Facilitation by ecosystem engineers enhances nutrient effects in an intertidal system

Britas Klemens Eriksson,[1,†] Joëlle Westra,[1] Imke van Gerwen,[1] Ellen Weerman,[2] Els van der Zee,[3] Tjisse van der Heide,[4] Johan van de Koppel,[1,5] Han Olff,[1] Theunis Piersma,[1,5] and Serena Donadi[6]

[1]Groningen Institute for Evolutionary Life Sciences, University of Groningen, Nijenborgh 7, 9747 AG Groningen, The Netherlands
[2]HAS Den Bosch, University of Applied Sciences, Onderwijsboulevard 221, 5223 DE 's-Hertogenbosch, The Netherlands
[3]Altenburg and Wymenga Ecological Consultants, Suderwei 2, 9269 TZ Feanwâlden, The Netherlands
[4]Aquatic Ecology & Environmental Biology Group, Institute for Water and Wetland Research at the Radboud University, Heyendaalseweg 135, 6525 AJ Nijmegen, The Netherlands
[5]NIOZ Royal Netherlands Institute for Sea Research, Department of Coastal Systems, Utrecht University, PO Box 59, 1790 AB Den Burg, Texel, The Netherlands
[6]Department of Aquatic Resources (SLU Aqua), Swedish University of Agricultural Sciences, Stångholmsvägen 2, SE-178 93 Drottningholm, Sweden

Abstract. Ecosystem engineering research has recently demonstrated the fundamental importance of non-trophic interactions for food-web structure. Particularly, by creating benign conditions in stressful environments, ecosystem engineers create hot beds of elevated levels of recruitment, growth, and survival of associated organisms; this should fuel food webs and promote production on the ecosystem scale. However, there is still limited empirical evidence of the influence of non-trophic interactions on the classical food-web processes that determine energy transfer, that is, consumer–resource interactions. On the basis of a biomanipulation experiment covering 600 m^2 of an intertidal flat, we show that ecosystem engineers influence resource uptake efficiency and the accumulation of algae following nutrient enrichment in a soft-sediment food web. Nutrient additions increased chlorophyll a concentrations in the sediment by 90%, but only in plots where we also introduced high densities (2000 per m^2) of a burrowing bivalve, the common cockle *Cerastoderma edule*. The artificial cockle beds increased the nutrient uptake efficiency of the biofilm and promoted sediment accumulation, which suggests that the cockles facilitated the sediment-living algae by increasing sediment stability. This indicates that ecological interactions, rather than the availability of nutrients per se, set the limits for production in this coastal ecosystem. Our results emphasize the need to include facilitation theory and recognize that positive interactions between species are key to understand, manage, and restore ecosystems under human influence.

† **E-mail:** b.d.h.k.eriksson@rug.nl

INTRODUCTION

The ecological importance of species that physically modify the abiotic environment for associated species, the so-called ecosystem engineers, was realized much earlier (sensu Jones et al. 1994) than the first integrative attempts to include non-trophic interactions into the ecology of species interactions (Goudard and Loreau 2008, Olff et al. 2009, Kéfi et al. 2012). Interactions beyond the

trophic ones are now considered important and ecosystem engineering a highly influential class of non-trophic interactions that determine the structure and composition of many different types of ecosystems (Wright and Jones 2004, Hastings et al. 2007, Kéfi et al. 2015, van de Koppel et al. 2015, van der Zee et al. 2016). Interestingly, recent studies suggest that ecosystem engineers will also strongly affect energy transfer in food webs (Sanders et al. 2014, van der Zee et al. 2015).

Ecosystem engineers influence ecological networks by modifying habitat conditions (Olff et al. 2009), that is, by alleviating stress or increasing habitat complexity. Non-trophic interactions that modify local exposure to abiotic factors and thereby reduce environmental stress are well documented (e.g., Aguiar and Sala 1994, Bertness and Callaway 1994, Bertness et al. 1999, Bruno et al. 2003, Fogel et al. 2004). From a food-web perspective, this is a very important mechanism because lower stress should increase resource uptake rates from primary producers and also allow consumers to forage more effectively (Menge 1978, Menge and Sutherland 1987). Another aspect of ecosystem engineering is that many ecosystem engineers increase habitat complexity by providing physical structures for associated organisms. Increasing the abundance of habitat and organic material associated with colonizing organisms should increase resource availability and thereby promote nutrient uptake, prey consumption, and food-web production. However, the physical structure may also limit nutrient uptake by shading associated primary producers (Eriksson et al. 2006, 2007) or change consumption rates by providing refuge for prey and/or mesopredators (Crain and Bertness 2006, Wright et al. 2014).

