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Intertidal mussel reefs change the composition and size distribution of diatoms in the biofilm

Rosyta Andriana¹ · Friederike G. Engel² · Joao B. Gusmao³ · Britas Klemens Eriksson¹

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Abstract

Migrating diatoms are microscopic ecosystem engineering organisms that have functional consequences on the seascape scale by significantly contributing to the microphytobenthos biofilm. The microphytobenthos biofilm is a thin photosynthesising layer that covers the sediment on intertidal flats. It fuels the food web, increases sediment stability, and enhances the deposition of particles, providing ecosystem services to coastal communities. Here we tested the effect of another ecosystem engineering habitat, intertidal blue mussel reefs, on the composition and properties of migrating diatom communities. Small-scale reefs constructed in the intertidal mimicked and reinforced the natural pattern in diatom community composition and function that we documented in the field. The field experiment adding small reefs to the intertidal ran from 30 April to 10 June 2015 and the field samples were collected around a natural blue mussel bed on the same tidal flat on 7 October 2015 (N 53.489°, E 6.230°). Both the constructed small-scale reefs and the natural reef changed the community composition of diatoms in the biofilm by promoting higher numbers of smaller-sized cells and species. Small diatoms have higher growth and gross photosynthesis rates, indicating that this explains the higher production and chlorophyll-a concentration of the biofilm measured on natural intertidal shellfish reefs. Our results showed that shellfish reefs have a large impact on biofilm functioning. However, biofilms are also fuel for the shellfish, indicating that the two very different ecosystem engineers may facilitate coexistence on tidal flats through a positive feedback loop.

Introduction

Marine soft sediment diatoms are microscopic ecosystem engineers that fundamentally alter the function of intertidal flats. They are characterised by two main groups of motile pennate raphid diatoms: epipelonal, which is larger (length of > 10 µm) and moves freely in the muddy sediment, and

epipsammon with smaller size (length < 10 µm) that can be found closely associated with sandy grains (Barnet et al. 2015; Ribeiro et al. 2013). The intertidal diatom community is easily recognised through their significant contribution to a thin-brown photosynthesising layer, the biofilm, that forms on top of the sediment during low tide (Aleem 1950; Kromkamp and Foster 2006). The biofilm consists of a complex matrix of extracellular polymeric substances (EPS) that surrounds the biofilm-associated organisms. The EPS is produced in large quantities by motile diatoms and has an important ecosystem function by binding particles together; stabilising shallow sediment; and influencing sediment transport on the seascape scale (Grant et al. 1986; Vos et al. 1988; Paterson 1989; Hoagland et al. 1993; Malarkey et al. 2015).

Primary productivity of the biofilm on the intertidal flat is a result of hydrodynamic disturbance (regulating sediment erosion), sediment characteristics (organic matter content, grain size), the elevation of the tidal flat (influencing the photoperiod), species composition (influencing pigment composition and growth rates), and vertical migration of the benthic diatoms themselves (Heip et al.

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1995; De Jong and Jonge 1995; Paterson 1986). The diatom biofilm contributes to a significant part of the total primary production on intertidal flats and fuels the benthic food web by supporting higher trophic levels, including blue mussels, oysters, birds, and fish (Admiraal 1984; Underwood and Kromkamp 1999; Christianen et al. 2017).

Intertidal blue mussel reefs (*Mytilus edulis* L.) are themselves important ecosystem engineers that mitigate hydrodynamic disturbance, change sediment properties, and provide habitat for a variety of associated organisms across trophic levels (Widdows et al. 1998, Widdows and Brinsley 2002; Gutiérrez et al. 2003; van der Zee et al. 2012). The engineering effects of blue mussels manifest both within the reefs and on a larger scale; blue mussel reefs modify the surrounding up to several hundred meters across the tidal flat (Donadi et al. 2013a; van de Koppel et al. 2015). The mussels remove suspended particulate matter from the water column and deposit this as nutrient-rich faeces and pseudofaeces (Biodeposit: Verwey 1952; Kautsky 1987; Asmus and Asmus 1991). Biodeposit from bivalves display elevated nutrient and chlorophyll *a* concentrations, indicating that the enrichment of the sediment contributes to an increased diatom production (Giles and Pilditch 2006; Donadi et al. 2013b). In addition, shelter provided by intertidal blue mussel reefs can initiate the development of diatom films in areas where the energy is otherwise too high (Donadi et al. 2013b; Engel et al. 2017). When a biofilm starts to develop in an area, EPS accumulation of the surface of the sediment will bind smaller sediment particles and decrease the sediment erosion threshold, increasing diatom production and organic matter content (van de Koppel et al. 2008). Diatoms grow better in sediment with high organic matter content because of the correlation with low sediment erosion and high nutrient availability (Stal and Brouwer 2003). Van de Koppel et al. (2008) showed that diatoms exude EPS that stabilises the sediment, which further promotes diatom growth and even more EPS production, leading to a positive feedback that promotes a stable and productive biofilm. Similarly, bivalves are favoured by low sediment erosion because this promotes the settlement and establishment of their juveniles (Donadi et al. 2014; van de Koppel et al. 2015). Because of resuspension, the microphytobentos is also an important food-source for blue mussel reefs on tidal flats. Thus, while blue mussel reefs are fuelled by the biofilm, they also facilitate the biofilm, indicating that positive feedbacks between these very dissimilar ecosystem engineers may reinforce each other (Eriksson et al. 2010).

