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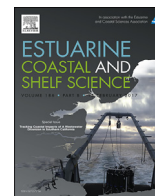
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## Mussel beds are biological power stations on intertidal flats



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### ABSTRACT

Intertidal flats are highly productive areas that support large numbers of invertebrates, fish, and birds. Benthic diatoms are essential for the function of tidal flats. They fuel the benthic food web by forming a thin photosynthesizing compartment in the top-layer of the sediment that stretches over the vast sediment flats during low tide. However, the abundance and function of the diatom film is not homogeneously distributed. Recently, we have realized the importance of bivalve reefs for structuring intertidal ecosystems; by creating structures on the intertidal flats they provide habitat, reduce hydrodynamic stress and modify the surrounding sediment conditions, which promote the abundance of associated organisms. Accordingly, field studies show that high chlorophyll *a* concentration in the sediment co-vary with the presence of mussel beds. Here we present conclusive evidence by a manipulative experiment that mussels increase the local biomass of benthic microalgae; and relate this to increasing biomass of microalgae as well as productivity of the biofilm across a nearby mussel bed. Our results show that the ecosystem engineering properties of mussel beds transform them into hot spots for primary production on tidal flats, highlighting the importance of biological control of sedimentary systems.

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### 1. Introduction

Benthic microalgae are important primary producers in intertidal soft-sediment habitats where they contribute up to 50% of total primary production (Underwood and Kromkamp, 1999). In these highly productive areas that have a great ecological and economical value across the globe (Heip et al., 1995), benthic microalgae fuel the benthic food web by forming extensive biofilms that support a vast array of organisms (Decho, 2000; Stal, 2003; Kromkamp et al., 2006; Markert et al., 2013; Rigolet et al., 2014). Resource availability and grazing play important roles in regulating benthic microalgae (Underwood and Kromkamp, 1999; Weerman et al., 2011a, 2011b). However, on tidal flats, large-scale heterogeneity in the abundance and productivity of benthic microalgae is commonly attributed to abiotic conditions, where increasing hydrodynamic stress decrease benthic microalgae biomass by resuspension of the sediment (de Jonge and van Beusekom, 1995; van

der Wal et al., 2010). Recently, we have recognized the importance of biological control over local hydrodynamic conditions on intertidal flats (van der Zee et al., 2012; Donadi et al., 2013a) and shown that the high abundances of benthic microalgae correlate strongly with the occurrence of mussel beds (Donadi et al., 2013b; Nieuwhof et al., 2016 personal communication).

Organisms that modify their habitats can facilitate complex food-webs by providing structural complexity and improving environmental conditions for many organisms (Olf et al., 2009; Kéfi et al., 2015; van der Zee et al., 2016). On tidal flats, above-ground aggregations of bivalves such as mussels or oysters can build extensive habitat-forming reefs (e.g. mussel beds). These structures are of fundamental importance for biological control of ecosystem structure and properties (Commito et al., 2008; Gutiérrez et al., 2011; van der Zee et al., 2012; Donadi et al., 2013a, 2015). By creating large emergent structures in the otherwise predominantly flat and soft-bottomed landscape, bivalve reefs generate habitat for many other species that live in or on the sediment (van der Zee et al., 2012; Nieuwhof et al., 2015). The reefs physically protect the surface sediment against erosion and resuspension, and furthermore increase organic matter content via

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suspension feeding and biodeposition (Widdows and Brinsley, 2002). The habitat modifying properties and ecosystem effects extend up to several hundred meters around reefs (van der Zee et al., 2012; Donadi et al., 2013a, 2013b, 2015; van de Koppel et al., 2015), which is reflected by a conspicuous increase of benthic microalgae biomass in the vicinity of intertidal mussel beds (Donadi et al., 2013b). Due to these impacts on a large spatial scale, it can be assumed that the interaction between benthic microalgae and intertidal bivalve reefs contribute significantly to coastal production. However, the assumed regulatory importance of bivalve reefs for microalgae biomass is based on observational data and statistical modelling only, while the causal link of (living) bivalves facilitating benthic diatoms have not been extensively examined. Consequently, we lack conclusive empirical evidence of the facilitation effect. In addition, due to limited measurements of actual productivity, we have a poor understanding of how the increased biomass of microalgae (commonly estimated by chlorophyll *a* concentration) around reefs relate to productivity of the system.

In this study, we tested the hypothesis that mussel beds increase the local biomass of benthic microalgae on a tidal flat. First, we showed that the biomass of benthic microalgae was consistently elevated across a mussel bed over several years and related this to higher primary productivity. We then used empirical evidence from a small-scale field experiment to demonstrate that the addition of live mussels to bare plots facilitates benthic microalgae.

## 2. Material and methods

### 2.1. Set-up transects

We set up two parallel transects spanning a distance of 1 km each on a tidal flat south of the island Schiermonnikoog (latitude 53.47° N, longitude 6.23° E, Friesland, The Netherlands; Fig. A.1a–b; Table A.1). This tidal flat is a mudflat with varying sediment grain types ranging from fine mud to sand. During low tide, the flat falls completely dry and the tidal range is about 3.5 m. The two transects were 300 m apart and perpendicular to the coast. One transect crossed a *Mytilus edulis* (blue mussel) reef that was ca. 100 m wide and extended for approximately 250 m along the coast; the other one was in a habitat without mussels present. The mussel bed is elevated and exhibits spatial self-organization on two scales: (1) a banded pattern with mussels on top of several meter large hummocks of accumulated sediment and small pools of 1–2 m in diameter, that are void of mussels and retain water during low tide (Liu et al., 2012), and (2) a labyrinth-like banded pattern of small mussel clusters that aggregate on the 5–10 cm scale (van de Koppel et al., 2008), but that changes into a thick homogenous cover of mussels at peak densities on the hummocks. We established the first point of each transect 350 m coastward of the mussel bed (about 500 m from the shore) and placed subsequent points every 50 m in seaward direction up to 100 m behind the mussel bed (last point ca. 1000 m from the shore). The transect points were selected to cover a visible plume of muddy sediment that extended around the mussel bed.