In this study, we used field biomanipulations to test the effects of sediment burrowing ecosystem engineers on resource uptake efficiency of microphytobenthos. Microphytobenthos indicates the community of microorganisms, often diatoms and cyanobacteria, that dominate primary production and fuel food webs on intertidal flats (Wolff 1983, Guarini et al. 2004, Méléder et al. 2007). They inhabit the upper millimeters of the sediment where they create a photosynthesizing biofilm. Intertidal flat communities have long been regarded to be shaped by hydrodynamic forces that act on the sediment, creating a

dynamic and ever-changing habitat (Paterson and Black 1999). It is now clear that in this dynamic environment, biological non-trophic interactions that alleviate hydrodynamic stress are key processes that regulate food-web structure, feeding behavior, and recruitment processes (Donadi et al. 2013a, 2014, 2015b, van der Zee et al. 2013, van der Heide et al. 2014). Microphytobenthos is strongly limited by sediment erosion, and we have earlier shown that non-trophic interactions that decrease hydrodynamic stress or increase sediment cohesion promote microphytobenthos biomass (Donadi et al. 2013b). Here, we demonstrate that non-trophic interactions facilitate growth of the microphytobenthos in response to nutrient enrichment, thus indirectly fueling intertidal food webs.

METHODS

Study system

The experiments were performed on the intertidal flats south of the island of Schiermonnikoog, Wadden Sea, the Netherlands (53°28′ N; 6°10′ E). This intertidal system is dominated by three sessile ecosystem engineers that modify the habitat on different scales: the blue mussel (*Mytilus edulis* L.), the cockle (*Cerastoderma edule* L.), and the lugworm (*Arenicola marina* L.; Donadi et al. 2015b). Intertidal mussels form beds that have a large influence on the sediment environment, increasing sediment organic content for 100s of meters across the tidal flat by (1) decreasing water flow rates, which decrease sediment erosion and thereby exercise long-range facilitation of associated fauna and microphytobenthos biomass (Donadi et al. 2013a, b, 2014, van de Koppel et al. 2015); and (2) depositing feces and pseudofeces (Graf and Rosenberg 1997, Bergfeld 1999). Cockles and lugworms live in the sediment and have local effects on sediment stability (de Paoli et al. 2015). Lugworms are upward conveyors: strong bioturbators that process the sediment in their gut and thereby de-stabilize the sediment and decrease microphytobenthos biomass. Cockles are biodiffusers, organisms that cause moderate bioturbation by random mixing of the sediment and thereby should increase sediment resuspension (Flach and Debruin 1994). However, in high abundances cockles stabilize the sediment, increasing the abundance of

microphytobenthos and sediment accumulation at sites with relatively high hydrodynamic stress (Donadi et al. 2013b).

Biomanipulation nutrient experiment

We set up a biomanipulation (BM) experiment in May–June 2010, by placing four experimental blocks in the intertidal 500 m from the shore (Appendix S1). To subject the experimental treatments to a gradient of environmental context, two of the four blocks were placed 200 m coastward of an intertidal mussel bed where the sediment was visibly influenced by plumes of muddy sediment radiating from the mussel aggregations. Earlier work has shown that the mussel beds in this area, by decreasing hydrodynamic stress and producing pseudofeces, contribute to increased organic matter content, higher nutrient concentrations, and lower oxygen availability in the sediment (Donadi et al. 2013a, 2014, 2015b). The other two blocks were placed at the same tidal elevation; ~500 m to the east of the mussel bed. These blocks were outside the influence of the mussel bed. In this way, our experimental treatments were placed in a natural gradient in hydrodynamic stress and critical sediment characteristics, increasing the generality of the experimental results across different common intertidal habitat types (Appendix S1: Fig. S1A).

In all four blocks, we performed three BM treatments by (1) adding high densities of adult cockles, (2) adding high densities of adult lugworms, or (3) not adding any organisms (no addition control), to six 5 × 5 m large BM experimental areas (24 in total). We added 25,000 cockles, of 2–4 yr age, to each cockle addition area (mean length of 32.40 mm ± 0.78 SE). To each lugworm addition area, we added 2000 lugworms with a mean dry weight of 0.97 g ± 0.12 SE. The cockle addition corresponded to high densities of ~1000 cockles per m^2 found in the field (Donadi et al. 2013a), and the lugworm addition was meant to double naturally occurring abundances (Beukema and Devlas 1979). The additions were made on top of the natural background of cockles (0–100 per m^2 depending on block) and lugworms (40–80 per m^2 depending on block). The BM treatments were randomly assigned to a BM area within each block. Each BM area was separated from each other by at least 5 m, and there were two replicates of each BM treatment in each block

(Appendix S1: Fig. S1B). The abundance of both cockles and lugworms was still significantly elevated in respective addition treatments compared to the no addition treatment, during the extent of the current BM × nutrient experiment from early May to late July in 2011 (see next paragraph; Appendix S2: Table S2, Fig. S2).

