1.7 \pm 3.1	18.6 \pm 27.0
<i>A. prostrata</i>	0.07 \pm 0.27	0.14 \pm 0.36	0.07 \pm 0.27	10.2 \pm 15.0	180.0 \pm 133.2
<i>S. anglica</i>	0	0	0	0.07 \pm 0.28	0.6 \pm 0.7
<i>Salicornia</i> spp.	0.36 \pm 0.84	0.14 \pm 0.36	0.79 \pm 1.72	42.2 \pm 46.5	20.8 \pm 28.4
<i>P. australis</i>	0.07 \pm 0.27	0	0	0	0
Seeds unid.	0.36 \pm 0.74	0	0.21 \pm 0.58	0.43 \pm 0.94	0.91 \pm 1.45
Total seeds (246 cm ⁻³)	0.5 \pm 0.94	0.5 \pm 1.09	0.93 \pm 1.86	54.2 \pm 49.7	219.9 \pm 129.8
Sample size	14	14	14	14	11

environmental variables had the highest correlation with the benthic species data. The association of the benthic community with the selected combination of environmental variables was tested for significance using a Mantel test. This test related the benthic distance matrix with the environmental distance matrix with Spearman's correlation using a bootstrapping procedure with 999 iterations.

All analyses and design of the figures were performed with the statistical program R (R Core Team, 2018) using additional packages vegan 2.5–1, ggplot 2 3.1.0, MASS 7.3–51.1, cowplot 09.2, tidyverse 1.3.0, raster 3.0–7, sf 0.8–0 and tmap 2.3–1.

3. Results

3.1. Abiotic environmental variables

Elevation decreased from the low marsh at 1.5 m above NAP towards the tidal flat at around 0.95 m above NAP and the elevation differed significantly between zones (ANOVA, $F_{df=4} = 103.5$, $p < 0.001$). The post hoc Tukey HSD test showed that the low marsh and pioneer zone differed significantly compared to the other zones (Fig. 2). Soil oxygenation differed between zones (ANOVA, $F_{df=4} = 88.3$, $p < 0.001$). It declined sharply between the pioneer and transition zone, from 214 ± 59 mm to 31 ± 44 mm. Oxygenation was significantly higher in the vegetated zones than at the transition zone and intertidal flat, which