In June 2012, we sampled chlorophyll *a* concentration at six transect points in both transects (–300 m, –200 m, –100 m, 0 m, +100 m, +150 m distance to the mussel bed/the corresponding tidal elevation in the no mussel bed habitat, where negative values mean distances coastward of the mussel bed/the corresponding tidal elevation in the no mussel bed habitat and positive distances seaward of the mussel bed/the corresponding tidal elevation in the no mussel bed habitat). Distance to the mussel bed is hereafter referred to collectively as distance to the mussel bed in both habitats. Sampling was replicated spatially, by including samples 50 m to the right and 50 m to the left of each

transect point (N = 36). In 2015–16, we took chlorophyll *a* and organic matter samples at five similar transect points in both transects (–350 m, –200 m, –100 m, 0 m, +100 m distance to the mussel bed, where negative distances are coastward and positive distances are seaward of the mussel bed), but instead of two spatial replicates we repeated the sampling six times in total (October 2015, October 2016, April 19, 2016, April 29, 2016, May 2016, June 2016). Due to unexpected weather conditions, we could not sample the two last transect points for the no mussel bed habitat in October 2015 (0 m, +100 m) and had to abandon the last sampling point (+100 m) in both habitats in April 2016 (N = 56).

We measured photosynthetic yield of the sediment as proxy for benthic microalgae productivity at two different time points. In June 2012 and June 2016, we took triplicate samples in five transect points per habitat.

### 2.2. Set-up field experiment

We designed an experiment to analyze the local effects of mussel presence on benthic microalgae in small-scale plots of 0.5 m<sup>2</sup> (Fig. A.2a). Thus, we did not simulate the hierarchical spatial structure of intertidal mussel beds (as described above; see also Snover and Commito, 1998; Kostylev and Erlandsson, 2001; Commito et al., 2006), or their long-range effects (Donadi et al., 2013a, 2013b; van de Koppel et al., 2015). Our experiment allowed us to assess the effect of mussels at the plot scale, avoiding possible confounding effects that different environmental factors could have when including multiple spatial scales in the experimental design (Wiens, 1989; Commito et al., 2006).

We set up the mussel facilitation experiment in three different sites on the same tidal flat as the transects, south of Schiermonnikoog island (Fig. A.1a, c). The three sites were placed at the same tidal elevation, meaning that all plots fell dry at the same time during low tide, but along a gradient of influence by mussel beds which also means that they differ in sediment erosion, organic matter content and infauna community composition (van der Zee et al., 2012; Donadi et al., 2013b, 2015). Site 1 was placed in the transect without a mussel bed; Site 2 coastward of the mussel bed included in the mussel bed transect (300 m to the east of Site 1); and Site 3 coastward of another larger mussel bed that is 100–200 m wide and extends almost 1000 m along the coast (2000 m east of Site 1; Fig. A.1a).

In each site, we tested the effects of adding mussels on benthic microalgae biomass. For this, we prepared four different treatments with three replicates in each site, leading to 36 experimental units (plots) in total. Each individual plot had an area of 0.25 m<sup>2</sup> (plot dimension: 0.5 m by 0.5 m) and each corner of the plot area was marked with a plastic pole. The poles were 66 cm long and inserted about 30 cm deep into the sediment. The distance between the plots was 5 m on each side.

The experiment combined two mussel addition treatments and two controls in a factorial design with: a fenced control (FC; Fig. A.2b), a fenced mussel addition treatment (FM; Fig. A.2c), a semi-caged control (CC; Fig. A.2d), and a semi-caged mussel addition treatment (CM; Fig. A.2e). For the two mussel addition treatments (FM, CM), we collected live *Mytilus edulis* and distributed them evenly in the plots so that the surface of the entire plot was covered. After the addition, the mussels organized themselves in the plots overnight by creating a spatial pattern of 5–10 cm banded aggregations (Fig. A.2e). Placing experiments on an intertidal flat may lead to critical artifacts 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. This is critical when placing bivalves on the tidal flat, since they become islands of food for both birds and crabs that

quickly consume the treatments. We therefore constructed two types of experimental controls: one semi-caged control (CC) that excluded both crab and bird predation but may have strong effects on flow attenuation; and one fenced control that only excluded bird predation but with minimal effects on flow. The semi-caged control consisted of a coarse plastic coated metal net that was wrapped around the marking poles. The net (mesh size: 1.2 cm) was placed directly on the seafloor with no space below it and had a height of 25 cm. The fenced control (FC) had a string attached to the poles at about 25 cm height, wrapping around the plots as protection against predation from birds (van Gils et al., 2012). The distribution of the plots was randomized within sites. Deployment of all plots disturbed the sediment in the same way, as all treatments had the same basic built with the four plastic poles in each corner. The experimental plots were set-up in three rows perpendicular to the tidal currents; the most seaward row of treatments was hit by the tides first in all three sites (Fig. A.1a,c, Fig. A.2a).

To test for experimental artifacts, we also included a control without a fence that was only marked in the corners of the plot (no string between the poles; randomized among the other treatments at each site;  $N = 9$ ; Appendix B).

Deployment of the experimental plots took place in the end of April 2015. After more than a month, in June 2015, we took chlorophyll *a* and organic matter samples in all experimental plots. We also collected data on hydrological conditions by using dissolution plasters as proxy for hydrodynamic stress (for methods see Donadi et al., 2013b). The dissolution plasters are cylindrical plaster molds which we expose to the hydrodynamic conditions of the study area during high tide and then estimate erosion by calculating the relative plaster weight loss. We deployed two dissolution plasters in each experimental plot for two tidal cycles before the final sampling.

Due to a severe storm in the end of May 2015, we lost three replicates of the semi-caged control (CC; two in Site 1 and one in Site 3), so that the total  $N = 6$  for this treatment.

### 2.3. Sampling and analysis

Chlorophyll *a* concentration in the sediment was measured as proxy for benthic microalgae biomass. For the chlorophyll *a* samples we collected three cores (diameter: 26 mm, depth: 2 mm) in the respective transect points. We pooled the sediment from all three cores and wrapped them onto a  $10 \times 10$  cm piece of aluminum foil to prevent exposure to light. The sediment in the foil was placed into small labeled plastic bags which were sealed and stored on ice in the dark immediately after collection. The samples were transported in cool boxes to the laboratory (<24 h). Samples were taken within a few hours on the same day in all plots. Once returned to the lab, we freeze-dried the samples and determined chlorophyll *a* content by acetone extraction (90%, dark,  $-20$  °C, 48 h) and methods described by Jeffrey and Humphrey (1975). The 2012 chlorophyll *a* concentration was measured with a spectrophotometer, whereas the 2015–16 samples were measured with a fluorometer.