We tested the joint effects of BM and nutrient enrichment on the biomass of microphytobenthos by adding a garden fertilizer treatment to the BM areas in 2011; the second year of BM. The nutrient treatment consisted of two smaller plots placed in opposite corners of the larger BM areas (nutrient treatment plots; Appendix S1: Fig. S1B). To one of the two smaller plots, the nutrient addition plot, we added 120 g of slow releasing fertilizer pellets (Plantacote Depot 6M), while the other plot served as the ambient nutrient control. The pellets continuously enrich the sediment porewater with nitrogen (14%, as NH_4–N and NH_3–N), phosphorus (9%, as P_2O_5), and potassium (15%, as K_2O), and spreading them in the sediment is a standard method to mimic marine eutrophication on soft bottoms from agriculture (Worm et al. 2000). To add the nutrients in the sediment, a metal square (0.25 × 0.25 m) was pushed into the sediment (5–7 cm deep). The sediment within the square was lifted up with a shovel, and the nutrients were spread out evenly in the exposed area. Then, the sediment was carefully put back on top of the fertilizer and the metal frame removed. The ambient nutrient plot was treated the same way, but without adding the fertilizer. The depth of the treatment of 5–7 cm ensured that the physical structure of the pellets did not interfere with the shallow biofilm. The nutrient treatments were added to all 24 BM areas on the 3 May 2011 (Appendix S1: Fig. S1B). The nutrient treatment experiment was terminated almost three months later, on the 28th of July in 2011.

Sampling and data analyses

We sampled chlorophyll *a* concentrations from the top layer of the sediment as a proxy for biomass of the microphytobenthos (Honeywill et al. 2002). From each nutrient treatment plot (both the nutrient addition and the ambient nutrient plots in each BM area), two subsamples of the upper 2 mm of the sediment layer were collected using a cutoff disposable syringe (2.4 cm internal

diameter). Samples from all sampling sites were collected within a few hours the same day and stored on ice in darkness. After freeze-drying the sediment, the chlorophyll *a* concentration was determined using acetone extraction following Jeffrey and Humphrey (1975). Chlorophyll *a* samples were collected on the 22nd of July 2011, 12 weeks after the start of the BM nutrient experiment.

To estimate the nutrient background, detect the effectiveness of the nutrient treatment, and to be able to calculate nutrient (resource) use efficiency (RUE), we sampled and analyzed total nitrogen ($NH_4 + NO_2^- + NO_3^-$) and ortho-phosphate (PO_4^{-3}) concentrations of the porewater. Porewater samples were collected from each nutrient treatment plot (both the nutrient addition and the ambient nutrient plots in each BM area), using ceramic soil moisture samplers (Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands) attached to airtight 50-mL syringes. Resource use efficiency (RUE) was calculated as the ratio between chlorophyll *a* concentrations in the sediment, divided by the porewater concentration of the limiting nutrient. In microphytobenthos communities, N:P ratios in non-limited conditions between 13 and 22 indicate a balance between nitrogen and phosphate (Hillebrand 1999). The ratio of total nitrogen to ortho-phosphate concentrations was 4.4 (±1.3 SD) in the ambient nutrient plots, which indicate strong nitrogen limitation under natural conditions (N:P ratios below 13 indicate a nitrogen-limited community, Hillebrand 1999). We therefore calculated RUE using total nitrogen concentrations of the porewater as the limiting nutrient. Porewater samples were also collected on the 22nd of July 2011.

To estimate effects of BM on sediment stability in the different habitat types, we measured bed elevation in the BM areas using a Trimble Spectre Precision LL500 Laser Level (Trimble, Sunnyvale, California, USA). Five replicate measurements were randomly taken in all BM areas in March 2011 and then in September 2011 to assess changes in bed level over the course of the summer when the bioengineering activity of cockles and lugworms is most conspicuous. The change in bed level shows net sediment accumulation or erosion. For statistical analyses of bed level change, we averaged the five samplings per BM area and calculated the difference

between March and September. The average standard deviation of the five measurements within a single plot was ±0.62 cm.