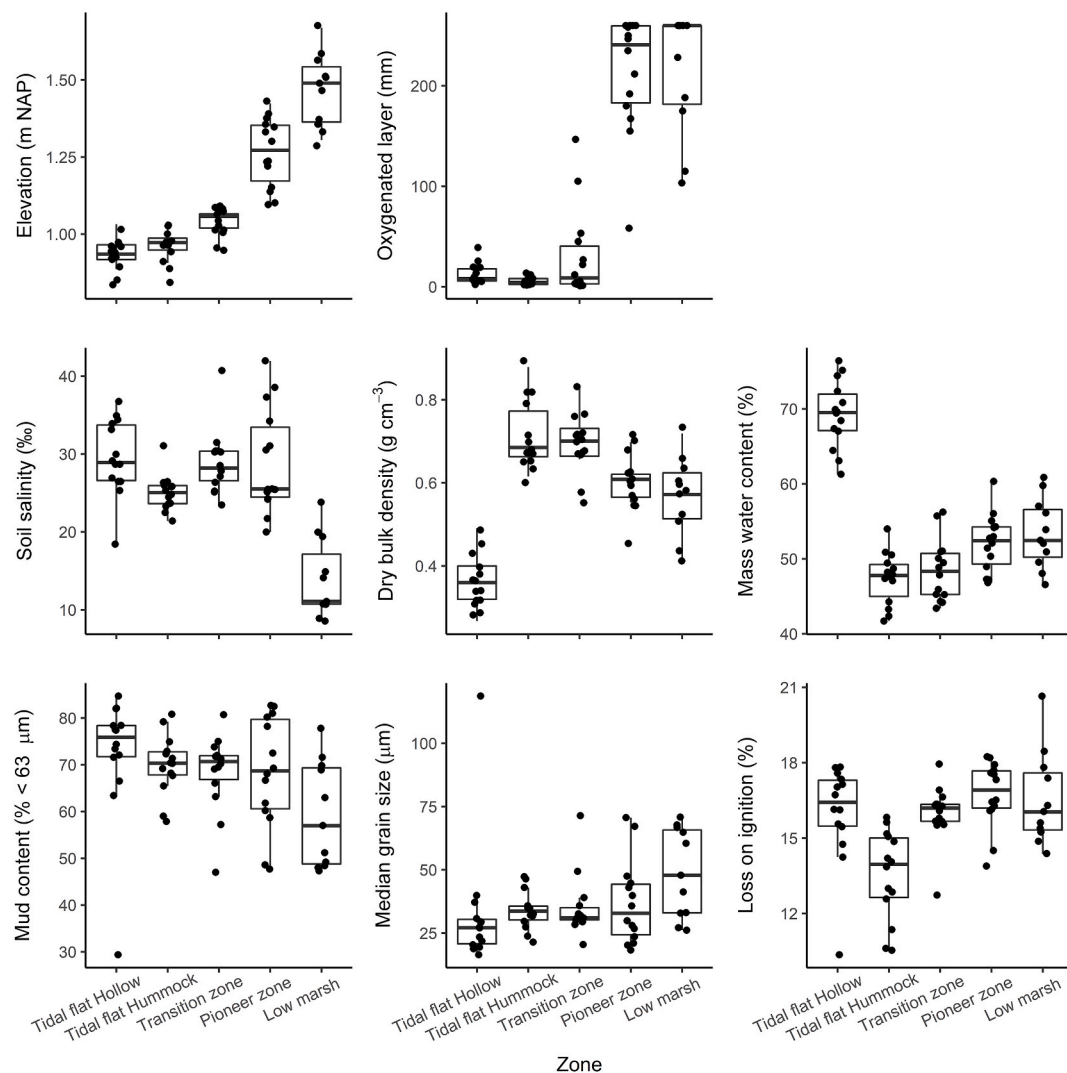


Fig. 2. Boxplots of abiotic variables that characterise different zones along the tidal flat to salt marsh gradient. Dots, jittered for clarity, indicate the individual measurements.

were not significantly different from each other. Soil oxygenation was on average 12 ± 10 mm for the tidal flat hollows and 6 ± 4 mm for the tidal flat hummocks.

Soil salinity varied significantly between zones (ANOVA, $F_{df=4} = 21.7$, $p < 0.001$). It was much lower at the low marsh but comparable

across the other zones (Tukey HSD, Fig. 2 and Supplementary Table 1). Overall, the soil was silty with a mud content between 50% and 80% and median grain size (d_{50}) of $37 \pm 17 \mu\text{m}$. A marginally significant difference in mud content was found between the tidal flat hollows and the low marsh (ANOVA, $F_{df=4} = 2.5$, $p = 0.048$, Tukey HSD, Fig. 2 and