For the organic matter samples, we took one sediment core (diameter: 2.6 cm, depth: 5 cm) at each transect point and in each experimental plot. The samples were placed into labeled plastic bags and stored in cool boxes on ice until they were transported to the laboratory. Upon return to the laboratory, the samples were frozen at  $-20$  °C until further processing. Organic matter content was measured by Loss on Ignition (LOI; 4 h, 550 °C) of oven dried (48 h, 60 °C) samples.

The dissolution plasters were collected after exposure to two tidal cycles and allowed to dry in air for a week. The relative plaster loss was then calculated by subtracting the dry weight of the

plaster after exposure to the tides from the dry weight before exposure. Submersion time in the different plots only varied marginally, therefore no standardization was deemed necessary.

Photosynthetic yield was measured as the maximum quantum yield of photosystem II with a Pulse Amplified Modulation fluorometer (Mini-PAM, Walz) as a proxy for benthic microalgae productivity. To avoid differences caused by different light conditions and changes in weather and timing of the tide, we collected triplicate samples of sediment at each transect point and transported them back to the lab (some were lost in 2012, reducing replication to duplicates at some points,  $N = 30$ ). The samples were placed in petri dishes which were randomly distributed on the same shelf in a climate room and allowed to acclimatize for half a day (light level:  $11 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ; temperature: 16 °C). The photosynthetic yield was then measured using the PAM after 30 min of dark adaptation. The PAM sensor was fixed 10 mm above the sediment.

### 2.4. Statistical analysis

All statistical analyses were performed and graphs were created in R v 3.3.1 (R Core Team, 2017). If necessary, the data were log-transformed to meet the assumptions of homogeneity of variances and normality of data distribution.

#### 2.4.1. Transects

Since the methods for the transect chlorophyll *a* data varied slightly between 2012 and 2015–16, we analyzed them separately. 2012 transect chlorophyll *a* concentration did not meet the assumption of homogeneity of variances, even after data transformation. Therefore, we analyzed differences in chlorophyll *a* concentration between the habitat types (mussel bed, no mussel bed) using a Kruskal-Wallis rank sum tests. We then performed two separate Spearman rank correlations for each habitat type, testing if chlorophyll *a* concentration changed with distance to the mussel bed (300 m, 200 m, 150 m, 100 m, 0 m). We averaged all three transects per habitat for the analysis. For the 2015–16 transect data of chlorophyll *a* and organic matter content, we performed analyses of covariance (ANCOVA) considering the factor habitat type (mussel bed, no mussel bed) and the covariable distance to the mussel bed (Table C.1 and Table E.1). For the analyses, we used data from all the six different sampling times in 2015–16. We pooled the 2012 and 2016 PAM data for the analysis of photosynthetic yield, because lost samples of 2012 led to poor replication. We performed an ANCOVA, considering year (2012, 2016) and habitat type (mussel bed, no mussel bed) as the two factors and distance to the mussel bed as the covariable (Table C.1). We used the triplicates per sampling point.

#### 2.4.2. Field experiment

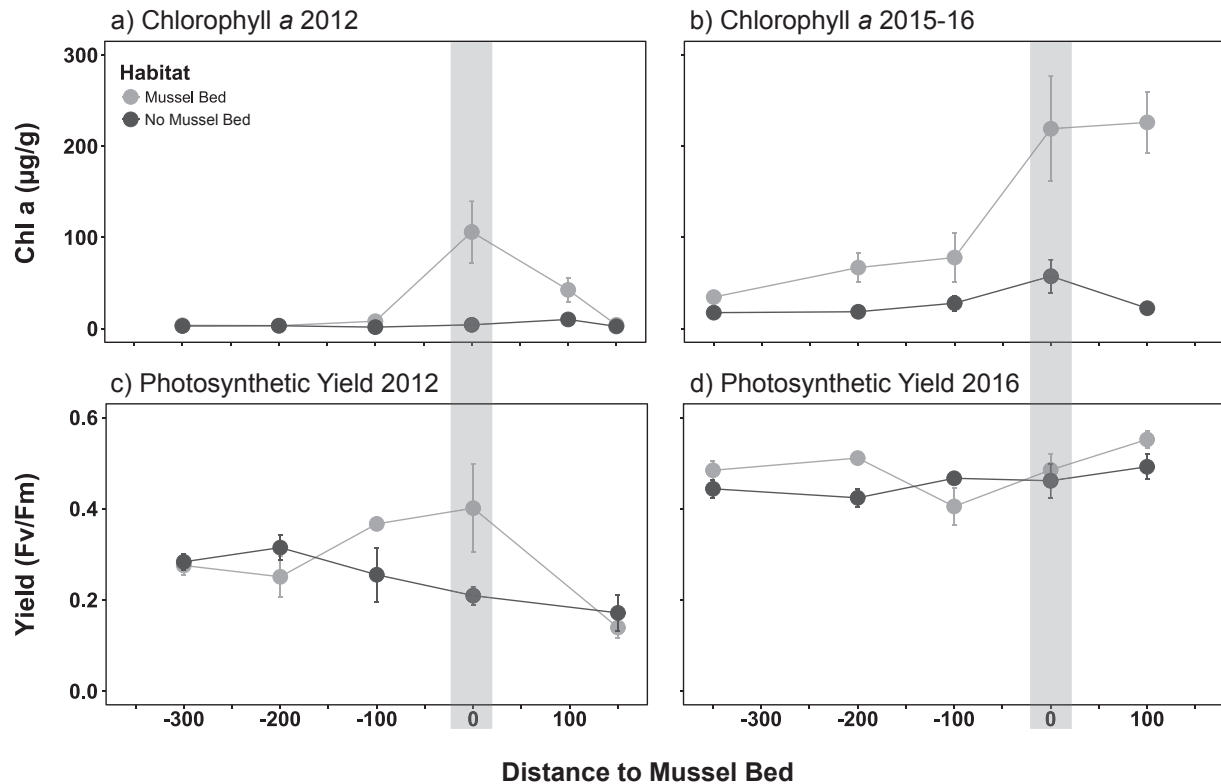
For the experiment, we analyzed the effect of the two different controls and mussel addition treatments on chlorophyll *a* concentration, organic matter content and hydrodynamic stress (plaster loss) using a fully crossed three-factorial ANOVA with site, cage and mussel addition as fixed factors.