To test for confounding effect of the nutrient treatments on the biomanipulations of cockles and lugworms, and on the biomass dominant grazer the common periwinkle (*Littorina littorea* L.), we counted cockles, lugworms heaps (as a proxy for lugworm numbers), and periwinkles in the nutrient treatment plots (both the nutrient addition and the ambient nutrient plots in each BM area). Lugworm heaps and periwinkles were counted on six occasions from the 3rd of May to the 28th of July in 2011. For statistical analyses of lugworm heap and periwinkle numbers, we used the average of the six samplings. On the 28th of July in 2011, we ended of the BM nutrient experiment by digging out the nutrient treatment plots and counting all cockles.

General effects of the BM treatments were analyzed using a mixed-model ANOVA with a full factorial combination of BM (cockle addition, lugworm addition, no addition control) and the random factor block (four levels). Effects on nutrient concentrations, chlorophyll *a* concentrations, and RUE were analyzed for the ambient and nutrient addition plots separately. Effects on cockle, lugworm, and periwinkle numbers were analyzed by summing the numbers in the ambient nutrient and nutrient-enriched plots together. Effects on the bed level were analyzed for the whole BM area. Data were log10 or square root (for counts) transformed when needed to fulfill model assumptions, and significant contrasts were tested using Tukey's HSD post hoc test.

Specific effects of the nutrient treatments on cockle, lugworm heap, and periwinkle numbers, as well as porewater nutrients (tot-N and PO_4^{-3}) and chlorophyll *a* concentration data, were analyzed by calculating the log-response ratio (LRR) of the response variables to the nutrient additions within each of the BM areas, using the formula:

$$LRR = LN \text{ (response variable in nutrient addition plots/response variable in ambient nutrient plots)}$$

Log-response ratio is preferred over simple ratios when comparing effects between treatments, sites, or across studies, since negative and positive effects are symmetrically distributed
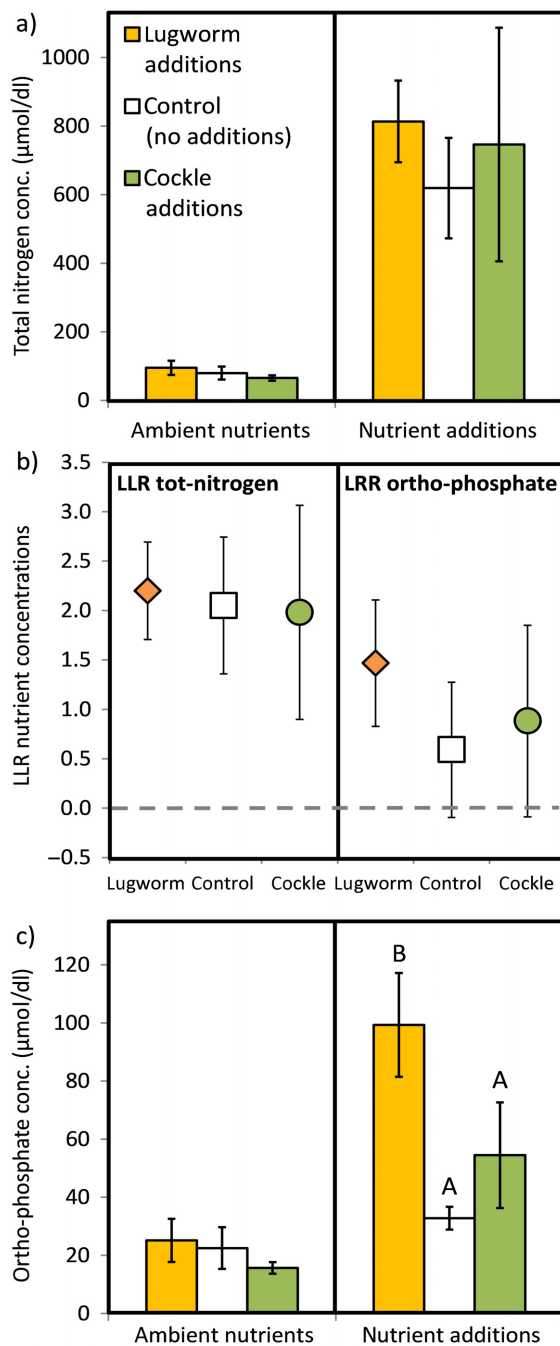
Fig. 1. Effects of biomanipulation (BM) and nutrient treatments on nutrient concentrations in the sediment porewater. The concentration of (a) total nitrogen and (b) ortho-phosphate in the sediment porewater; in ambient nutrients (left panel, $n = 24$) and nutrient addition (right panel, $n = 23$) treatments; and in three BM treatments (orange bars = lugworm addition; green bars = cockle addition; white bars = no addition

around zero. This enabled us to account for the paired design of the nutrient treatment plots. The LRR were then analyzed using the same mixed-model ANOVA as described above, with the full factorial combination of BM (cockle addition, lugworm addition, no addition control) and the random factor block (four levels) as explanatory variables. In addition, 95% confidence intervals were calculated to evaluate whether the nutrient treatment had significant effects in specific treatment combinations.