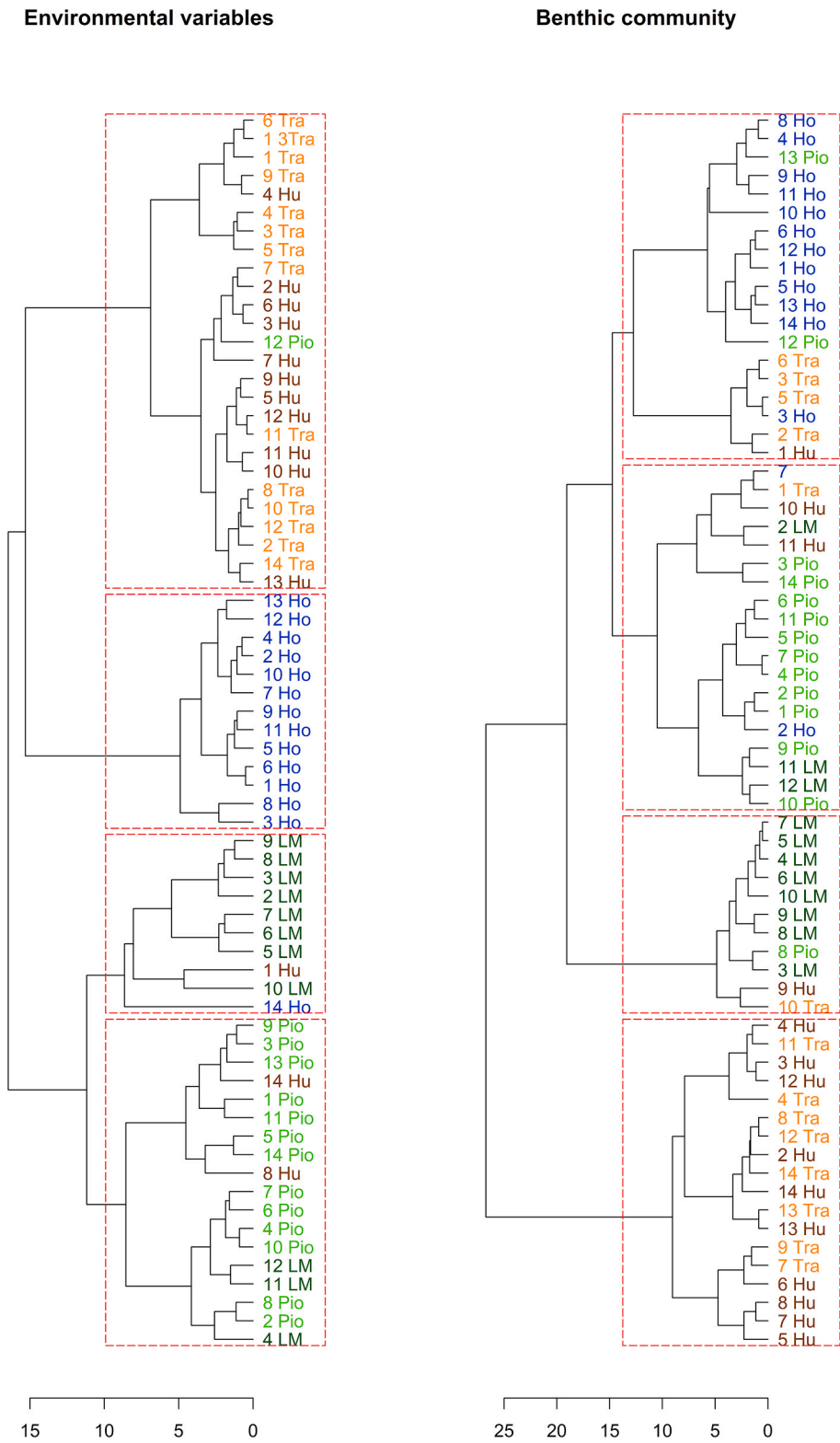


Fig. 3. Cluster analysis of abiotic environmental variables (left) and the benthic community (right). The dashed red boxes indicate the clustering. The predefined zonation is indicated in colour with transect number and zone abbreviation: low marsh dark green, LM; pioneer zone light green, Pio; transition zone orange, Tra; tidal flat hummocks brown, Hu; tidal flat hollows blue, Ho.

Supplementary Table 1). Median grain size was similar across all zones (ANOVA, $F_{df=4} = 1.7$, $p = 0.17$).

Mass water content differed between the zones (ANOVA, $F_{df=4} = 64.8$, $p < 0.001$). On average, the hollows on the tidal flat were distinctly different from all other zones with a higher mass water content of $\pm 70\%$ vs. $\pm 50\%$ (Fig. 2, Tukey HSD). Consequently, a reversed pattern occurred for dry bulk density, which varies across zones (ANOVA, $F_{df=4} = 48.8$, $p < 0.001$). Dry bulk density was lower in the tidal flat hollows compared to all other zones (Fig. 2, Tukey HSD). Organic and carbonate content (LOI) varied across zones (ANOVA, $F_{df=4} = 8.5$, $p < 0.001$) and was significantly lower on the tidal flat hummocks, but similar for all other zones (Fig. 2, Tukey HSD).

The cluster analysis for the abiotic environmental variables did not reflect the same five zones as classified in the field based on vegetation cover and topographic heterogeneity, i.e. hummock and hollows (Fig. 3). The cluster analysis indicated that the transition zone and tidal flat hummocks shared similar environmental conditions. Together with the cluster for tidal flat hollows, these two clusters were distinctly different from the two clusters of the vegetated zones (Fig. 3). The low marsh and pioneer zone were more alike and some low marsh samples were clustered with the pioneer zone.

3.2. Benthic community distribution

The soil samples were dominated in abundance by three species groups: oligochaetes, collembolans and harpacticoid copepods (Fig. 4). These three groups hardly occurred in the low marsh, where insects were most common. Insects differed significantly across zones (ANOVA, $F_{df=4} = 11.1$, $p < 0.001$) and were significantly more abundant on the low marsh (Tukey HSD). The insects included multiple species of fly pupae, spiders, chironomids, aphids and one thrips. In the pioneer zone, oligochaetes and harpacticoid copepods were the most common benthic fauna. The oligochaetes numbers contained 95% juveniles and 5% adults. Adults were identified up to species level and belonged to *Paranais litoralis* (most common), *Baltidrilus costatus*, *Nais elinguis* and *Enchytraida* sp. Few adults but many juvenile oligochaetes were present in May, which differed significantly across zones (ANOVA, respectively $F_{df=4} = 6.7$, $p = 0.0002$ and $F_{df=4} = 31.3$, $p < 0.001$). Post hoc tests indicated that oligochaetes occurred in significantly higher abundance in the pioneer zone (Fig. 4). The pioneer zone contained on average 365 oligochaetes per sample, which corresponds to approximately 148×10^3 oligochaetes per m^2 . Oligochaete juveniles also occurred in all other zones, with the second highest abundance in the low marsh (Fig. 4).

Benthic copepods were found in distinct zones (ANOVA, $F_{df=4} = 11.0$, $p < 0.001$). Copepod abundance was significantly higher in the