## 3. Results

### 3.1. Transect results

The mussel bed increased benthic microalgae biomass and productivity across the intertidal flat; both in the 2012 and the 2015–16 samplings (Fig. 1a–d).

On average, the chlorophyll *a* concentration was significantly higher in the mussel bed habitat compared to the no mussel bed habitat in both sampling periods (Fig. 1a–b; 2012:  $\chi^2 = 9.62$ ,  $df = 1$ ,



**Fig. 1.** a) Chlorophyll *a* concentration 2012, b) chlorophyll *a* concentration 2015–16, c) photosynthetic yield 2012, and d) photosynthetic yield 2016 in the transects. Negative distances to the mussel bed indicate positions coastward of the mussel bed, whereas positive values indicate a position seaward of the mussel bed. In the no mussel bed habitat, the distances represent similar tidal elevations as in the mussel bed habitat. The gray circles represent averages of transects in the mussel bed habitat and the black circles represent averages of transects in the no mussel bed habitat. Error bars denote standard errors of the mean.

$p = 0.002$ ; 2015–16:  $F_{1,52} = 25.55$ ,  $p < 0.01$ , Table C.1). In 2012, the chlorophyll *a* concentration significantly increased with increasing proximity to the mussel bed in the mussel bed transect (Fig. 1a;  $n = 18$ , Spearman  $R = 0.82$ ;  $t = 5.8$ ,  $p < 0.001$ ). In the no mussel bed habitat in 2012, there was no similar increase of chlorophyll *a* with distance to the tidal level of the mussel bed (Fig. 1b;  $n = 18$ , Spearman  $R = 0.24$ ;  $t = 0.97$ ,  $p = 0.35$ ). The increase in benthic microalgae biomass directly on the mussel bed was on average more than 25 times stronger in the mussel bed transect compared to points at the same tidal elevation in the habitat without a mussel bed (2012; Fig. 1a). In 2015–16, the chlorophyll *a* concentration significantly increased with increasing proximity to the mussel bed and corresponding tidal level in both transects (Fig. 1b; significant main effect of distance:  $F_{1,52} = 21.12$ ,  $p < 0.01$ , Table C.1). The interaction between habitat type and distance to the mussel bed was statistically not significant (2015–16:  $F_{1,52} = 1.76$ ,  $p = 0.19$ , Table C.1), but the increase was nearly four times stronger in the mussel bed transect compared to points at the same tidal elevation in the habitat without a mussel bed (Fig. 1b).

The effect of the mussel bed on photosynthetic yield depended on the year (Fig. 1c–d), as we detected a significant interaction between year, habitat type and distance to mussel bed ( $F_{1,50} = 5.10$ ,  $p = 0.03$ , Table C.2). In 2012, the photosynthetic yield decreased gradually in seaward direction across the transect without a mussel bed while it increased strongly with proximity to the mussel bed in the mussel bed habitat (Fig. 1c). In contrast, in 2016 the photosynthetic yield increased gradually in seaward direction across the no mussel bed transect (Fig. 1d). The yield in the mussel bed transect in 2016 was in general higher than in the no mussel bed transect, but had an abrupt minimum 100 m coastward of the

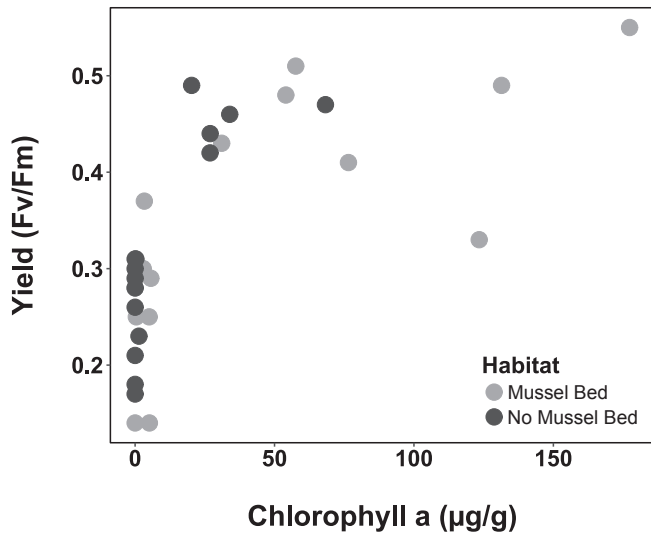
mussel bed, where the yield was even lower than in the no mussel bed habitat at a comparable tidal elevation (Fig. 1d). This area was characterized by the presence of macrofaunal structures (tube worms) that covered the entire area and possibly disturbed the biofilm. Overall, photosynthetic yield was higher in 2016 compared to 2012 (Fig. 1c–d; Year:  $F_{1,50} = 36.41$ ,  $p < 0.01$ , Table C.2) and there was a marginal trend towards an average higher yield in the mussel bed habitat in both years (Habitat type:  $F_{1,50} = 3.30$ ,  $p = 0.08$ , Table C.2). The highest yield overall was recorded in the mussel bed habitat for both years: in 2012 the highest yield was measured directly on the mussel bed (Fig. 1c); whereas in 2016 it was measured 100 m seaward of the mussel bed (Fig. 1d).

The positive effect of the mussel bed on both biomass and productivity of the benthic microalgae resulted in a strong correlation between chlorophyll *a* concentration and photosynthetic yield across the transects in June 2012 and June 2016 (including all matching chlorophyll *a* and yield measurements sampled from the same transect points in June 2012 and June 2016: Spearman rank correlation  $\rho = 0.76$ ,  $N = 30$ ,  $p < 0.05$ ; Fig. 2).