In this study, we tested the effect of intertidal blue mussel reefs on the biodiversity and composition of the migrating diatom community, to increase the understanding of the interaction between bioengineering of the mussels and the

function of the biofilm. In earlier studies, we have observed that the production and chlorophyll-*a* content of the biofilm increase on mussel reefs compared to bare areas on an intertidal flat (Engel et al. 2017). The size of diatoms (measured as biovolume) is inversely related to their growth rates and the chlorophyll-*a* specific absorption coefficient, which is an important property determining gross photosynthesis rates (Geider et al. 1986; Morin et al. 2008). Small diatoms also have a relatively large surface to volume ratio, which allows them to absorb nutrients rapidly and compete successfully in nutrient rich environments (Geider et al. 1986). We therefore hypothesised that the increase in biofilm production observed on mussel reefs (Engel et al. 2017) relates to a shift in diatom community composition towards smaller species and a higher total biovolume caused by an increased number of cells. Changes in the growth rate, composition of species, and size distribution of the diatom community influence EPS production (Smith and Underwood 1998; Underwood and Smith 1998; Brouwer et al. 2005) and therefore should have consequences for the sediment stabilising function of the biofilm.

Intertidal blue mussel reefs often form large aggregations that span 100s of meters, and consist of a thick and elevated mussel matrix interspersed by smaller patches of bare substrate and water-filled pools. Since it is difficult to construct or manipulate such a complex and large structures as natural blue mussel beds in a realistic way, we instead created small plot-sized (1 m²) artificial mussel reefs to causally link the changes in the migrating diatom community to the presence of mussels. Since blue mussel reefs enrich the sediment and decrease sediment erosion, we hypothesised that the small-scale additions of mussels would promote local production and development of the biofilm: generating a biofilm with higher diatom biovolume, with higher diatom abundances, and with a higher fraction of smaller diatom species, compared to plots where we did not add mussels (e.g. Asmus and Asmus 1991; Geider et al. 1986; Giles and Pilditch 2006; Donadi et al. 2013a). However, the small-scale artificial reefs do not mimic the large-scale hydrodynamic effects and complexity of natural blue-mussels reefs (e.g. Donadi et al. 2013a). To make a realistic inference of the results, we therefore compared the results from the small-scale experiment with the natural species composition and size distribution of the migrating diatom community around a large intertidal shellfish reef.

Material and methods

Field experiment

We conducted the field experiment on an intertidal flat in the Netherlands, south of the island of Schiermonnikoog (N 53.489°, E 6.230°, Fig. S1). The intertidal flats in this area are characterised by vast stretches of bare sediment that are occasionally broken up by blue mussel reefs that can cover several hectares. In the past decade, the Pacific oyster (*Crassostera gigas* [Thunberg]) has increased strongly on the blue mussel reefs (Andriana et al. 2019). Today, the older parts of the reefs consist of a mixed matrix of oysters and blue mussels, while the younger parts are still completely dominated by blue mussels. Previous studies have shown that benthic habitat characteristics (e.g. sediment type) vary depending on proximity to shellfish reefs (Donadi et al. 2013a, b). We therefore replicated the experimental set-up in two sites with visibly different sediment character, one on the coastal side of the mussel reef (along field study transect MB, see field study below; site “wake”) and one at the same tidal elevation in the sandy area adjacent to the reef (along field study transect S, see field study below; site “sand”) (Fig. S1). The “wake” site was more sheltered, and the sediment type (mud/silt, high organic matter content; see results) had a characteristic of cohesive sediment (plaster erosion loss: $12.8 \pm 1.3\%$ per tide, $n = 4$; relative shear strength of the sediment: 7.5 ± 2.7 , $n = 7$; mean \pm SD). The “sand” site was more exposed and had a sediment type characteristic of sandy less cohesive sediment (plaster erosion loss: $14.9 \pm 1.2\%$ per tide, $n = 4$; relative shear strength of the sediment: 2.5 ± 2.2 , $n = 7$; mean \pm SD). Erosion was measured as weight loss of model plaster (Knauf Modelgips, Knauf B.V., Utrecht, The Netherlands) moulded in plastic cylinders (6.3 cm long; 2.4 cm diameter) with a pin through them. The pin was inserted in the sediment so that the plaster cylinders were fully exposed to the incoming water. The cylinders were left on the tidal flat for two tidal cycles and then dried in the laboratory until constant weight. We measured the undrained shear strength of the sediment using a handheld shear vane. A shear vane consists of a rod with vanes mounted to one end and a torque gauge in the other end. The vane is inserted in the sediment and slowly rotated until the cohesiveness of the sediment fails, so that the vane rotates in the sediment (Grabowski 2014). However, vanes are normally adapted for soil with high shear strength. We therefore used longer home-made and non-calibrated vanes in all our samplings, which are more suitable for softer intertidal sediment (pers comm Tjeerd Bouma, NIOZ). Thus, we did not transform the shear strength to an absolute unit. The measurements

are therefore limited to show differences in relative cohesion between sites. The measurements were collected across the whole tidal flat on two different occasions in September and October by different persons. We, therefore, min–max normalised the data to be between 0 and 10 to make it comparable.