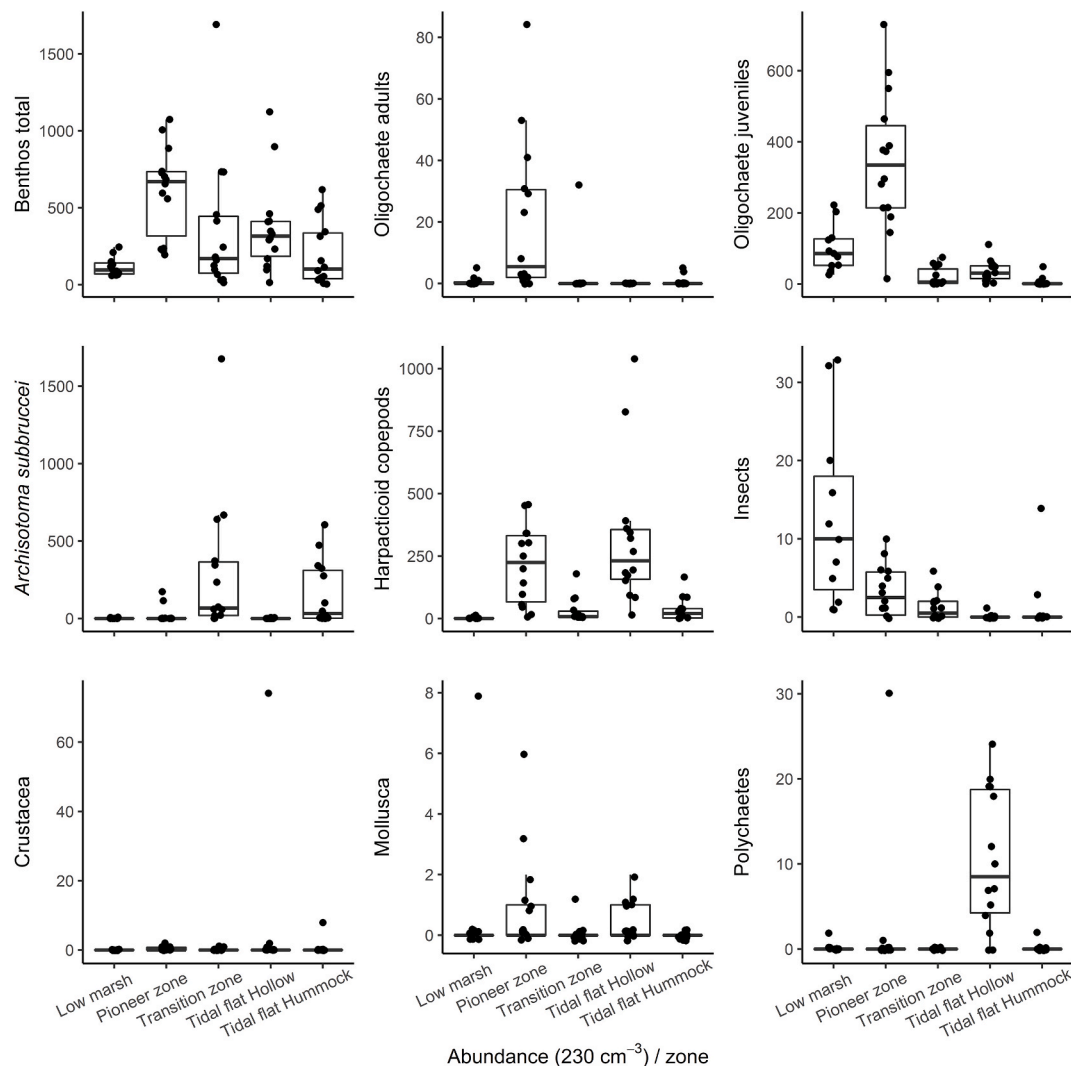


Fig. 4. Boxplots of total benthos abundance and abundance per benthic group that characterise different zones along the tidal flat to the salt marsh gradient. Dots, jittered for clarity, indicate the individual measurements. Soil samples were taken 10 cm deep and species abundance is given per 246 cm^3 . Note: the vertical axes contain different scales.

pioneer zone and tidal flat hollows (Tukey HSD). Remarkably, they occurred most frequently in two non-bordering zones (Fig. 4). Springtails (collembola) *Archisotoma* sp. (likely all *Archisotoma subbrucei*) were dominant in the transition zone and the hummocks on the tidal flat (ANOVA, $F_{df=4} = 4.3$, $p = 0.004$, Tukey HSD). The transition zone contained on average 299 individuals per sample, which corresponds to 120×10^3 collembolans per m^2 . The tidal flat hummocks contained on average 157 individuals per sample, corresponding to 63×10^3 collembolans per m^2 . Springtails and harpacticoid copepods hardly ever co-occurred. The pioneer zone contained on average 215 copepods per sample, which corresponds to 86×10^3 individuals per m^2 . The tidal flat hollows contained on average 317 individuals per sample, equivalent to 127×10^3 copepods per m^2 .

Polychaetes were present in the tidal flat hollow samples (Fig. 4). Polychaetes contained individuals of *Nereidinae* (often juveniles, most common), *Streblospio benedicti*, *Aphelochaeta marioni*, *Eteone longi*, *Heteromastus filiformis*, and *Pygospio elegans* and differed in zonal abundance (ANOVA, polychaetes, $F_{df=4} = 10.7$, $p < 0.001$). Mollusca were scarce and consisted of mostly *Hydrobia* sp. in the pioneer zone and a few juvenile mussels, *Mytilus edulis*, in the tidal flat hollows. Crustaceans were found in all zones in low abundance, consisting of calanoid copepods, amphipods, ostracoda, one *Crangon crangon* and one hollows sample with 66 *Corophium volutator* juveniles. Molluscan and crustacean abundances did not differ significantly between zones (ANOVA, respectively, $F_{df=4} = 1.5$, $p = 0.21$ and $F_{df=4} = 0.9$, $p = 0.46$). *C. volutator* and juvenile flatfish were observed in the tidal flat hollows but often successfully avoided the sampling gear. Macrofauna (>1 mm) were scarce throughout the study site. The total abundance of benthic animals differed between zones (ANOVA, $F_{df=4} = 5.1$, $p = 0.0014$) and was highest in the pioneer zone (Fig. 4).

For the benthic community, four clusters were distinguished. The clusters of the benthic community contained more variability regarding the predefined zonation than the clustering of environmental variables (Fig. 3). Generally, separate clusters were observed for the low marsh, the pioneer zone, the tidal flat hummocks and the tidal flat hollows. Part of the transition zone samples, mainly the northern transects, formed a cluster together with the tidal flat hollows. The other transition zone samples clustered with the tidal flat hummocks. Similar to the environmental variables, the benthic community of the transition zone predominantly clustered with the tidal flat hummocks (Fig. 3).