### 3.2. Results field experiment

The mussel additions doubled chlorophyll *a* concentration in the sediment (Fig. 3a; Table 1). There was an indication of a stronger effect in the caged treatments, but there was no significant effect of caging, and no interaction effect between caging and mussel addition (Fig. 3a; Table 1). The mussel additions had no significant effects on organic matter content or plaster loss (Fig. 3b–c; Table 1). There was a marginal trend for the cage treatment to decrease plaster loss (Fig. 3c; Table 1), indicating that the cages decreased





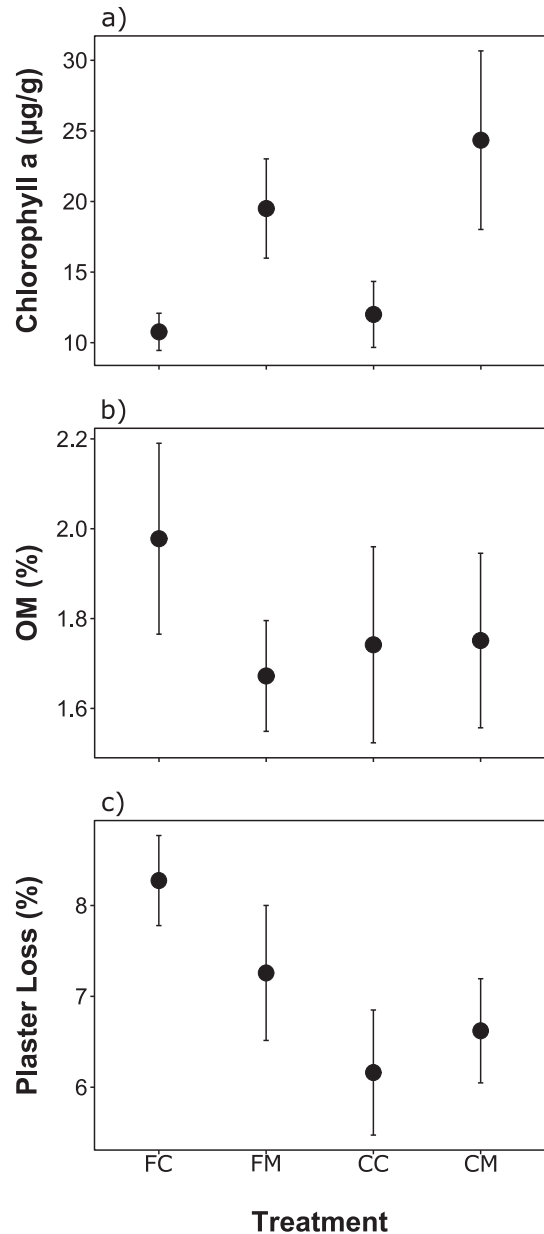
**Fig. 2.** Correlation between photosynthetic yield and chlorophyll *a* concentration for both years (2012 and 2016). The gray circles represent data points in the mussel bed habitat, whereas the black points represent data points in the habitat without a mussel bed. We show data for all transect points that had corresponding chlorophyll *a* and yield measurements (N = 30).

hydrodynamic stress in a way that the mussels alone did not.

The chlorophyll *a* concentration of the sediment was higher at site 2 coastward of the mussel bed, compared to site 1, without a mussel bed (Fig. D.1a; Tukey HSD post hoc test: site 2 > site 1,  $p < 0.05$ ); but there was no interaction between the experimental treatments and site, demonstrating that mussels increased chlorophyll *a* concentration across habitat types (Table 1). The organic matter content was highest at site 2 (behind the smaller mussel bed) and lowest at site 1, without a mussel bed (Fig. D.1b; Tukey HSD post hoc test: site 2 > site 3 > site 1,  $p < 0.05$ ). There was a non-significant trend for decreased hydrodynamic stress at site 3 compared to the other sites (Fig. D.1c).

#### 4. Discussion

We demonstrate that mussel beds increase the biomass of primary producers. Previous research had suggested this facilitative effect of mussel beds (Donadi et al., 2013b), but did not provide conclusive evidence. Our results are also the first to actually link a long-distance interaction of up to 200 m in the vicinity of mussel beds, to both increasing biomass of primary producers and higher levels of productivity. There was a clear inter-annual variation in both chlorophyll *a* and productivity, where the long-range effect of the mussel bed shifted in magnitude and direction between 2012 and 2015–16. However, in general, the areas on the tidal flat with the highest biomass of benthic microalgae also had high values of photosynthetic yield and these were always the sampling points in proximity of the mussel bed; indicating that the positive effect of the mussel bed prevailed through the annual fluctuations in environmental conditions. These results confirm and highlight the importance of reef-building bivalves for the functioning of coastal ecosystems. Specifically, in soft-sediment habitats reef-building bivalves act as ecosystem engineers and thereby are essential for associated communities (van de Koppel et al., 2015). By creating a three-dimensional habitat structure, bivalve reefs are important structuring components of many coastal ecosystems. In soft-bottom intertidal areas, bivalve reefs are often the only hard surfaces that can be used by other sessile organisms for attachment, favoring habitat forming algae and rich communities of epifauna



**Fig. 3.** a) Average chlorophyll *a* concentration, b) average organic matter content (OM), and c) average plaster loss in the different treatments (FC = fenced control, FM = fenced mussel addition, CC = semi-caged control, CM = semi-caged mussel addition) of the field experiment. Bars denote standard errors of the mean.

**Table 1**

Results of three-factorial ANOVA for field experiment testing effects of mussel additions (two levels – no mussel addition, mussel addition), cage (two levels – cage present, no cage present) and site (three levels); on chlorophyll *a* concentration (Chl *a*), organic matter content (OM) and plaster loss (PL) in the experimental plots.