At each site, we constructed 15 experimental plots with an area of 0.25 m² each (sides 0.5 by 0.5 m), placed in three parallel rows with three meters between each of the plots (to all sides). To six randomly chosen plots at each site, we added live blue mussels (*M. edulis*) (treatment “reef”), while six other plots were left unaltered (treatment “bare flat”). Placing experiments on an intertidal flat may lead to critical artefacts because of changes to water flow caused by equipment rather than treatments, but also because of changed predation rates since the experiment may hinder or attract natural predators. Isolated clumps of blue mussels are highly prone both to crab and bird predation and may quickly be completely removed when placed on the tidal flat. We therefore protected the mussels by constructing two types of experimental controls with different effects on predation and exposure. Half of the plots in both the bare flat and reef treatments had a fence mounted around them consisting of a plastic net (“plastic fence”). The plastic net protects the mussels from both crab and bird predation but may have strong effects on flow. The other half of the plots had a fence consisting of a rope placed around the perimeter that only hinder birds from eating the mussels but have minimal effects on flow (“rope fence”). The ropes were fastened in a 66-cm-long plastic pole marking each corner of the plot, inserted 30 cm into the sediment. In the bare flat treatments, the same types of fences (cage and rope) were used, to have a comparable control to the mussel reef treatment. In addition, three plots at each site were left completely unmanipulated (no addition of mussels and no addition of a fence), to separately test for “fence” effects. We did this by comparing the effects of fence on the diatom community in the plots without mussel additions: thus comparing the “no mussel additions and no fence” treatment (unfenced control) with the “bare flat and plastic fence” and “bare flat and rope fence” treatments, respectively (see Supplement 2). The experiment was set out on the 30 April 2015. We sampled the sediment in the plots to analyse diatom abundance and species composition, chlorophyll-*a* concentrations, and organic matter content, on the 10 June 2015, approximately 1 month after the mussel addition and at the height of biofilm development. Diatom abundance as estimated by chlorophyll-*a* concentrations in the sediment normally peaks in the month of June in the Wadden Sea (Staats et al. 2001; Van der Wal et al. 2010). For more details on the experimental set-up, see Engel et al. 2017.

Field study

We performed the field study by setting up four parallel transects, spanning from the coast seaward, on the same intertidal flat as the field experiment (Fig. S1). The transects were 200 m apart from each other and 400 m long. One transect crossed an intertidal blue-mussel reef (transect MB), two transects crossed the flat 50 m on each side of the shellfish reef (transects ME and MW), and one transect crossed a bare part of the flat 250 m from the shellfish reef (transect S). Previous analyses demonstrate a small spatial variability among sampling points based on their physical locations in relation to the mussel bed (Engel et al. 2017). We therefore divided all sampling points into either “reef front”: sites on the seaside close to the reef (50 m away; $n=3$); “reef wake”: sites in the sheltered wake close to or on the reef (0–75 m; $n=4$); or “bare sand”: sites more than 100 m from the reef with visibly much sandier sediment than the sites closer to the reef ($n=10$). We sampled sediment from each of the 17 transect points on the 7 October 2015; to analyse diatom abundance and species composition, chlorophyll-*a* concentration, and organic matter content (for “Material and methods” see below).

Sediment sampling and sample processing

At each transect point and in each experimental plot, we took sediment cores (diameter: 26 mm) for measuring chlorophyll-*a* concentration and organic matter content in the sediment, as well as to determine benthic diatom composition and biomass. For chlorophyll-*a* and species composition, we pooled the sediment from three cores of 0.2 cm (chlorophyll-*a*) and 2 cm (diatom composition) depth, respectively. The cores were sampled 1 dm from each other in a triangular pattern, and the sediment was collected in a piece of aluminium foil to avoid exposure to light. Organic matter was sampled with a 5-cm-deep core (once in each plot/transect point). All samples were placed in sealed plastic bags and transported back to the laboratory on ice in a cool box.

Further sample processing took place in the laboratory. Chlorophyll-*a* concentration was determined by a method derived from Jeffrey and Humphrey (1975): After freeze-drying the sediment, we extracted the chlorophyll with 90% acetone (dark, -20°C , 48 h) and measured concentration with a fluorometer (Trilogy, Turner Design). We determined organic matter content of the sediment by Loss on Ignition (burning oven dried samples in a muffle kiln at 550°C for 4 h). For diatom species identification and biovolume calculation, the live motile benthic microalgae were extracted from the sediment by spreading out the sediment in petri dishes and placing two layers of lens cleaning tissue on top of the sediment. After 5 h of exposure to light, we removed the top layer of the tissues, which now contained the diatoms

that migrated to the surface. We rinsed the diatoms off the tissues with sterile North Sea water and collected the communities in brown glass bottles. We fixed the samples with Lugol’s iodine solution and stored them in the dark until further processing. To determine species composition and biovolume of the diatoms, we used the Utermöhl technique (Utermöhl 1958) and counted diatom cells under an inverted microscope ($400\times$ magnification). We identified diatom groups to the highest resolution possible, which for some diatoms was the genus level. We calculated species-specific biovolumes based on methods described by Hillebrand et al. (1999), which uses simple geometric shapes to approximate diatom cell volume by measuring multiple dimensions of several cells per species.