RESULTS

The nutrient addition treatment increased both the nitrogen and phosphate concentrations in the sediment porewater strongly compared to the ambient nutrient treatments (Fig. 1). There were no significant differences in total nitrogen concentrations between blocks or BM treatments (Fig. 1a, Table 1). Nutrient addition increased the total nitrogen concentrations across all treatments by an average of eight times compared to the ambient nutrient treatment (LRR of tot-N concentrations: 95% confidence intervals do not cross zero, Fig. 1b). The enrichment effect did not depend on BM, but was different between blocks (significant main effect of block on LRR of tot-N: $F_{3,11} = 8.5$, $P < 0.05$; Table 1). The difference between blocks was subtle (Tukey's HSD test: no significant contrasts between blocks) and depended on that plots with lower organic matter contents and background nitrogen concentrations showed a stronger response to the enrichment (Pearson correlation of organic matter content of the sediment and LRR of nitrogen concentrations: $r = -0.44$, $P < 0.05$; and of tot-N concentration in the ambient plots and LRR of

(Fig. 1. *Continued*)
control). Error bars in (a) and (b) show SE. Letters indicate significant different contrasts between bars within a panel from Tukey's HSD post hoc test. (c) Log-response ratios of nutrient additions on total nitrogen (left panel, $n = 23$) and ortho-phosphate (right panel, $n = 23$) in three BM treatments (orange diamond = lugworm addition; green circle = cockle addition; white squares = no addition control). Error bars in (c) show 95% confidence intervals; if the confidence intervals do not cross the zero line (striped line), the effect of nutrient addition is statistically significant.

Table 1. Factorial ANOVA results of biomanipulation (BM; cockle addition, lugworm addition, no addition control) and a random factor block (four levels) on total nitrogen (tot-N) and ortho-phosphate (PO_4^{-3}) concentrations in ambient and nutrient addition plots, and the log-response ratio (LRR) of tot-N and PO_4^{-3} concentrations to the nutrient enrichment treatment.

Effect	df	tot-N conc. in ambient nutrient plots		tot-N conc. in nutrient addition plots		LRR tot-N		PO_4^{-3} conc. in ambient nutrient plots		PO_4^{-3} conc. in nutrient addition plots		LRR PO_4^{-3}	
		F	P	F	P	F	P	F	P	F	P	F	P
Intercept	1	26.2	<0.05	1531.4	<0.001	61.0	<0.01	17.0	<0.05	1362.0	<0.001	12.4	<0.05
Block	3	2.7	0.141	1.4	0.320	8.3	<0.05	2.1	0.203	0.5	0.689	3.5	0.086
BM	2	0.8	0.488	1.5	0.293	0.6	0.596	0.6	0.593	5.4	<0.05	2.9	0.126
Block × BM	6	1.6	0.234	0.6	0.715	0.2	0.980	1.6	0.250	0.9	0.546	0.7	0.625
Error	†	df = 12		df = 11		df = 11		df = 11		df = 12		df = 11	

† Due to loss of one porewater NH_4, one PO_4, and one chlorophyll a sample, df in the error term vary between analyses.
$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

nitrogen concentrations: $P < 0.05$, and -0.49). There were no significant differences in ambient phosphate concentrations between blocks or BM treatments, but only in the lugworm addition plots did the nutrient additions increase phosphate concentrations significantly (main effect of BM on phosphate concentrations in the nutrient addition plots: $F_{2,12} = 5.4$, $P < 0.05$; Tukey's HSD test: lugworm > no addition treatment, $P < 0.01$; lugworm > cockle addition treatment, $P = 0.07$; Table 1; Fig. 1b, c). There were no significant effects of the nutrient addition treatment on the number of cockles or lugworm heaps (Appendix S2), but the number of periwinkles responded positively to the nutrient additions in the cockle addition treatment (main effect of BM on LRR of periwinkles: $F_{2,12} = 14.4$, $P < 0.01$; Tukey's HSD test: cockle > lugworm addition treatment, $P < 0.05$; cockle addition > no addition control, $P = 0.07$; Appendix S2: Table S2; the 95% confidence intervals around average LRR do not cross zero in the cockle addition treatment, Appendix S2: Fig. S2 vi).