Factor	df	Chl <i>a</i> (µg/mg)		OM (%)		PL (%)	
		F	p	F	p	F	p
Mussel addition (M)	1	12.59	<0.01	1.71	0.20	0.66	0.43
Cage (C)	1	0.52	0.48	0.22	0.65	4.53	0.05
Site (S)	2	5.45	0.01	16.00	<0.01	0.88	0.43
MxC	1	0.37	0.55	3.14	0.09	1.46	0.24
MxS	2	0.21	0.81	1.28	0.30	0.92	0.41
CxS	2	0.73	0.49	0.70	0.51	3.40	0.05
MxCxS	2	0.66	0.53	0.82	0.46	0.23	0.80
Residuals	21						

(Albrecht and Reise, 1994). Bivalve reefs are also important nursery grounds for many economically important organisms such as fish (Kristensen et al., 2015). In addition to locally modifying the habitat with their physical presence, they also have long distance effects by creating sediment conditions around them that are beneficial for many benthic organisms including infauna species (van der Zee et al., 2012; Donadi et al., 2015). This shows that bivalves are autogenic and allogenic ecosystem engineers at the same time (Jones et al., 1994). Our study demonstrates that mussel beds not only facilitate animal biodiversity on many different trophic levels as shown in previous studies (Albrecht and Reise, 1994; van der Zee et al., 2012; Donadi et al., 2015; Kristensen et al., 2015), but they also provide excellent growing conditions for benthic primary producers across the intertidal. Together, this demonstrates that mussel beds are hot spots that fuel primary production in intertidal areas and support a productive and diverse food web far beyond its physical borders.

The abundance of benthic microalgae in intertidal sediments strongly depends on sediment erosion. Increased hydrodynamic stress leads to higher sediment erosion, which in turn re-suspends the microalgae (de Jonge and van Beusekom, 1995). Larger biofilms of benthic microalgae are therefore often found in areas that are protected from hydrodynamic stress. Previous research shows that mussel beds reduce hydrodynamic stress (Donadi et al., 2013b) and therefore productivity of benthic primary producers is expected to increase in the vicinity of these structures. Our transect results confirm an increase in primary producer biomass closer to the mussel bed, but in our experiment we found that protection from hydrological forces alone does not increase the standing stock of primary producers. Although shelter by the cages decreased the hydrodynamic stress more than the mussels, it was only when mussels were physically present in the plots we could demonstrate an increase in primary producer biomass. Generally, in addition to reducing hydrodynamic stress, mussels excrete feces and pseudofeces that are rich in organic matter and nutrients which may promote growth of the biofilm (van Broekhoven et al., 2015). The physical changes by the mussels may also hinder infauna that feed on benthic microalgae and/or affect sediment stability via bioturbation. For example, the amphipod *Corophium volutator* and the polychaete *Arenicola marina*, are common infauna found in the Wadden Sea that negatively affect benthic microalgae via grazing and bioturbation, respectively (Gerdol and Hughes, 1994; Chennu et al., 2015). Thus, the exclusion of infauna grazers and bioturbators may also have promoted the biofilm (Gerdol and Hughes, 1994; Brustolin et al., 2016). However, the reefs also support higher abundances of epifauna (Norling and Kautsky, 2007), which includes dominant grazers such as *Littorina littorea* (common periwinkle) that consume the biofilm. Consequently, in our experiment, on a local scale, the increase in microphytobenthos biomass by the physical presence of the mussels may have been caused through a combination of: (1) reduced hydrodynamic stress, (2) increased nutrient availability, and (3) changes to the associated invertebrate community.

Scale is important for the magnitude of engineering effects. Our experiment was designed to analyze effects at small scales and therefore did not simulate the hierarchical spatial structure of mussel beds or manipulate long-range effects (Liu et al., 2012; van de Koppel et al., 2015). The hierarchical structure of the mussel patches possibly play a major role on the overall engineering effect of mussel beds on the tidal flats systems. Indeed, comparing the experimental results of mussel addition to the field measurements across the mussel bed indicates that the facilitative effect on primary producers was dependent on the extent of the mussel aggregation. Our small addition plots doubled the biomass of benthic microalgae, but this was still less than half of the biomass on the

natural mussel bed. The importance of the size of mussel patches was previously observed for associated macrofauna (Norling and Kautsky, 2007). Even though the presence of single mussels was shown to increase biomass and species richness of associated macrofauna, the increase was much higher in larger patches of *Mytilus edulis* (Norling and Kautsky, 2007). The temporal scale is probably also very important; the mussel bed from the transect data had been present in the same location for multiple years before we took the measurements. This means that there was much more time for organic matter to be accumulated in the sediment and we saw a large increase of organic matter content on the mussel bed (Fig. E.1). In contrast, we did not see any significant effect of the mussel additions on organic matter content in the experimental plots, where the mussels only had about one month time to produce and accumulate organic matter. Worldwide, we have observed a decrease in native bivalve reefs due to overfishing, habitat degradation, or invasion of non-native species (Jackson et al., 2001; Edgar and Samson, 2004; Lotze et al., 2006; Eriksson et al., 2010), which can have detrimental effects on coastal ecosystems. Protection and restoration efforts should not only focus on the presence of the species but also needs to consider the scale of reefs.

Our results demonstrate the importance of bivalve reefs for primary production on intertidal flats. On a global scale, we see a decline in ecosystem engineering species in coastal areas (Jackson et al., 2001; Lotze et al., 2006). Bivalves are therefore already of an importance to conservation and restoration efforts in many areas of the world (e.g. Schulte et al., 2009; McLeod et al., 2012; de Paoli et al., 2015). Our research shows that establishing and protecting reefs in soft-bottom habitats is a key conservation priority and an essential strategy to restore and manage coastal production.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.ecss.2017.04.003>.

## References

- Albrecht, A., Reise, K., 1994. Effects of *Fucus vesiculosus* covering intertidal mussel beds in the Wadden Sea. *Helgol. Mar. Res.* 48, 243–256. <http://dx.doi.org/10.1007/BF02367039>.
- van Broekhoven, W., Jansen, H., Verdegem, M., Struyf, E., Troost, K., Lindeboom, H., Smaal, A., 2015. Nutrient regeneration from feces and pseudofeces of mussel *Mytilus edulis* spat. *Mar. Ecol. Prog. Ser.* 534, 107–120. <http://dx.doi.org/10.3354/meps11402>.
- Brustolin, M.C., Thomas, M.C., Mafra Jr., L.L., da Cunha Lana, P., 2016. Bioturbation by the sand dollar *Encope emarginata* (Echinoidea, Mellitidae) changes the composition and size structure of microphytobenthic assemblages. *Hydrobiologia*. <http://dx.doi.org/10.1007/s10750-016-2815-6>.
- Chennu, A., Volkenborn, N., de Beer, D., Wetthey, D.S., Woodin, S.A., Polerecky, L., 2015. Effects of bioadvection by *Arenicola marina* on microphytobenthos in permeable sediments. *PLoS One*. <http://dx.doi.org/10.1371/journal.pone.0134236>.