3.3. Seed abundance and plant cover

The low marsh contained the most diverse plant community as well as the highest cover. On average, 5.5 different plant species were present and the cover was nearly 90%. It was dominated by *Puccinellia maritima* and *Aster tripolium*, with a combined cover of 66% on average (Table 1).

Salicornia spp. and *Atriplex prostrata* were often present, though in lower abundance. *Spergularia* spp., *Elymus athericus*, *Triglochin maritima*, *Agrostis stolonifera* and *Spartina anglica* were sparsely present in the low marsh. The low marsh was followed by the pioneer zone with 3.7 species on average and cover close to 40% (Table 1). The pioneer plants *Salicornia* spp. and *S. anglica* dominated the pioneer zone. *Suaeda maritima*, *P. maritima* and *A. tripolium* were often present in low cover in the pioneer zone. In the transition zone, solely the annual pioneer *Salicornia* spp. was present and its cover was a maximum of 1%. The tidal flat was unvegetated with the exception of one *Salicornia* spp. seedling on a hummock.

Seed abundance differed across the zones (ANOVA, $F_{df=4} = 32.4$, $p < 0.001$). The post hoc Tukey HSD test indicated that the low marsh and pioneer zone differed significantly from each other and all other zones. Seeds abundance in the soil was lowest at the tidal flat and significantly increased towards the low marsh (Table 2), which resulted in a similar pattern as the plant cover (Fig. 5). Microphytobenthos abundance (cyanobacteria and diatoms) was similar throughout the tidal flat to salt marsh zones (ANOVA, $F_{df=4} = 1.5$, $p = 0.22$, Fig. 5).

The seed bank did not represent all the species that were present aboveground. Seeds from six different plant species were identified, of which five occurred aboveground in our sampling plots. The sixth seed species belonged to *Phragmites australis*, which was present higher up the marsh and disperses by wind. Five plant species present in the vegetation were absent from the seedbank. In the low marsh (and in total), the most common seed species found was *A. prostrata* with on average 180 seeds per sample (246 cm^3). However, with only a 5% cover, this was not the dominating species aboveground (Table 1). In the pioneer zone, *Salicornia* spp. seeds prevailed with over 40 seeds per 246 cm^3 , corresponding to the dominant vegetation aboveground. The total number of seeds in the transition zone and the tidal flat was, on average, less than 1 seed per 246 cm^3 , mostly *Salicornia* spp. (Fig. 5 and Table 3).

Salicornia spp. and *A. prostrata* seeds were found throughout all zones, ranging from salt marsh to tidal flat (Table 3). The wind-dispersed *A. tripolium* occurred in all zones as well, although in lower abundance. *S. anglica* seed abundance was remarkably low: only six seeds in the low marsh and one in the pioneer zone, even though in the pioneer zone *S. anglica* was the second most common species aboveground. The total number of seeds of all marsh plants combined was four times higher in the low marsh than in the pioneer zone, whereas the vegetation cover was two times higher in the low marsh than in the pioneer zone (Tables 1 and 3).

3.4. The benthic community associated with abiotic environmental variables

The clustering of the benthic community was largely reflected by the

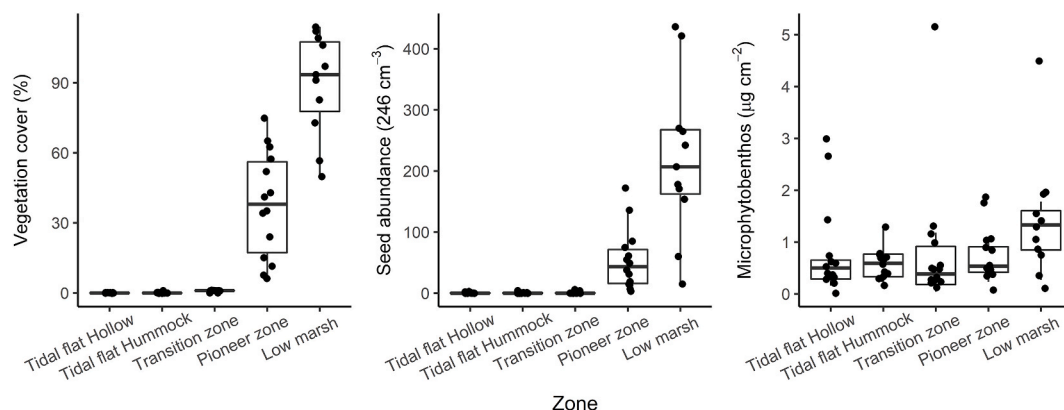


Fig. 5. Boxplots of biotic variables that characterise different zones along the tidal flat to salt marsh gradient. Dots, jittered for clarity, indicate the individual measurements. Information on vegetation species, cover and seed species can be found in Tables 1 and 3.

grouping in the nMDS analysis (Figs. 3 and 6). The first nMDS ordination axis was associated mostly with elevation and soil salinity. The low marsh, pioneer zone and tidal flat hollow samples follow a gradient along this axis. The transition zone and tidal flat hummock samples were more spread out, as they were not restricted on the first axis and show overlap between the two. The second ordination axis was mainly influenced by dry bulk density (and thus, as negatively correlated, mass water content), with the transition zone and tidal flat hummocks being less moist. The most common species occurring in both these two zones were the collembolans. The tidal flat hollows were clearly moistest and associated with polychaetes (Fig. 6).