The microphytobenthos thrived in the artificial cockle beds: The cockle additions generated significant nutrient effects, promoted sediment accumulation, and increased RUE (Fig. 2). There were no significant effects of block or BM on chlorophyll a concentrations in the ambient nutrient plots, but in the nutrient addition plots, there was a trend toward higher chlorophyll a concentrations in the cockle addition areas compared to the lugworm addition areas (main effect of BM: $F_{2,12} = 4.3$, $P = 0.067$; Fig. 2a; Tukey's

HSD test: cockle addition > lugworm addition, $P < 0.01$; Table 2). Accordingly, in the cockle addition areas, the nutrient addition treatment increased chlorophyll a concentrations significantly by 1.9 times compared to ambient nutrient conditions (Fig. 2b), a nutrient effect that was consistent across blocks (LRR of chlorophyll a concentrations: Only the main effect of BM was significant; $F_{2,11} = 5.8$, $P < 0.05$; Table 2). In the lugworm addition and no addition control areas, there was no significant effect of nutrient enrichment on chlorophyll a concentrations in the sediment (only in the cockle addition treatment, the confidence intervals around the average LRR of chlorophyll a concentrations do not cross zero; Fig. 2b). The increase in chlorophyll a concentrations by adding nutrients in the cockle addition areas coincided with a significant accumulation of sediment over the summer, which increased the bed level with an average of 1.4 cm ±0.41 SD, while the sediment did not accumulate in the lugworm addition and no addition areas (main effect of BM: $F_{2,12} = 24.5$, $P < 0.01$; Tukey's HSD test: cockle > lugworm and no addition control $P < 0.01$, Table 2; only in the cockle addition treatment, confidence intervals around the average bed level change do not cross zero, Fig. 2b; see also Donadi et al. 2015b). Accordingly, the positive effect of adding nutrients on the microphytobenthos correlated significantly with an increase in average bed level (Pearson correlation of 2011 data: $r = 0.43$, $P < 0.05$, Fig. 2b). In the nutrient addition treatment, the cockle additions also increased RUE of the microphytobenthos
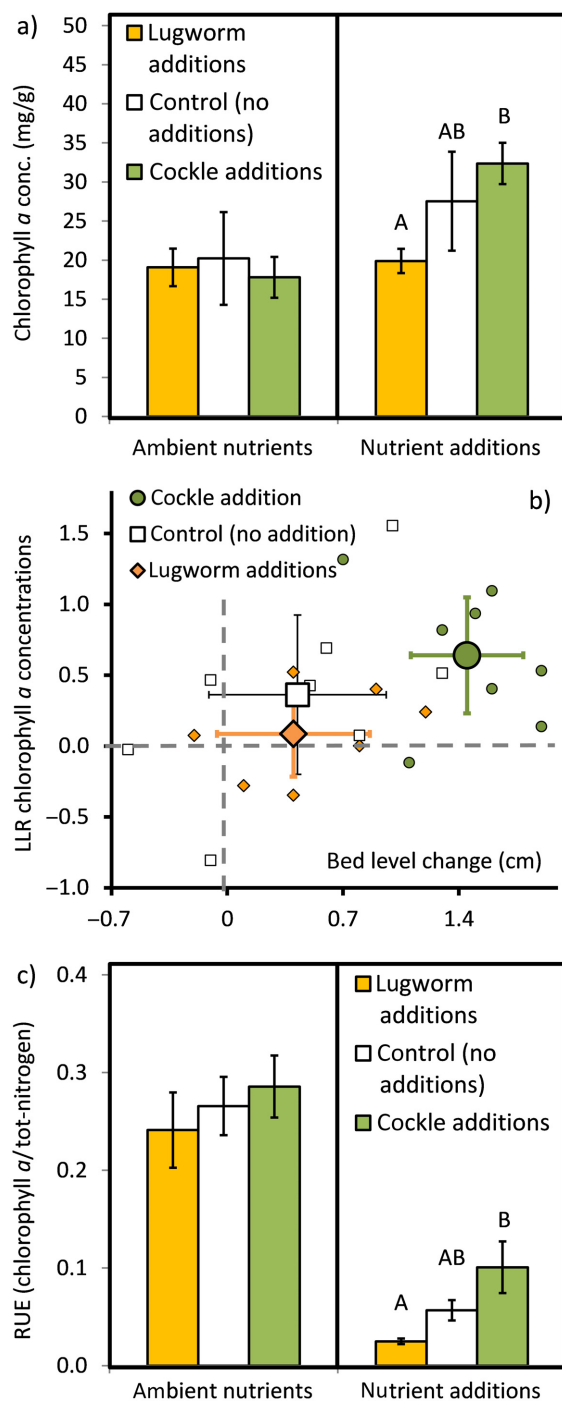
significantly compared to the lugworm addition treatment (main effect of BM: $F_{2,11} = 5.6$, $P < 0.05$; Tukey's HSD test: cockle > lugworm addition treatment, $P < 0.05$; Table 2; Fig. 2c). In ambient nutrient conditions, there were no effects of the experimental treatments on RUE (Table 2; Fig. 2c).