- Commito, J.A., Como, S., Grupe, B.M., Dow, W.E., 2008. Species diversity in the soft-bottom intertidal zone: biogenic structure, sediment, and macrofauna across mussel bed spatial scales. *J. Exp. Mar. Bio. Ecol.* 366, 70–81. <http://dx.doi.org/10.1016/j.jembe.2008.07.010>.
- Commito, J.A., Dow, W.E., Grupe, B.M., 2006. Hierarchical spatial structure in soft-bottom mussel beds. *J. Exp. Mar. Bio. Ecol.* 330, 27–37. <http://dx.doi.org/10.1016/j.jembe.2005.12.015>.
- Decho, A.W., 2000. Microbial biofilms in intertidal systems: an overview. *Cont. Shelf Res.* 20, 1257–1273. [http://dx.doi.org/10.1016/S0278-4343\(00\)00022-4](http://dx.doi.org/10.1016/S0278-4343(00)00022-4).
- Donadi, S., van der Heide, T., Piersma, T., others, 2015. Multi-scale habitat modification by coexisting ecosystem engineers drives spatial separation of macrobenthic functional groups. *Oikos* 124, 1502–1510. <http://dx.doi.org/10.1111/oik.02100>.
- Donadi, S., van der Heide, T., van der Zee, E.M., others, 2013a. Cross-habitat interactions among bivalve species control community structure on intertidal flats. *Ecology* 94, 489–498. <http://dx.doi.org/10.1890/12-0048.1>.
- Donadi, S., Westra, J., Weerman, E.J., others, 2013b. Non-trophic interactions control benthic producers on intertidal flats. *Ecosystems* 16, 1325–1335. <http://dx.doi.org/10.1007/s10021-013-9686-8>.
- Edgar, G.J., Samson, C.R., 2004. Catastrophic decline in mollusc diversity in eastern Tasmania and its concurrence with shellfish fisheries. *Conserv. Biol.* 18, 1579–1588. <http://dx.doi.org/10.1111/j.1523-1739.2004.00191.x>.
- Eriksson, B.K., van der Heide, T., van de Koppel, J., Piersma, T., van der Veer, H.W., Olf, H., 2010. Major changes in the ecology of the Wadden Sea: human impacts, ecosystem engineering and sediment dynamics. *Ecosystems* 13, 752–764. <http://dx.doi.org/10.1007/s10021-010-9352-3>.
- Gerdol, V., Hughes, R.G., 1994. Effect of *Corophium volutator* on the abundance of benthic diatoms, bacteria and sediment stability in two estuaries in south-eastern England. *Mar. Ecol. Prog. Ser.* 114, 109–115.
- van Gils, J.A., van der Geest, M., Jansen, E.J., Govers, L.L., de Fouw, J., Piersma, T., 2012. Trophic cascade induced by molluscivore predator alters pore-water biogeochemistry via competitive release of prey. *Ecology* 93, 1143–1152. <http://dx.doi.org/10.1890/11-1282.1>.
- Gutiérrez, J.L., Jones, C.G., Byers, J.E., others, 2011. 7.04—physical ecosystem engineers and the functioning of estuaries and coasts. In: *Treatise on Estuarine and Coastal Science*, pp. 53–81.
- Heip, C.H.R., Goosen, N.K., Herman, P.M.J., Kromkamp, J., Middelburg, J.J., Soetaert, K., 1995. Production and consumption of biological particles in temperate tidal estuaries. *Oceanogr. Mar. Biol.* 33, 1–149.
- Jackson, J.B., Kirby, M.X., Berger, W.H., others, 2001. Historical overfishing and the recent collapse of coastal ecosystems. *Science* 293, 629–637. <http://dx.doi.org/10.1126/science.1059199>.
- Jeffrey, S.W., Humphrey, G.F., 1975. New spectrophotometric equations for determining chlorophylls *a*, *b*, *c*<sub>1</sub> and *c*<sub>2</sub> in higher plants, algae and natural phytoplankton. *Biochem. Physiol. Pflanz.* 191–194.
- Jones, C.G., Lawton, J.H., Shachak, M., 1994. Organisms as ecosystem engineers. *Oikos* 69, 373–386. <http://dx.doi.org/10.2307/3545850>. Doi.
- de Jonge, V.N., van Beusekom, J.E.E., 1995. Wind- and tide-induced resuspension of sediment and microphytobenthos from tidal flats in the Ems estuary. *Limnol. Oceanogr.* 40, 776–778. <http://dx.doi.org/10.4319/lo.1995.40.4.0776>.
- Kéfi, S., Berlow, E.L., Wieters, E.A., Joppa, L.N., Wood, S.A., Brose, U., Navarrete, S.A., 2015. Network structure beyond food webs: mapping non-trophic and trophic interactions on Chilean rocky shores. *Ecology* 96, 291–303. <http://dx.doi.org/10.1890/13-1424.1>.
- van de Koppel, J., van der Heide, T., Altieri, A.H., others, 2015. Long-distance interactions regulate the structure and resilience of coastal ecosystems. *Annu. Rev. Mar. Sci.* Vol. 7 (7), 139–158. <http://dx.doi.org/10.1146/annurev-marine-010814-015805>.
- van de Koppel, J., Gascoigne, J.C., Theraulaz, G., Rietkerk, M., Mooij, W.M., Herman, P.M.J., 2008. Experimental evidence for spatial self-organization and its emergent effects in mussel bed ecosystems. *Science* 322, 739–742. <http://dx.doi.org/10.1126/science.1163952>.
- Kostylev, V., Erlandsson, J., 2001. A fractal approach for detecting spatial hierarchy and structure on mussel beds. *Mar. Biol.* 139, 497–506. <http://dx.doi.org/10.1007/s002270100597>.
- Kristensen, L.D., Stenberg, C., Stottrup, J.G., others, 2015. Establishment of blue mussel beds to enhance fish habitats. *Appl. Ecol. Environ. Res.* 13, 783–798. [http://dx.doi.org/10.15666/aeer/1303\\_783798](http://dx.doi.org/10.15666/aeer/1303_783798).