The BioEnv analyses showed that the benthic community was best correlated with elevation, LOI and dry bulk density ($r = 0.49$; Mantel test $p = 0.001$, Table 2). Elevation was the single variable that correlated best with the benthic community ($r = 0.29$). Elevation was collinear to soil oxygenation and dry bulk density to water content (Supplementary Table 1).

4. Discussion

Based on the environmental cluster analysis, four zones were identified: low marsh, pioneer zone, tidal flat hollows and a combined zone consisting of the transition zone and the tidal flat hummocks. Similarly, the benthic community formed four general clusters, with the benthic community of the transition zone partially overlapping with both the tidal flat hummocks and hollows. Strong changes in some environmental variables, most importantly soil oxygenation and seed abundance, implicate that there is a distinct difference between the vegetated parts (low marsh and pioneer zone) and the sparsely or un-vegetated parts (transition zone and tidal flat). The waterlogged hollows on the tidal flat formed a separate cluster, located within the elevated tidal flat (transition zone and tidal flat hummocks combined).

4.1. Abiotic variables along the tidal flat to marsh interface in relation to seedling establishment

Our first hypothesis was that abiotic characteristics show gradients from the intertidal flat to the marsh, with the transition zone being intermediate between the two. Elevation did show a gradient but other

variables showed sharp changes between zones, such as oxygenation and water content. Other factors remained similar across zones, such as mud content and median grain size, therefore this hypothesis is rejected.

In contrast to current knowledge we did not find a shift in d50 between the vegetated and unvegetated areas (van der Wal and Herman, 2012). Median grain size and mud content largely overlapped (Supplementary Table 1), indicating that grain size distribution is not a constraining factor for seedling establishment. This may be different in areas where soils are less silty (van der Wal and Herman, 2012). Inundation is an important factor in the windows of opportunity concept, which provides a framework of physical boundary conditions, such as an inundation-free and hydrodynamically quiet period for seeds, to germinate, root and establish successfully (Balke et al., 2011; Wiehe, 1935). When these windows of opportunity take place on the bare tidal flat, sudden massive colonization of pioneers may occur (Balke et al., 2014). Inundation characteristics are linked to elevation and influence pioneer seedling establishment (Wiehe, 1935). Elevation formed a gradient from tidal flat to salt marsh, although some locations with typical low marsh vegetation occurred at lower elevations than pioneer zone stations. These were mainly situated at the extremities of the marsh (north-eastern and south-western stations) because marsh growth originated from the middle where it is currently at its widest (Fig. 1) (Baptist et al., 2019a). Because all stations lie above the required minimum elevation (Wang and Temmerman, 2013), this implies that elevation is neither a limiting factor for seedling establishment in this study.

Surface soil salinity was significantly lower in the low marsh, which enables the establishment of non-pioneer marsh species (Erfanzadeh et al., 2010c; Veldhuis et al., 2019). As salinity was similar across the vegetated pioneer zone and more seaward zones in this marsh, high soil salinity is probably not an inhibiting factor for vegetation establishment in the transition zone and tidal flats. There was a sharp decline in soil oxygenation from pioneer to transition zone. As pioneer species are usually tolerant towards limited soil oxygenation (Davy et al., 2011), that is not expected to hinder pioneer seedling establishment. Vegetation can transport oxygen into the soil through their root system (Holmer et al., 2002; Lee, 2003), although this is currently contradicted, especially for muddy soils (Koop-Jakobsen et al., 2017). Additionally, adequate drainage proves more determinant in sediment oxygenation than vegetated vs. bare areas (Mossman et al., 2020). Oxygen rich soils can also originate from bioturbation by benthic fauna (Reise, 2002), for example by oligochaetes (van Regteren et al., 2017). In our set-up, it was not possible to discriminate between the effects of elevation and oxygenation (due to collinearity) but this distinction is essential (Davy et al., 2011; Mossman et al., 2020). For example to understand underlying processes such as whether oxygenated soils precede or follow from vegetation establishment. This is an interesting topic for future research.

4.2. Variation in the benthic community and relation to seedling establishment

The second hypothesis was that the benthic fauna of the transition zone would consist of a mixture of intertidal flat and salt marsh species. This hypothesis is rejected as four zones had their own distinct community. The transition zone was dominated by springtails, followed by copepods and oligochaetes in much lower abundance. Mollusca and polychaetes (dominant in the hollows) were practically absent as well as insects (dominating the low marsh). Many benthic groups occurred in one or two specific zone. This may originate from our predefined zonation in the sampling design (Fig. 4). The hypothesis may be better answered using consistent distance sampling intervals from the marsh vegetation edge. As this marsh previously expanded both laterally and horizontally (Baptist et al., 2019b) the distances between zones within each transect differ. Distances between sampling stations are much shorter at the extremities of the marsh (Fig. 1), which may also explain the clustering of some low marsh benthic communities with the pioneer zone (Fig. 3).