DISCUSSION

The results of our BM experiment, demonstrating positive effect on the biofilm from creating beds of burrowing bivalves (cockles), establish once again the fundamental importance of ecosystem engineers for coastal marine production (Donadi et al. 2013a, b, van der Zee et al. 2013, 2016, Kéfi et al. 2015). The artificial cockle beds enabled the microphytobenthos living in the sediment to use excess nutrients more effectively, and the higher resource uptake efficiency was related to more microphytobenthic biomass

(Fig. 2. *Continued*)
BM treatments (orange bars = lugworm addition; green bars = cockle addition; white bars = no addition control). Error bars in (a) show SE, and letters indicate significant different contrasts between bars within a panel from Tukey's HSD post hoc test. (b) The relation between log-response ratios ($n = 23$) of chlorophyll a concentrations to nutrient additions (y-axis) and bed level change over the summer (March to September, $n = 24$) (x-axis) in three BM treatments (orange diamond = lugworm addition; green circle = cockle addition; white squares = no addition control). The smaller symbols show individual BM areas; the larger symbols show averages with 95% confidence intervals for the three different BM treatments; if the confidence intervals do not cross the zero lines (striped line), the effect of nutrient addition is statistically significant. (c) Resource use efficiency (RUE) of the microphytobenthos estimated as the ratio of chlorophyll a in the sediment and concentrations of the limiting nutrient nitrogen in the porewater; in ambient nutrient (left panel = no nutrients added) and nutrient addition (right panel) treatments; and in three BM treatments (orange bars = lugworm addition; green bars = cockle addition; white bars = no addition control). Error bars in (c) show SE, and letters indicate significant different contrasts between bars within a panel from Tukey's HSD post hoc test.

Fig. 2. Effects of biomanipulation (BM) and nutrient treatments on chlorophyll a concentrations, bed level change, and resource uptake efficiency (RUE). (a) Chlorophyll a concentrations of the sediment in ambient nutrients (left panel = no nutrients added) and nutrient addition (right panel) treatments and in three

Table 2. Factorial ANOVA results of biomanipulation (BM; cockle addition, lugworm addition, no addition control) and a random factor block (four levels); on chlorophyll *a* concentrations in ambient and nutrient-enriched plots; the log-response ratio (LRR) of chlorophyll *a* concentrations to the nutrient enrichment treatment; resource uptake efficiency (RUE) of the limiting resource nitrogen in ambient and nutrient-enriched plots; and on bed level change from March to September 2011.

Effect	df	Chl *a* conc. in ambient nutrient plots		Chl *a* conc. in nutrient addition plots		LRR of chl *a* concentrations		RUE tot-N ambient nutrient plots		RUE tot-N nutrient addition plots		Bed level change	
		F	P	F	P	F	P	F	P	F	P	F	P
Intercept	1	1681.7	<0.001	804.2	<0.001	13.5	<0.05	107.1	<0.01	499.8	<0.001	37.2	<0.01
Block	3	0.6	0.615	3.3	0.099	1.8	0.241	4.0	0.069	1.5	0.308	3.2	0.107
BM	2	0.1	0.927	4.3	0.067	5.8	<0.05	1.0	0.413	5.6	<0.05	24.5	<0.01
Block × BM	6	0.9	0.522	1.4	0.291	0.3	0.939	0.4	0.871	0.7	0.639	0.3	0.913
Error	†	df = 12		df = 11		df = 11		df = 12		df = 11		df = 12	

† Due to loss of one porewater NH_4, one PO_4, and one chlorophyll *a* sample, df in the error term vary between analyses.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

under nutrient enrichment. Thus, by creating benign conditions, bed-forming ecosystem engineers create hotspots of production on the intertidal which fuel the food web by increasing the transition of nutrients into organic tissue. Our results indicate that ecological interactions, rather than the availability of nutrients per se, may set the limits for production in this coastal ecosystem.