Data analyses

We analysed experimental effect on species composition based on both biovolume and cell numbers, using permutational multivariate analysis of variance (PERMANOVA) (analysing distance matrices using the `adonis2` function in the `vegan` package, version 2.5–6; R Core Team 2020; Oksanen et al. 2019). We analysed experimental effect on total biovolume, total cell number, average cell size, and species richness of the diatom community, as well as organic matter content of the top 5 cm of the sediment using analysis of variance (ANOVA). All models contained the fixed explanatory factors mussel addition (“bare flat” or “mussel reef”), type of fence (plastic net or rope), and site (reef “wake” or “sand”). However, we lost two of the fenced replicates at the sandy site creating an unbalanced design. We therefore compared AICc values for the univariate models including different levels of interactions (full factorial model: site \times fence \times mussel addition; two-way factorial model: site \times fence + fence \times mussel addition + site \times mussel addition; main effects model: site + fence + mussel addition) using ANOVA for unbalanced designs (Type III ANOVA using the `car` package in R, version 3.0–10). In the ANOVA analyses of total biovolume, total number of diatom cells, size distribution, and organic matter content, none of the interaction effects were statistically significant and the reduced model without any interactions was consistently the best model according to AICc (lowest AICc values; Table S3). For all univariate models (ANOVAs), except for species richness, we therefore present results from the most reduced model (main effects model). For species richness, we present results from the two-way factorial model, which was the best model according to AICc (Table S3). If needed to fit assumptions of the models, data were sqrt- or log-transformed. We did not analyse experimental effects on chlorophyll-*a* concentrations in the sediment, since these were analysed and presented for a larger subset in Engel et al. (2017).

For the field study, the species composition based on biovolume data was analysed with Nonmetric Multidimensional Scaling (NMDS) using Bray Curtis dissimilarities (monoMDS in the vegan package, version 2.5–6; R Core Team 2020; Oksanen et al. 2019). We tested if there was a difference in community composition between different habitats (“reef front”, “reef wake”, or “sand”) using analysis of similarities (ANOSIM; vegan package, version 2.5–6; R Core Team 2020; Oksanen et al. 2019). The relation between different covariates (total biovolume, cell numbers, average cell size and species richness of the diatom community, chlorophyll-*a* concentration, and organic matter content of the sediment) and the species composition of the diatom community was evaluated using the envfit function, which correlates the covariates onto the ordination axes. To get a visualisation of changes in species composition, we also plotted the weighted averages for each species along the ordination axes, based on the biovolume at each site (species scores). We also tested differences in the covariates between different habitats (“reef front”, “reef wake”, or “sand”) using one-way analysis of variance (ANOVA). If needed to fit assumptions of the models, data were sqrt- or log-transformed.

Results

Field experiment

The experimental blue mussel reefs changed the species composition and size distribution of motile diatoms in the biofilm (Fig. 1). The community included 22 species groups of motile diatoms (Table S4). There was a difference in diatom species composition between the plots with artificial mussel reefs compared to the plots without reefs (“bare flat”), and between the reef wake and sand plain habitats, both for the multivariate analyses based on biovolume and cell numbers (PERMANOVA; biovolume—mussel addition: $F_{1,14}=2.8$, $p=0.036$; site, $F_{1,14}=7.1$, $p<0.001$; cell number—mussel addition: $F_{1,14}=2.8$, $p=0.048$; site: $F_{1,14}=8.0<0.001$; Fig. 1a, b). The change in community composition was mainly caused by the mussels promoting a higher number of diatoms with small cell sizes (biovolume $<51 \mu\text{m}^3$; [CYL, NAV I&II, NITZ I&II]; Table S4; Fig. 1a, b). The PERMANOVAs showed no significant interaction effects ($F_{1,14}<1.5$, $p>0.17$) and no significant effects of different types of fencing around the experimental plots (biovolume—fence: $F_{1,14}=1.6$, $p=0.18$; cell number—fence: $F_{1,14}=1.9$, $p=0.102$). In addition, there were no significant effects of the ropes or the plastic fence compared to the unmanipulated control (“fence” effects; Supplement 2).

The experimental reefs increased the total number of cells and decreased the average cell size of the diatoms

(ANOVA, main effect of mussel additions—total cell number: $F_{1,18}=4.6$, $p=0.046$; average cell size: $F_{1,18}=7.4$, $p=0.014$), but had no significant effects on total biovolume, organic matter content of the sediment, or species richness (ANOVA, main effect of mussel additions—total biovolume: $F_{1,18}=0.7$, $p=0.417$; organic matter content: $F_{1,18}=0.1$, $p=0.768$; species richness: $F_{1,18}=0.7$, $p=0.417$; Table S5, Fig. 1). The total biovolume, total cell numbers, organic matter content of the sediment, and species richness were higher in the wake of the mussel bed compared to the sand plain habitat (ANOVA, main effect of site—total biovolume: $F_{1,18}=11.1$, $p=0.004$; total cell number: $F_{1,18}=17.1$, $p<0.001$; organic matter content: $F_{1,18}=36.7$, $p<0.001$; species richness: $F_{1,18}=30.7$, $p<0.001$; Fig. 1). Site had no effect on average cell size (ANOVA: $F_{1,18}=0.8$, $p=0.373$). The type of fence only had significant effects on species richness (Table S5), where the plastic fence increased species richness in the more sheltered wake of the mussel bed (ANOVA, significant interaction effect between fence type and site: $F_{1,18}=11.0$, $p=0.005$). There were no significant “fence” effects on any of the response variables, comparing different fences to the unmanipulated control (Supplement 2).