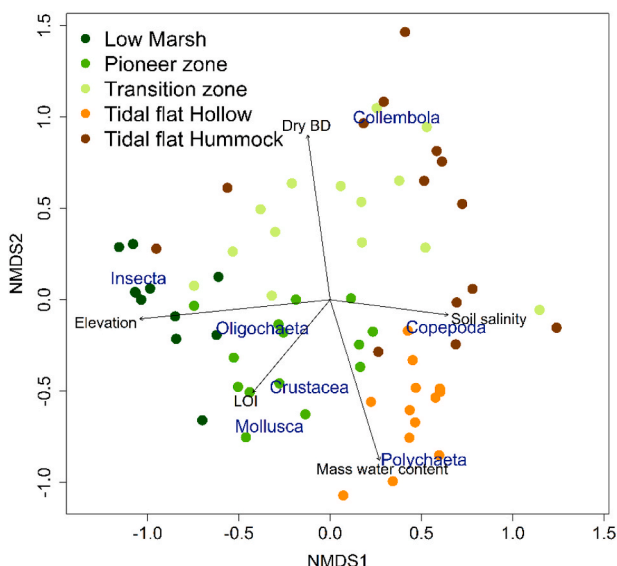


Fig. 6. nMDS ordination plot based on the benthic community of sampling stations, shown with coloured dots for the 5 zones. Dominant benthic groups are displayed in blue and an overlay of environmental variables is added to the graph in black. The stress was 0.146, indicating a good 2d-representation of the data.

Tidal polychaetes were, unsurprisingly, confined to the moistest areas, the tidal flat hollows. Harpacticoid copepods were also abundant in the hollows. Harpacticoid copepods found in muddy environments are well capable of coping with soil anoxia (Grego et al., 2014). Remarkably, they prevailed in the pioneer zone as well, which did not border the tidal flat hollows. Harpacticoids were identified to order level. Because variation in harpacticoid copepods is considerable in Dutch estuaries, up to 21 species are found (van Damme et al., 1984), the two peaks in abundance may originate from different copepod species. Collembolans (*Archisotoma subbrucei*) occurred most frequently in the transition zone and the tidal flat hummocks. This overlap is not surprising, as these zones were also clustered together in terms of abiotic variables (Fig. 3).

Overall, oligochaetes were the most abundant species found, with a clear peak in the pioneer zone. In an earlier study, it was suggested that the increase of oligochaetes at the marsh edge is related to grain size (van der Wal and Herman, 2012) as oligochaetes favour fine sediment (Seys et al., 1999). However, a decline in grain size at the vegetated marsh edge was not observed in our study: the median grain size was similar in all zones (d₅₀ 32–49 µm). The increase in density of oligochaetes in the vegetated area relative to the bare flat has been observed previously (Bouma et al., 2009; Rader, 1984; van der Wal and Herman, 2012; Whaley and Minello, 2002). Possibly, the evasion of predators such as juvenile flatfish, dunlins and common shelducks may explain the lower density of oligochaetes on the tidal flat (Quammen, 1984; Reible et al., 1996; Seys et al., 1999). Additionally, one has to bear in mind that all sampling was performed in May 2016 (spring). Seasonal distribution patterns may occur because oligochaetes can show increased abundances multiple times per year (Chapman and Brinkhurst, 1981). Juvenile oligochaetes did not follow the adult distribution pattern (Fig. 4). Although not fully quantitatively sampled due to their small size, oligochaete eggs were abundant in other areas than juveniles (apart from the pioneer zone). Indicating that juveniles may disperse from the tidal flat towards the vegetated areas.

The benthic community can influence seedling establishment through bioturbation (van Wesenbeeck et al., 2007). The most abundant groups, harpacticoids, collembolans and oligochaetes, have bioturbative capacity (Bonaglia et al., 2014; Christian, 1989; van Regteren et al., 2017). When bioturbating organisms were summed, the highest number of bioturbators, on average 241×10^3 individuals per m², existed in the pioneer zone, coinciding with the area where yearly establishment of *Salicornia* spp. is highest. Suggesting that interference of bioturbation with seedling establishment as shown in lab experiments (van Regteren et al., 2017) may not occur on the marsh scale for small bioturbators. A significant drop was observed in benthic abundance from the pioneer to low marsh zone. A meshwork of roots in the low marsh may inhibit burrowing infauna to successfully colonize this area (Reise, 2002).

Bioturbation affects the biogeochemistry of the soil, increasing fluxes, stimulating soil oxygenation and mineralisation of organic matter content (Bonaglia et al., 2014; Nascimento et al., 2012; Reise, 2002). Well oxygenated soils are favoured for the establishment of non-pioneer marsh species (Davy et al., 2011; Mossman et al., 2012a) and the presence of benthos may aid in creating abiotic conditions appropriate for the vegetation establishment of later successional stages. Additionally, bioturbation may have a positive effect due to the burial and thus retention of seeds (Delefosse and Kristensen, 2012; van Regteren et al., 2019), protecting them from erosive forces (Zhu et al., 2014).