- Kromkamp, J.C., de Brouwer, J.F.C., Blanchard, G.F., Forster, R.M., Créach, V., 2006. Functioning of microphytobenthos in estuaries. In: *Proceedings of the Colloquium. Royal Netherlands Academy of Arts and Sciences*.
- Liu, Q.-X., Weerman, E.J., Herman, P.M.J., Olf, H., van de Koppel, J., 2012. Alternative mechanisms alter the emergent properties of self-organization in mussel beds. *Proc. R. Soc. B Biol. Sci.* 279, 2744–2753. <http://dx.doi.org/10.1098/rspb.2012.0157>.
- Lotze, H.K., Lenihan, H.S., Bourque, B.J., others, 2006. Depletion, degradation, and recovery potential of estuaries and coastal seas. *Science* 312, 1806–1809. <http://dx.doi.org/10.1126/science.1128035>.
- Markert, A., Esser, W., Frank, D., Wehrmann, A., Exo, K.-M., 2013. Habitat change by the formation of alien *Crassostrea*-reefs in the Wadden Sea and its role as feeding sites for waterbirds. *Estuar. Coast. Shelf Sci.* 131, 41–51. <http://dx.doi.org/10.1016/j.ecss.2013.08.003>.
- McLeod, I.M., Parsons, D.M., Morrison, M.A., Le Port, A., Taylor, R.B., 2012. Factors affecting the recovery of soft-sediment mussel beds in the Firth of Thames, New Zealand. *Mar. Freshw. Res.* 63, 78–83. <http://dx.doi.org/10.1071/MF11083>.
- Nieuwhof, S., Herman, P., Dankers, N., Troost, K., van der Wal, D., 2015. Remote sensing of epibenthic shellfish using synthetic aperture radar satellite imagery. *Remote Sens.* 7, 3710–3734. <http://dx.doi.org/10.3390/rs70403710>.
- Norling, P., Kautsky, N., 2007. Structural and functional effects of *Mytilus edulis* on diversity of associated species and ecosystem functioning. *Mar. Ecol. Prog. Ser.* 351, 163–175. <http://dx.doi.org/10.3354/meps07033>.
- Olf, H., Alonso, D., Berg, M.P., Eriksson, B.K., Loreau, M., Piersma, T., Rooney, N., 2009. Parallel ecological networks in ecosystems. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 364, 1755–1779. <http://dx.doi.org/10.1098/rstb.2008.0222>.
- de Paoli, H., van de Koppel, J., van der Zee, E., others, 2015. Processes limiting mussel bed restoration in the Wadden-Sea. *J. Sea Res.* 103, 42–49. <http://dx.doi.org/10.1016/j.seares.2015.05.008>.
- R Core Team, 2017. *R: a language and environment for statistical computing*.
- Rigolet, C., Thiébaud, E., Dubois, S.F., 2014. Food web structures of subtidal benthic muddy habitats: evidence of microphytobenthos contribution supported by an engineer species. *Mar. Ecol. Prog. Ser.* 500, 25–41. <http://dx.doi.org/10.3354/meps10685>.
- Schulte, D.M., Burke, R.P., Lipcius, R.N., 2009. Unprecedented restoration of a native oyster metapopulation. *Science* 325, 1124–1128. <http://dx.doi.org/10.1126/science.1176516>.
- Snover, M.L., Commito, J.A., 1998. The fractal geometry of *Mytilus edulis* L. spatial distribution in a soft-bottom system. *J. Exp. Mar. Bio. Ecol.* 223, 53–64.
- Stal, L.J., 2003. Microphytobenthos, their extracellular polymers, and the morphogenesis of intertidal sediments. *Geomicrobiol. J.* 20, 463–478. <http://dx.doi.org/10.1080/0143851126>.
- Underwood, G.J.C., Kromkamp, J., 1999. Primary production by phytoplankton and microphytobenthos in estuaries. *Adv. Ecol. Res.* 29, 93–153. [http://dx.doi.org/10.1016/S0065-2504\(08\)60192-0](http://dx.doi.org/10.1016/S0065-2504(08)60192-0).
- van der Wal, D., Wielemaker-van den Dool, A., Herman, P.M.J., 2010. Spatial synchrony in intertidal benthic algal biomass in temperate coastal and estuarine ecosystems. *Ecosystems* 13, 338–351. <http://dx.doi.org/10.1007/s10021-010-9322-9>.
- Weerman, E.J., Herman, P.M.J., van de Koppel, J., 2011a. Top-down control inhibits spatial self-organization of a patterned landscape. *Ecology* 92, 487–495. <http://dx.doi.org/10.1890/10-0270.1>.
- Weerman, E.J., Herman, P.M.J., van de Koppel, J., 2011b. Macrobenthos abundance and distribution on a spatially patterned intertidal flat. *Mar. Ecol. Prog. Ser.* 440, 95–103. <http://dx.doi.org/10.3354/meps09332>.
- Widdows, J., Brinsley, M., 2002. Impact of biotic and abiotic processes on sediment dynamics and the consequences to the structure and functioning of the intertidal zone. *J. Sea Res.* 48, 143–156. [http://dx.doi.org/10.1016/S1385-1101\(02\)00148-X](http://dx.doi.org/10.1016/S1385-1101(02)00148-X).
- Wiens, J.A., 1989. Spatial scaling in ecology. *Funct. Ecol.* 3, 385–397.
- van der Zee, E.M., Angelini, C., Govers, L.L., others, 2016. How habitat-modifying organisms structure the food web of two coastal ecosystems. *Proc. R. Soc. B Biol. Sci.* 283, 20152326. <http://dx.doi.org/10.1098/rspb.2015.2326>.
- van der Zee, E.M., van der Heide, T., Donadi, S., Eklöf, J.S., Eriksson, B.K., Olf, H., van der Veer, H.W., Piersma, T., 2012. Spatially extended habitat modification by intertidal reef-building bivalves has implications for consumer-resource interactions. *Ecosystems* 15, 664–673. <http://dx.doi.org/10.1007/s10021-012-9538-y>.