Field study

We found a clear difference in the diatom community composition between the sand habitat, and the front and wake of the mussel reef (Fig. 2). According to the analysis of similarities (ANOSIM), the variation in species composition was statistically significantly different between different habitats ($R=0.43$, $p=0.004$; Fig. 2a). *Navicula* spp. dominated the biofilm, but the reef front was additionally characterised by the largest species: 56% of the total biovolume consisted of species with a biovolume $>1300 \mu\text{m}^3$ (*Pleurosigma aestuarii*, *Entomoneis paludosa*, *Stauroneis* spp., and the largest species of *Navicula* [NAF and NAA; Table S4; Figs. 2b, 3a). The reef wake was characterised by high numbers of the smaller species: 57% of the counted individuals had a biovolume $<51 \mu\text{m}^3$ (*Cylindrotheca closterium*, and the smallest species of *Navicula* [NAV I&II] and *Nitzschia* [NITZ I&II]; Figs. 2b, 3b). The sand communities were completely dominated by medium sized *Navicula* species: 92% of the biovolume (NAV III&IV, Fig. 3a). There was a significant correlation between total biovolume, cell size, chlorophyll-*a*, organic matter content of the sediment, and species richness, and the composition of the diatom community along the ordination axes (Fig. 2a) (NDMS, envfit function results: total biovolume: $r^2=0.57$, $p=0.004$; cell size: $r^2=0.53$, $p=0.006$; chlorophyll-*a*: $r^2=0.38$, $p=0.046$; organic matter: $r^2=0.41$, $p=0.031$; species richness: $r^2=0.67$, $p<0.001$; Fig. 2a). Statistical analyses of differences between habitats supported some of the apparent