4.3. Seed availability limited seedling establishment in the transition zone

The third hypothesis, that seed abundance will be highest at the low marsh and decreases towards the tidal flat, was confirmed by our data. However, the near absence of seeds (even of pioneer species) from the transition zone was not foreseen. Seed abundance in the low marsh was four times higher than in the pioneer zone while vegetation cover only doubled. Just indicative due to low numbers, *S. anglica* was most

abundant in the pioneer zone whereas 86% of seeds were retrieved in the low marsh. Suggesting that these were transported landwards (Chang et al., 2007; Huiskes et al., 1995) and retained in this zone due to dense vegetation (Chang et al., 2007, 2008; Huiskes et al., 1995). Transport of seeds out of the marsh is lower but also occurs (Huiskes et al., 1995; Zhu et al., 2014), proven by the colonization of bare restoration sites by seed dispersal from nearby marshes (Erfanzadeh et al., 2010a; Mossman et al., 2012a; Wolters et al., 2008). Transport both ways can explain the high seed numbers in the low marsh and a depletion of seeds in the transition zone found in this study, as seeds are either transported further up the marsh or out the marsh system.

Establishment of early pioneer colonizers, such as *S. anglica* and *Salicornia* spp., can initiate marsh formation and thus expansion (Smit van der Waaij et al., 1995). However, seeds of both species were rare, forming a limitation to seedling establishment. Seeds may arrive in the transition zone but subsequently wash away again with the tide or through sediment erosion (van Regteren et al., 2019; Zhu et al., 2014). The establishment of *S. anglica* from seeds in the transition zone is improbable, as *S. anglica* seeds were not present in the seed bank. This is common for this area (van Regteren et al., 2019) as well as for other European marshes (Erfanzadeh et al., 2010b; Wolters and Bakker, 2002). This low abundance of *S. anglica* seeds may partially originate from the fungus *Claviceps purpurea* which invades the reproductive spikelets and prevents germination (Marks and Truscott, 1985). Additionally, *S. anglica* seeds may arrive in the transition zone but subsequently wash away again with the tide or through sediment erosion (van Regteren et al., 2019; Zhu et al., 2014). *S. anglica* is able to expand clonally through rhizomes (Zhu et al., 2014) and this species was present in the pioneer zone. Clonal expansion rates of perennial species are low in exposed sites (Silinski et al., 2016a) and may contribute to marsh expansion in a slow pace. Few seeds of *Salicornia* spp. were found in the transition zone and seedling establishment occurred only sparsely. *Salicornia* spp. is an annual species that relies on seed dispersal for its yearly establishment. Our results suggest that a lack of seeds forms an important limitation for vegetation establishment in the transition zone and thus for marsh expansion.

4.4. Marsh expansion potential

The fourth and last hypothesis states that the transition zone is an appropriate area for seedling establishment due to ameliorated stressful conditions and a seed source close by. This would result in an area suited for salt marsh expansion. Although sparsely vegetated, the abiotic soil conditions in the transition zone were extremely similar to the elevated intertidal flat (hummocks). Seeds were practically absent in the transition zone and the benthic community clustered either with the tidal flat hollow or hummocks. This area lacks a clearly identifiable transition zone based on abiotic and biotic factors.

Despite having the appropriate conditions for vegetation establishment in terms of elevation (Wang and Temmerman, 2013) and a local seed source (Rand, 2000), the marsh has not expanded laterally in the last few years (Baptist et al., 2019a). Unfavourable soil conditions are mentioned as an inhibiting factor for vegetation establishment (Erfanzadeh et al., 2010a; Wolters et al., 2008). Whether abiotic soil conditions have to improve before vegetation can establish or the vegetation itself alters the soil conditions remains an interesting topic for future research. The potential limiting effects of inappropriate windows of opportunity (Balke et al., 2011) or unfavourable abiotic soil conditions (Mossman et al., 2012a; Wolters et al., 2008) will only become apparent when sufficient seeds are available. High and frequent sediment dynamics, occurring multiple times each year the past four years, can cause seed loss after dispersal (Baptist et al., 2019a; van Regteren et al., 2019). Even though a seed source was near, seed availability in the transition zone, evidently, constituted a prominent bottleneck for marsh expansion.

5. Conclusions

The sparsely vegetated transition zone in between the tidal flat and salt marsh has similar physical and biological characteristics as the bare tidal flat zone and is distinctly different from the salt marsh pioneer zone. The dissimilarity is mainly determined by soil oxygenation, the benthic community and seed availability. Our data shows that there is no real transitional zone from intertidal flat to salt marsh, implying that establishment of pioneer plants is the first stage in changing local environmental conditions. Bioturbation of small benthos does not seem to inhibit the establishment of pioneer plants, as the highest density of bioturbators was found in the vegetated pioneer zone. Even though a seed source was in proximity, the main constraining factor for vegetation establishment in the transition zone was the availability of seeds, which inhibited lateral marsh expansion. The growth of the marsh area may be driven by stochastic events of seed retention in the transition zone and therefore lead to stepwise opposed to gradual marsh expansion.

CRediT authorship contribution statement

M. van Regteren: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization, Project administration. **D. Amptmeijer:** Formal analysis, Writing - review & editing, Visualization. **A.V. de Groot:** Methodology, Investigation, Funding acquisition. **M.J. Baptist:** Writing - review & editing, Funding acquisition, Supervision. **K. Elschot:** Writing - review & editing, Supervision.

Declaration of Competing Interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ecss.2020.106909>.

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