Elevated nutrient use efficiency and increased biomass of the microphytobenthic biofilm under nutrient enrichment correlated with sediment accumulation. This suggests that the artificial beds of burrowing bivalves—cockles—facilitated the biofilm production and development by stabilizing the sediment and decreasing sediment erosion. High abundances of sediment-living bivalves may stabilize the sediment by providing a network of physical structures with their shells and mucus production that immobilizes the sediment surface. However, cockles may also enhance the chemical environment for the microphytobenthos by increasing the exchange of nutrients, carbon dioxide, and oxygen through the sediment (Aller 1982). The microphytobenthos in our study system are dominated by diatoms (own observation), and diatoms increase sediment cohesion significantly by producing extracellular polysaccharides (Paterson 1989, Paterson et al. 1990, Yallop et al. 1994, Bishop et al. 2012, McLeod et al. 2012). Thus, the cockles may initially have facilitated the microphytobenthos by improving the chemical properties of the sediment, and the lower sediment erosion was then caused by the developing biofilm itself. Most likely, both facilitation processes interact and reinforce each other, together creating benign conditions under which the biofilm can flourish. Interestingly, cockle recruitment was also facilitated by the increased sediment stabilization in the cockle addition plots (Donadi et al. 2014), suggesting a positive feedback where the cockles and the biofilm improve habitat conditions for each other and thereby increase both primary production and secondary production. On the contrary, creating habitats, dominated by a burrowing polychaete that bioturbates the sediment, did not improve the resource uptake efficiency of the biofilm.

Cockles may also influence the microphytobenthos by true food-web interactions, either by direct consumption or, indirectly, by changing the grazer community through ingestion of invertebrate larvae. Benthic algae contribute largely to the diet of cockles (Kang et al. 1999); thus, it is likely that the cockles preyed on and actually gained from the increased biofilm in the cockle addition areas, which would indicate that they support their own food source (Donadi et al. 2013*b*). The artificial cockle beds also increased the abundance of deposit feeders (such as *Corophium* ssp.) and grazers that feed on the biofilm (Donadi et al. 2015*b*), which would further increase the grazing rates. Our results also showed that periwinkles were attracted to the high biomass of the biofilm in the nutrient

addition plots in the cockle addition areas. However, the positive non-trophic effects seem to have outweighed any negative trophic effects in our experiment, highlighting the importance and strength of facilitation.

We have earlier demonstrated positive feedbacks and long-range interactions between bivalves on the tidal flats, where mussel reefs promote communities of burrowing cockles hundreds of meters away by reducing hydrodynamic stress (Donadi et al. 2013a, van de Koppel et al. 2015). This study indicates a positive non-trophic feedback between the burrowing cockles and the biofilm that have a large influence on nutrient cycling and energy transfer. Together, this suggests that we may have a strong linkage between the different intertidal ecosystems through a long-range facilitation cascade, where the engineering function of mussels promotes cockles across the tidal flat, which in turn facilitate the local production of the biofilm. Facilitation is an significant ecological process, especially in stressful environments (Bertness and Callaway 1994, Bruno et al. 2003), and the relative importance of positive ecosystem engineering effects is therefore assumed to increase with increasing environmental stress (Wright and Jones 2004, Badano and Cavieres 2006, Crain and Bertness 2006, Wright et al. 2006). Soft-sediment systems, such as tidal flats, are largely regulated by adverse hydrodynamic conditions that control sediment erosion (De Jonge and Van Beusekom 1995, Herman et al. 2001, Donadi et al. 2013b, 2015a). Stabilizing engineers such as reef- and bed-forming bivalves therefore have a large impact on community structure (Donadi et al. 2013a, 2014, van der Zee et al. 2013). Thus, to predict effects of different human impacts and future environmental changes on natural ecosystems, it is key that we increase our understanding of the role of non-trophic interactions for ecosystem resilience (Olff et al. 2009, Eriksson et al. 2010, van de Koppel et al. 2015).

CONCLUSION

By demonstrating emergent properties that develop in the interaction between bivalve and biofilm, we highlight the importance of ecological complexity for ecosystem health (Olff et al. 2009) and provide support for that soft-sediment systems may be highly vulnerable to non-linear catastrophic shifts in ecosystem functioning (Eriksson et al. 2010). In combination with the artificially constructed beds of burrowing bivalves (cockles), the nutrient enrichment treatment promoted the biofilm. This indicates that by improving local conditions, the bivalves shifted the biofilm community from being regulated by environmental stress toward being regulated by nutrient limitation and competition for resources—in accordance with the stress gradient hypothesis (Menge 1978, Menge and Sutherland 1987). Thus, against the background of hydrodynamic conditions, biological facilitation may set the limit for coastal production. Global trends of degraded coastal ecosystems, resulting in decreased water quality, ecosystem services, and ecosystem functioning, have prompted a variety of restoration efforts. Our results suggest that a first priority must be to restore the ability of the coastal communities to use resources effectively by restoring ecosystem complexity and the natural wealth of ecological interactions.

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