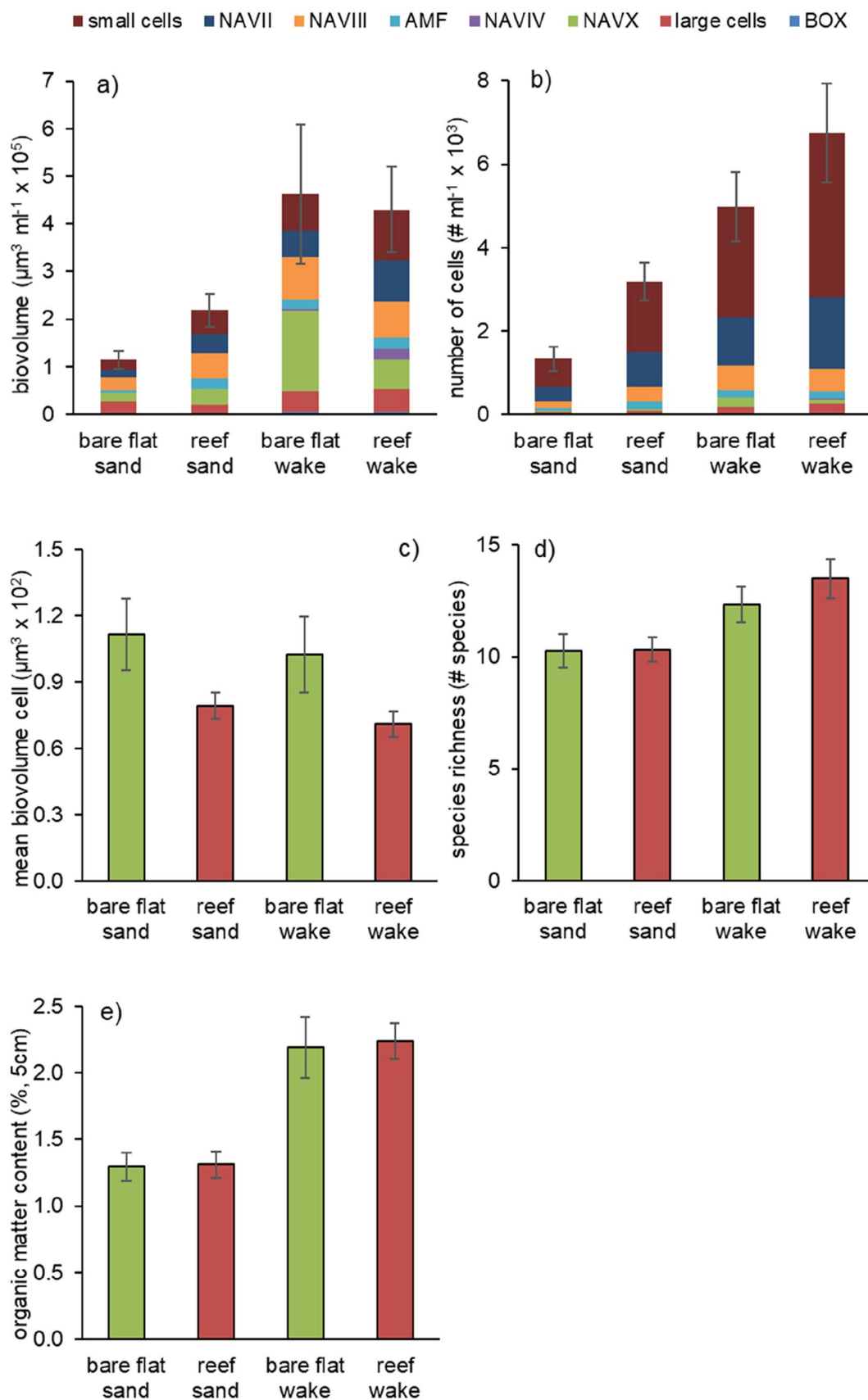


Fig. 1 Experimental effects on diatom community composition and properties of the biofilm by adding small artificial mussel reefs to an intertidal flat (comparing reef vs bare flat), in an exposed sandy habitat (sand) and a more sheltered wake of a blue mussel reef (wake). **a** biovolume of diatoms, **b** number of diatom cells, **c** mean biovolume of the individual cells, **d** species richness of the diatom community, and **e** the organic matter content of the upper 5 cm of the sediment. Error bars show standard error of the mean; in **a** and **b** the bars show SE for the total biovolume and the sum of all diatom cells, respectively. The legend on the top rank the species left to right from smallest to largest size. For abbreviations of species names, see Table S4

relations in the NDMS plot: the average size of the diatom cells and total biovolume was highest in the mussel reef front; the species richness of the diatom film was higher in both reef affected habitats (“reef front” and “reef wake”) compared to the sand flat; and there were no significant differences in total number of diatom cells between habitats (ANOVA: biovolume: $F_{2,14} = 4.2$, $p = 0.038$; cell number: $F_{2,14} = 0.8$, $p = 0.488$; cell size: $F_{2,14} = 5.0$, $p = 0.023$; species richness: $F_{2,14} = 8.8$, $p = 0.003$; Fig. 3). However, contrary to the expectations from the NMDS plot, there were also no significant differences between the habitats for the organic matter or chlorophyll-*a* content of the sediment (ANOVA: chlorophyll-*a*: $F_{2,14} = 1.9$, $p = 0.187$; organic matter content: $F_{2,14} = 1.6$, $p = 0.233$; Fig. 3).

Discussion

We show that mussel reefs can change species composition and the size distribution of the biofilm by promoting higher numbers of small motile diatoms. Constructing small mussel reefs increased diatom cell numbers and decreased the cell size, and this was caused by a strong increase in abundance of a few small species. The field study was sampled later in the season with lower general production and abundance. Still, the general decrease in cell size was apparent when comparing the front to the wake of the mussel reef, and this was caused by a change in species composition where a small diatom species (*Navicula* spp.) was more common in the wake of the reef, and one of the largest diatom species in the area (*Pleurosigma aestuarii*) prevailed in front of the mussel reef. At the same time, species richness and chlorophyll-*a* concentrations in the biofilm in general correlated positively with the species composition of diatoms around the mussel reef. The size of diatoms determines growth and gross photosynthesis rates where smaller cells have a higher productivity (Geider et al. 1986; Morin et al. 2008), and earlier published results showed that the artificial mussel reefs increase chlorophyll-*a* concentrations in the sediment (Engel et al. 2017). Thus, the small reefs mimicked and reinforced parts of the natural pattern in diatom community composition and function that we documented

in the field, indicating that the natural intertidal shellfish reef has a significant impact on the function of the biofilm in the study area. The function of the intertidal biofilm provides large ecosystem services by stabilising the surface of coastal sediments and increasing sedimentation (Grant et al. 1986; Vos et al. 1988; Paterson 1989; Hoagland et al. 1993; Malarkey et al. 2015); improving water quality by binding toxic metals and pathogens (Decho 2000); contributing to coastal production (Colijn and de Jonge 1984) and sustaining the intertidal food-web (Christiaan et al. 2017). This highlights the importance of intertidal shellfish reefs for intertidal ecosystems, supporting ecosystem functions and services for coastal populations by promoting the biofilm (Widdows and Brinsley 2002; van der Zee et al. 2012; Engel et al. 2017; Eriksson et al. 2017).

The experimental reefs were very small compared to natural reefs, and it is not clear how the small-scale experimental effects compare to the natural large-scale effects that mussel reefs have on their surroundings. Natural mussel reefs stabilise the sediment up to 100 m in the wake of their physical structures (Donadi et al. 2013a, b; van de Koppel et al. 2015). However, the species composition changed correspondingly when comparing small- and large-scale effects in the experiment: the artificial mussel reefs promoted smaller species, and the species were smaller in the wake of the mussel reef than on the sandy plain. Thus, the small-scale effect of the artificial mussel reef was similar to the large-scale effect of the natural mussel reef. The diatom community was, in general, dominated by small *Navicula* species, which is common for intertidal sediments in the Wadden Sea (Janssen et al. 1999). The sheltered wake of the mussel reef with less sediment erosion and more cohesive sediment was characterised by smaller species of diatoms (length < 10 µm) (Sabbe 1997), such as *Nitzschia* spp., *Cylindrotheca closterium*, and the smallest *Navicula* species. The sandy area, with high sediment erosion and more non-cohesive sediment, and the front of the mussel bed with strong hydrodynamic conditions, was characterised by larger species (mainly epipelon: length of > 10 µm), such as *Pleurosigma aestuarii*, *Entomoneis paludosa*, *Stauroneis* spp., and the largest species of *Navicula*. Earlier, field studies have highlighted the importance of sediment erosion for diatom community composition, suggesting that the adhesion capacity of the diatoms themselves and the sediment sorting (grain size) by hydrodynamic conditions determine the diatom assemblage structure (e.g. Colijn and Dijkema 1981; Sundbäck 1983; De Jonge 1985; Sabbe and Vyverman 1991; Ribeiro et al. 2013). Diatom cell size affects attachment capacity, and exposure to wave action determines the size distribution of periphyton diatom communities on hard substrates (Busse and Snoeijs 2003). Accordingly, Delgado et al. (1991) showed that large diatom species were favoured in

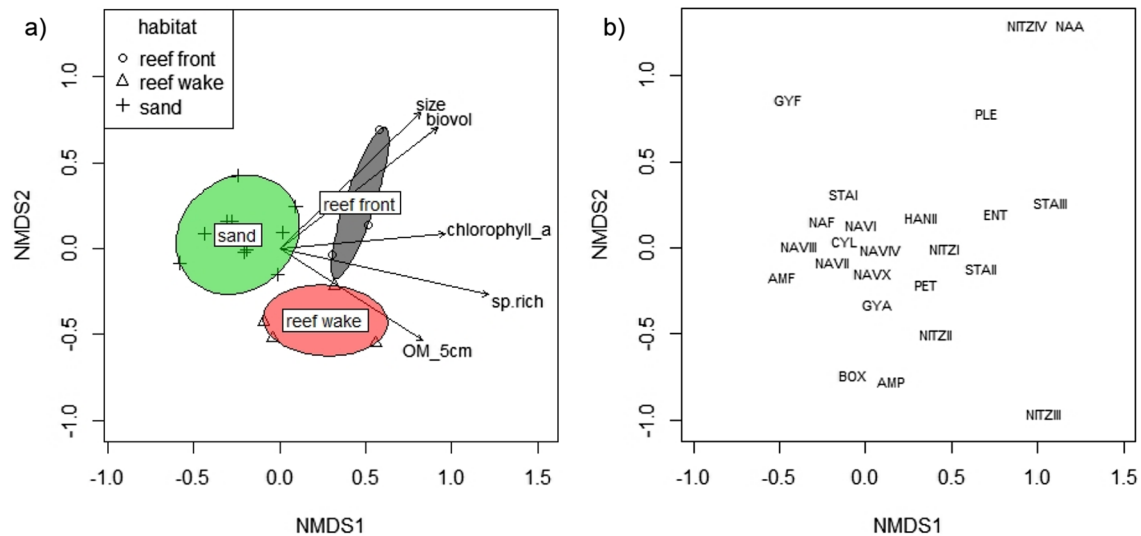


Fig. 2 Nonmetric Multidimensional Scaling (NMDS) results showing the **a** site and **b** species scores for the two first ordination axes. The ellipses are ellipsoid hulls that enclose all site scores of each habitat type (green: sand, red: wake of mussel reef, grey: front of mussel reef). The arrows show significant correlations with environmental and biological variables ($p < 0.05$). Abbreviations arrows: *size* average size of cells in the sample, *biovol* total biovolume of the sample,

chlorophyll-a chlorophyll-*a* content in the top 2 mm of sediment, *OM_5* organic matter content of top 5 cm of sediment, *sp. rich* number of species in the sample. The species scores are the weighted average of the site scores for each species along the ordination axes. The weighted average is constructed by weighing the biovolume of each species to corresponding value on the NMDS axes for each site. For abbreviations of species names, see Table S4

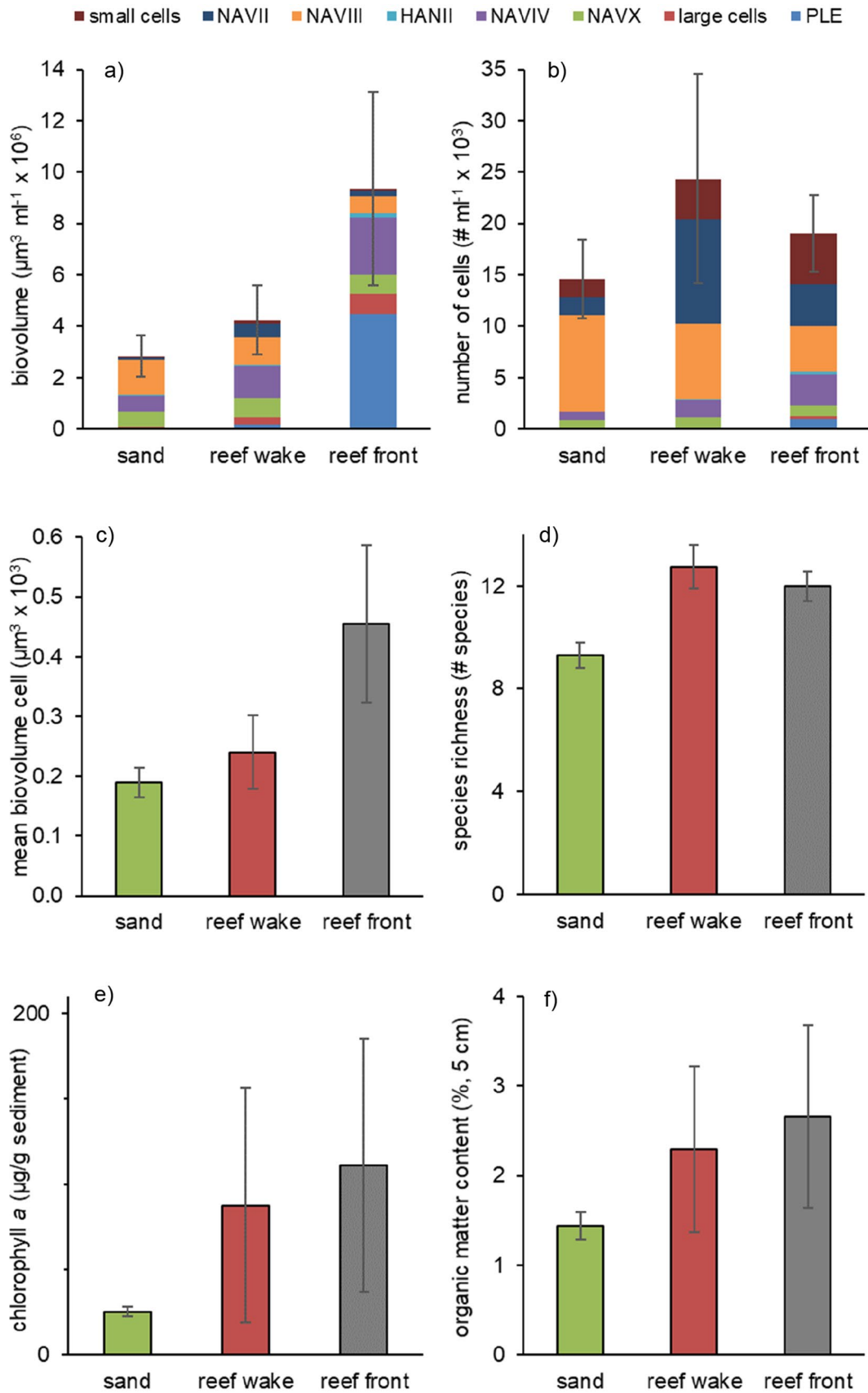
exposed sandy substrates with strong hydrodynamic conditions, suggesting they were less vulnerable in a harsh sedimentary environment. Our study indicates that the physical protection against wave exposure provided by the formation of shellfish reefs creates a habitat favouring small motile benthic diatoms sensitive to sediment erosion.

The vertical migrating behaviour of the pennate diatom community that moves around in the top layer of the sediment is characteristic for intertidal flats (Colijn and De Jonge 1984; de Jonge 1985). The migrating behaviour is also important for the development of the microphytobenthos biofilm. Motility is linked to the occurrence of extracellular polymeric substances (EPS), which is exuded from their raphe, a slit in their silicate exterior (frustule) (Lind et al. 1997). The migration of diatoms is strongly promoted by light, and increasing light leads to an accumulation of diatom cells on the sediment surface (Sauer et al. 2002). The EPS exuded by migratory diatoms contributes significantly to the biofilm matrix (Stal and Brouwer 2003). There is a negative correlation between grain size and the amount of EPS in the sediment (Brouwer et al. 2003), and diatom biomass is, in general, higher in cohesive than in non-cohesive sediment (in cohesive sediment, the grain size diameter is less than 63 μm) (De Brouwer and Stal 2001; Kromkamp and Foster 2006); indicating that biofilms probably can only develop in sediment characterised by low energy (Stal and Brouwer 2003). This further supports the hypothesis that it is the sediment stabilising effect that enhanced

the biofilm development on the artificial and natural shellfish reefs in our study, shown by an increasing number of diatom cells or biovolume in the sediment.

The interaction between the biofilm and shellfish reefs indicates the importance of facilitative processes between individual organisms and organism groups in harsh environments with high abiotic stress, such as tidal flats (Maestre et al. 2009; Eriksson et al. 2010). By ameliorating hydrodynamic disturbance, blue mussel reefs facilitate the biofilm and thereby create regions with high microphytobenthos biomass and primary production (Donadi et al. 2013a, b; Engel et al. 2017; Eriksson et al. 2017, this paper). At the same time, because of resuspension, the microphytobenthos play a major part in benthic–pelagic coupling and contribute strongly to the production in estuaries and shallow coasts (Underwood and Kromkamp 1999). As such, the biofilm is important both for deposit and suspension feeding organisms (Jonge and Beusekom 1995; Ubertaini et al. 2012), and an important food-source for the blue

Fig. 3 Composition of motile diatom species in samples collected from a sand flat, the wake of a mussel reef and the front of the same mussel reef, based on **a** biovolume, **b** number of individuals, **c** mean biovolume of the individual cells, **d** species richness of the diatom community, **e** chlorophyll-*a* concentrations in the upper 2 mm of the sediment, and **f** the organic matter content of the upper 5 cm of the sediment. Error bars show standard error of the mean; in **a** and **b** the bars show SE for the total biovolume and the sum of all diatom cells, respectively. The legend on the top rank the species left to right from smallest to largest size. For abbreviations of species names, see Table S4



mussel reefs. Thus, our results strongly suggest that by initiating small-scale diatom sediment feedbacks, intertidal blue mussel reefs create areas of high production and biodiversity that they themselves are favoured by, potentially promoting also larger-scale self-reinforcing feedbacks.

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Compliance with ethical standards

Conflict of interest The authors have no conflicts of interests or competing interests to declare.

Ethical standards This research was conducted in accordance with the Netherlands Code of Conduct for Scientific Practice. All applicable international, national, and institutional guidelines for sampling, care, and experimental use of organisms for the study have been followed.

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